THE AMERICAN SOCIETY OF HUMAN GENETICS

57th Annual Meeting

October 23–27, 2007 • San Diego, California

2007 Program Committee

Elizabeth R. Hauser, *Chair*

Hans C. Andersson
Leslie G. Biesecker
Katrina M. Dipple
David B. Goldstein
Susanne M. Gollin
Albert R. La Spada
Michael Lovett
Miriam Meisler
Hope Northrup
Mary E. Norton
Margaret A. Pericak-Vance
Joan A. Scott
Lisa G. Shaffer
Mark D. Shriver
Hongyu Zhao

ASHG Administrative Office

Elaine Strass	Joann Boughman
Executive Director	*Executive Vice President*
Barbara Abbott	*Membership Manager*
Sharon Adler	*Accounting Assistant*
Yimang Chen	*Information Services Manager*
Phyllis Edelman	*Administrative Assistant*
Karen Goodman	*Administrative Assistant/ Receptionist*
Krista Koziol	*Director of Publications*
Jane Nelson	*Media Manager*
Kenna Shaw	*Director of Education*
Mary Shih	*Membership Assistant*
Dayna Silberman	*Program Coordinator*
Katie Van Horne	*Educational Programs Associate*
Toney Vogel	*Exhibits Manager*
Ray Wolfe	*Web Site Coordinator*
Hubert Zhang	*Programmer/Analyst*

FASEB Office of Scientific Meetings and Conferences

Geri Swindle, *Director*

Pauline Minhinnett, CMP, *Senior Meetings Manager*

Crystal Hiner, *Meeting Manager*

THE AMERICAN SOCIETY OF HUMAN GENETICS

9650 Rockville Pike
Bethesda
Maryland 20814-3998

telephone: (301) 634-7300
fax: (301) 634-7079
http://www.ashg.org/

THE AMERICAN SOCIETY OF HUMAN GENETICS

was organized in 1948. Its purpose is to encourage and integrate research, scholarship and eduation in all areas of human genetics, to bring into close contact investigators in the many general fields of research that involve human genetics, and to encourage discourse on applications of human genetics to society at large.

CONTENTS

Please refer to the ASHG Annual Meeting Web site at http://ashg.org/ (click on ASHG Annual Meetings) for searchable abstracts and meeting-related information including San Diego Convention Center floor plans, meeting schedule and highlights, scientific session listings, awards, and exhibitor information.

The meeting resources CD available to registrants also provides complete meeting information and searchable abstracts.

SPONSORS

The American Society of Human Genetics gratefully acknowledges the following sponsors:

AFFYMETRIX®

High School Workshop
and
Session 1: Genome-wide Association Studies in the Era of Open Data Access and Collaboration

---

BECKMAN COULTER®

High School Workshop

---

illumina®

High School Workshop
and
Undergraduate Genetics Education Workshop

---

nature genetics

www.nature.com/naturegenetics

Session 23 Refreshment Break

---

nature REVIEWS GENETICS
www.nature.com/reviews/genetics

Session 5 Refreshment Break

SEQUENOM®

Meeting Tote Bag

Springer
the language of science

Trainee-Mentor Luncheon

ADVERTISERS

AAAS/SCIENCE
Affymetrix, Inc.
Applied Imaging Corporation
Combimatrix Molecular Diagnostics
Deerac Fluidics
Elsevier
GE Healthcare
Genzyme Corporation
Idaho Technology, Inc.
Molecular Devices, now part of MDS Analytical Technologies
Nature Publishing Group
Oxford Gene Technology
Roche Applied Science
SEQUENOM, Inc.
Shire
Signature Genomic Laboratories, LLC
Whatman, Inc.
Xceed Molecular

FUTURE MEETINGS

2008	November 11–15	Philadelphia, Pennsylvania
2009	October 20–24	Honolulu, Hawaii
2010	November 2–6	Washington, District of Columbia
2011	October 11–15	12th International Congress of Human Genetics Montreal, Quebec, Canada (ASHG Annual Meeting subsumed)
2012	November 6–10	San Francisco, California
2013	October 22–26	Boston, Massachusetts
2014	October 18–22	San Diego, California

INCLUDED IN THIS BOOK

Schedule of All Sessions and Meetings

A complete schedule of all scientific sessions, and ancillary and satellite meetings, is provided for easy reference, beginning on page 7. Sessions listed in bold are ASHG scientific sessions and are open only to scientific (i.e., paid) registrants. Asterisks (*) denote functions that the organizer has indicated are by invitation only. For changes made after publication of this book, please consult on-site signs and the separately printed Program Addendum.

Platform Session Listings

Listings for all platform sessions have been included in this book beginning on page 17 to provide details including session date, time of day and location, and each presentation's start time. Platform presentations are listed in chronological order from 1–286.

Abstracts

Abstracts begin on page 34 and are divided into two sections: platform and poster presentations. In each section the numbers located above the title match program numbers and poster board numbers wherever abstracts are referenced. Platform presentations are numbered 1–286; poster presentations are numbered 287–2814.

Abstracts are also included on the CD available to meeting registrants, and on the Web at http://ashg.org/ (click on Annual Meetings).

A separate Program Guide contains details of scientific presentations.

Speaker and Author Index

A speaker and author index begins on page 526. Names of all invited speakers, presenting authors and co-authors are listed followed by their session or abstract number(s). Presenting author names are noted with an asterisk.

Permuted Title Index

This index, beginning on page 580, was produced by a computerized permutation process that creates an entry for every significant term in the title of each abstract and of selected special/invited presentations. Terms are listed alphabetically followed by text to provide subject context. An abstract number or session notation appears at the end of each index line.

SCHEDULE OF ALL SCIENTIFIC SESSIONS AND EVENTS

Ancillary and satellite meetings are reunion parties or small meetings of editorial boards, committees, etc., and are not official Society functions. **Asterisks (*) denote meetings that the organizer indicated are by invitation only. Otherwise, attendance may be assumed to be open to all registrants.** Listings in bold-face type indicate the event is an ASHG-sponsored scientific session open only to scientific registrants. Please consult the on-site bulletin board in the registration area of the San Diego Convention Center for changes.

MONDAY, October 22		
*8:00 AM - 5:00 PM	P3G: Public Population Projects in Genomics Meeting *For further details, contact Irene Toffolo at itoffolo@p3g.org*	San Diego Marriott Hotel and Marina, Santa Rosa
12:00 noon - 5:30 PM	The Future of Genomic Medicine *By advance registration only*	The Neurosciences Institute Auditorium, 10640 John Jay Hopkins Drive, San Diego, CA 92121
*3:00 PM - 10:00 PM	ASHG Board of Directors Meeting #1	Off Site
*6:30 PM - 9:30 PM	ABMG Accreditation Committee	San Diego Marriott Hotel and Marina, Pacific
*6:30 PM - 9:30 PM	ABMG Credentials Committee Meeting	San Diego Marriott Hotel and Marina, Laguna
*6:30 PM - 9:30 PM	ABMG MOC Committee	San Diego Marriott Hotel and Marina, Oceanside
TUESDAY, October 23		
*7:30 AM - 3:00 PM	P3G: Public Population Projects in Genomics Meeting *For further details, contact Irene Toffolo at itoffolo@p3g.org*	San Diego Marriott Hotel and Marina, Santa Rosa
8:00 AM - 4:00 PM	ASHG High School Workshop	Convention Center, Room 33
8:00 AM - 4:00 PM	ASHG Undergraduate Genetics Education Workshop	Convention Center, Room 25B
*8:00 AM - 5:00 PM	ACMG Board of Directors Meeting	San Diego Marriott Hotel and Marina, Solana
*8:00 AM - 7:00 PM	ABMG Board of Directors Meeting	San Diego Marriott Hotel and Marina, Del Mar
8:30 AM - 8:00 PM	Human Genome Variation Society Scientific Meeting with a focus on “Massive Paralel DNA Sequencing” & Annual General Meeting	Convention Center, Room 29
9:00 AM - 10:00 AM	High Throughput & Automated Cytogenetics Seminar	San Diego Marriott Hotel and Marina, Marina G
*9:00 AM - 11:00 AM	ASHG Social Issues Committee	Convention Center, Room 22

TUESDAY, October 23 *(continued)*		
*11:00 AM - 2:00 PM	GeneTests Editorial/Advisory Board Meeting	San Diego Marriott Hotel and Marina, Irvine
*11:00 AM - 2:00 PM	International Federation of Human Genetics Societies Executive Committee Meeting	Convention Center, Room 23A
*11:00 AM - 4:00 PM	ASHG Program Committee Meeting #1	Convention Center, Room 25A
12:00 noon - 6:00 PM	The Society of Craniofacial Genetics 30th Annual Meeting and Symposium: Branching Out: Using Evolution and Development as Tools to Understand Normal and Abnormal Variations in Craniofacial Morphology	Convention Center, Room 25C
1:00 PM - 2:00 PM	Case Studies in Dry-State Biosample Management	San Diego Marriott Hotel and Marina, Laguna
1:00 PM - 3:00 PM	Biomarker Discovery and Validation using MassARRAY Technology	San Diego Marriott Hotel and Marina, Marina G
1:00 PM - 5:00 PM	Hereditary Hearing Impairment Consortium	San Diego Marriott Hotel and Marina, Point Loma
3:00 PM - 7:00 PM	**Registration Open**	**Convention Center, Hall D Lobby**
*3:00 PM - 8:00 PM	ASHG Board of Directors Meeting #2	Convention Center, Room 22
4:00 PM - 6:00 PM	Introduction to Galaxy and Rgenetics for Software Developers	San Diego Marriott Hotel and Marina, Leucadia
*4:30 PM - 7:30 PM	ASHG Information and Education Committee Meeting	Convention Center, Room 25A
5:00 PM - 6:30 PM	Publishing Your Science: Tips from the Editors of *Science* on Writing Successful Papers	San Diego Marriott Hotel and Marina, Santa Rosa
5:00 PM - 7:00 PM	The Genomic Basis of Disease: Clinical Implementation of High-Resolution Genome Analysis	San Diego Marriott Hotel and Marina, Point Loma
*6:00 PM - 9:00 PM	ACMG Lab QA Biochemical Subcommittee	San Diego Marriott Hotel and Marina, Oceanside
*6:00 PM - 9:00 PM	ACMG Lab QA Cytogenetics Subcommittee	San Diego Marriott Hotel and Marina, Newport Beach
*6:00 PM - 9:00 PM	ACMG Lab QA Molecular Subcommittee	San Diego Marriott Hotel and Marina, Pacific
6:30 PM - 8:30 PM	Cleveland Clinic Genomic Medicine Institute Reception	Convention Center, Center Terrace
*6:30 PM - 9:00 PM	*American Journal of Medical Genetics* Editorial Board Meeting	San Diego Marriott Hotel and Marina, Mission Hills

8:00 PM - 10:00 PM	**Opening Mixer**	**San Diego Marriott Hotel and Marina, Marina D/E**
WEDNESDAY, October 24		
7:30 AM - 6:00 PM	**Registration Open**	**Convention Center, Hall D Lobby**
8:00 AM - 9:30 AM	**Concurrent Education Sessions I:** **1. Genome-Wide Association Studies in the Era of Open Data Access and Collaboration** *Sponsored by Affymetrix, Inc.* **2. The Genetics and Evolutionary History of the MHC and KIR Region Genes and Their Impact on Human Health** **3. Molecular Biology of Genetic Diseases of the Skin: Progress and Perspectives**	**Convention Center,** **Room 20D** **Room 20B/C** **Room 20A**
8:00 AM - 9:30 AM	**Concurrent Social Issues Sessions I:** **4. Genetics Policy and Educational Issues in the Response to Hurricane Katrina: Future Implications for Mass Fatalities** **5. Understanding ART: The Ethics of Assisted Reproductive Technology**	**Convention Center,** **Room 30** **Room 28**
10:00 AM - 11:30 AM	**Concurrent Education Sessions II:** **6. Proteomics for Geneticists: New Tools and Applications from the NHLBI National Proteomics Centers** **7. The Scientist's Role in Improving Genetic Education and Awareness** **8. Designing Geneticists: Study Design Issues in Population-based Genetics and Genomics Research**	**Convention Center,** **Room 20B/C** **Room 28** **Room 20D**
10:00 AM - 11:30 AM	**Concurrent Social Issues Sessions II:** **9. Genomic Profiling: The Good, the Bad, and the Unknown!** **10. DNA as Unique Identifier: Privacy, Trust, and the Future of Genomic Biorepositories**	**Convention Center,** **Room 20A** **Room 30**
10:30 AM - 6:30 PM	**Posters and Exhibits Open**	**Convention Center, Exhibit Hall E**
11:30 AM - 1:00 PM	**Curbstone Consults: Review and Discussion of Unique Cases - Especially for Clinicians and Counselors**	**Convention Center, Room 24B**
11:30 AM - 1:00 PM	ABMG Business and Program Directors Meeting	Convention Center, Room 31A/B
11:30 AM - 1:00 PM	Affymetrix Seminar on Genetic Analysis: Learn How You Can Find it Fast with Affymetrix Products	San Diego Marriott Hotel and Marina, Marina E
11:30 AM - 1:00 PM	An Update on Human Genomic Resources at the NCBI	San Diego Marriott Hotel and Marina, Point Loma

WEDNESDAY, October 24 *(continued)*		
*11:30 AM - 1:00 PM	ASHG Trainee-Mentor Luncheon *By advance ticket purchase only*	Convention Center, Room 33
11:30 AM - 1:00 PM	Demonstration and Application of the Rosetta Syllego System v1.0 in Genetic Data Management and Analysis Workflows	San Diego Marriott Hotel and Marina, Marina D
11:30 AM - 1:00 PM	Genome-Wide Copy Number Variation Association Studies Including the unSNPable Genome	San Diego Marriott Hotel and Marina, Mission Hills
11:30 AM - 1:00 PM	Integrated Solutions for Optimal Sensitivity for Array CGH	San Diego Marriott Hotel and Marina, Santa Rosa
*11:30 AM - 1:00 PM	New Sample and Assay Technologies from QIAGEN	San Diego Marriott Hotel and Marina, Cardiff
*11:30 AM - 1:00 PM	The Genome Sequencer FLX System: The Second Generation of Sequencing Technology	San Diego Marriott Hotel and Marina, Marina F
11:30 AM - 1:00 PM	When to Screen and When to Treat in Lysosomal Storage Diseases?	San Diego Marriott Hotel and Marina, Marina G
1:00 PM - 1:30 PM	**11. Presidential Address: Who Is Under the Umbrella - and Why Are We Here?**	**Convention Center, Hall H**
1:30 PM - 3:30 PM	**12. Plenary Abstract Presentations**	**Convention Center, Hall H**
3:30 PM - 4:30 PM	**13. The Peter and Patricia Gruber Foundation's Gruber Prize**	**Convention Center, Hall H**
4:30 PM - 6:30 PM	**Poster Session I**	**Convention Center, Exhibit Hall E**
6:00 PM - 7:00 PM	Introduction to Rgenetics and Galaxy for Biologists	San Diego Marriott Hotel and Marina, Marina G
6:00 PM - 8:00 PM	Multiplex Gene Expression: Addressing Multiple Research Arenas	San Diego Marriott Hotel and Marina, Marina F
6:30 PM - 7:30 PM	Progeny LAB Software Presentation – Getting Personal with Genomic Data: Accomplish total integration of both clinical and genomic data along with any associated samples without compromising sensitive patient information.	San Diego Marriott Hotel and Marina, Marina E
6:30 PM - 8:00 PM	ABMG Open Forum - EMOC and New Portal	Convention Center, Room 31A/B
6:30 PM - 8:00 PM	Baylor College of Medicine Molecular and Human Genetics Reception	San Diego Marriott Hotel and Marina, Marina D
*6:30 PM - 8:00 PM	Careers/Job Fair Meet'n Greet	Convention Center, Hall D Lobby
*6:30 PM - 8:30 PM	ACMG Professional Practice & Guidelines Committee	Convention Center, Room 25A
*6:30 PM - 9:00 PM	Human Mutation Editorial Board Meeting	San Diego Marriott Hotel and Marina, Point Loma

*6:30 PM - 9:30 PM	ACMG Laboratory Quality Assurance Committee	Convention Center, Room 22
6:30 PM - 9:30 PM	CME Symposium	Convention Center, Room 33
6:30 PM - 10:00 PM	Neurofibromatosis Symposium - 2007	San Diego Marriott Hotel and Marina, Santa Rosa
8:00 PM - 10:00 PM	**14. Trainee Program: Can I Get There from Here? Different Career Paths for Scientists** Reception to follow on Plaza Terrace.	**Convention Center, Room 28**
THURSDAY, October 25		
*7:00 AM - 8:00 AM	ACMG Membership Committee Meeting	Convention Center, Room 22
7:00 AM - 8:00 AM	National MPS Society Scientific Advisory Board Meeting	San Diego Marriott Hotel and Marina, Point Loma
7:30 AM - 5:00 PM	**Registration Open**	**Convention Center, Hall D Lobby**
8:00 AM - 10:30 AM	**Concurrent Platform Sessions I:** **15. Fragile X: From Bench to Population** **16. Autoimmunity and Genetic Associations** **17. Statistical Analysis of Genome-Wide Association Studies** **18. Genomics** **19. Metabolic Disorders** **20. Perinatal and Reproductive Genetics** **21. Cancer Genetics and Cytogenetics**	**Convention Center,** **Hall H** **Room 20A** **Room 20B/C** **Room 20D** **Room 28** **Room 29** **Room 30**
8:00 AM - 6:30 PM	**Posters Open**	**Convention Center, Exhibit Hall E**
10:30 AM - 6:30 PM	**Exhibits Open**	**Convention Center, Exhibit Hall E**
11:00 AM - 1:00 PM	**Concurrent Invited Sessions I:** **22. Pathways of Brain Development: Genetic and Phenotypic Characterization of Malformations of Cortical and Cerebellar Development** **23. Is Linkage Dead? The Future of Linkage Analysis and Family Data in the Genome-wide Association Era** **24. Complex Human Disease Genes: Help from Animal Models** **25. Challenges with Expanded Newborn Screening Using Tandem Mass Spectroscopy** **26. RNA Interference and Human Disease** **27. The Ciliopathies: Diverse Phenotypes, Common Mechanisms, Unexpected Functions** **28. A Balanced Genome: X-autosome Dosage Compensation**	**Convention Center,** **Room 29** **Room 20A** **Room 20D** **Room 20B/C** **Hall H** **Room 28** **Room 30**

THURSDAY, October 25 *(continued)*		
11:30 AM - 1:00 PM	GOLD, Global Organisation for Lysosomal Diseases' Annual General Meeting	San Diego Marriott Hotel and Marina, Santa Rosa
1:00 PM - 2:00 PM	*AJHG* Editorial Board Meeting	Convention Center, Room 22
1:00 PM - 2:00 PM	CGH - New Applications	San Diego Marriott Hotel and Marina, Marina F
*1:00 PM - 2:00 PM	High-Resolution Melting and More on the LightCycler 480 Real-Time PCR System	San Diego Marriott Hotel and Marina, Mission Hills
*1:00 PM - 2:00 PM	Human Molecular Genetics Editorial Board Meeting	Convention Center, Room 25A
1:00 PM - 2:00 PM	Illumina Technology Workshop	San Diego Marriott Hotel and Marina, Marina G
*1:00 PM - 2:00 PM	Latest Advancements in the use of Whole Genome Amplification for Genotyping	San Diego Marriott Hotel and Marina, Marina E
1:00 PM - 2:00 PM	Pharmacological Chaperones: A Novel Investigational Approach for Treating a Broad Range of Human Genetic Diseases	San Diego Marriott Hotel and Marina, Marina D
1:00 PM - 2:00 PM	Solutions Enabling Personalized Medicine	San Diego Marriott Hotel and Marina, Del Mar
2:00 PM - 4:30 PM	**Concurrent Platform Sessions II:** **29. Feeling Left Out: What Do Deletions Really Mean?** **30. Complex Disease Mechanisms** **31. Neurogenetics** **32. Statistical Genetics and Genetic Epidemiology** **33. Molecular Basis of Mendelian Disorders I** **34. Genetic Counseling and Clinical Services** **35. Cardiovascular Genetics**	**Convention Center,** **Hall H** **Room 20A** **Room 20B/C** **Room 20D** **Room 28** **Room 29** **Room 30**
4:30 PM - 6:30 PM	**Poster Session II**	**Convention Center, Exhibit Hall E**
6:00 PM - 8:30 PM	Harvard Medical School Genetics Training Program Reception	San Diego Marriott Hotel and Marina, Cardiff
6:30 PM - 7:30 PM	Working with the HapMap Website (Tutorial)	San Diego Marriott Hotel and Marina, Pacific
6:30 PM - 8:00 PM	Christian Fellowship of Human Geneticists	Horton Grand Hotel, Courtyard Regency
6:30 PM - 8:00 PM	Cornelia de Lange Syndrome Open Forum	San Diego Marriott Hotel and Marina, Irvine

6:30 PM - 8:00 PM	Mutation Discovery and Genotyping via Hi-Res Melting using the LightScanner System. Reception sponsored by Idaho Technology, Inc.	San Diego Marriott Hotel and Marina, Warner Center
6:30 PM - 8:00 PM	National Institute of Mental Health (NIMH) New Investigator Outreach	San Diego Marriott Hotel and Marina, Torrance
6:30 PM - 8:00 PM	UCLA Intercamus Medical Genetics Training Program and UCLA Department of Human Genetics Reception	San Diego Marriott Hotel and Marina, Santa Rosa
6:30 PM - 8:30 PM	Emory University Department of Human Genetics Reception *For further information contact jgclark@emory.edu*	Stingaree, Rooftop Oasis, 454 6th Avenue, San Diego, CA 92101
6:30 PM - 8:30 PM	Pittsburgh Reunion	San Diego Marriott Hotel and Marina, Leucadia
6:30 PM - 8:30 PM	The Human Variome Project	Convention Center, Room 32A
6:30 PM - 8:30 PM	University of Alabama at Birmingham, Department of Genetics, Reception	San Diego Marriott Hotel and Marina, Point Loma
6:30 PM - 9:00 PM	Human Genetics and Epigenetics: High-Definition Microarray Analysis of DNA Copy Number Variation (CGH), Chromatin Structure (ChIP-chip), and Non-coding RNA Expression	San Diego Marriott Hotel and Marina, Marina G
6:30 PM - 9:30 PM	A Closer Look at MPS: Emerging Issues and Solutions	San Diego Marriott Hotel and Marina, Marina E/F
6:30 PM - 9:30 PM	Annual Meeting of the Association of Chinese Geneticists in America (ACGA), followed by a banquet *For further information contact wu_b@tch.harvard.edu*	Convention Center, Room 32B
*6:30 PM - 12:00 midnight	GE Healthcare Launch Party	Off Site
7:30 PM - 9:30 PM	University of Maryland School of Medicine Program in Genetics and Genomic Medicine	San Diego Marriott Hotel and Marina, Laguna
8:00 PM - 10:30 PM	Indiana University School of Medicine Department of Medical & Molecular Genetics Alumni Reception	San Diego Marriott Hotel and Marina, Mission Hills
8:00 PM - 11:00 PM	University of Michigan Department of Human Genetics Alumni Gathering	San Diego Marriott Hotel and Marina, Solana
9:00 PM - 11:00 PM	CHOP/PENN Reunion	San Diego Marriott Hotel and Marina, Del Mar

FRIDAY, October 26		
7:45 AM - 5:00 PM	**Registration Open**	**Convention Center, Hall D Lobby**
8:00 AM - 10:30 AM	**Concurrent Platform Sessions III:** **36. Noncoding RNAs** **37. Animal Models** **38. Psychiatric Genetics** **39. Letting the Genie Out of the Bottle: Genotype/ Phenotype Correlations** **40. Mitochondria and Disease** **41. Cytogenetics** **42. Methods in Gene Mapping**	**Convention Center,** **Hall H** **Room 20A** **Room 20B/C** **Room 20D** **Room 28** **Room 29** **Room 30**
8:00 AM - 1:30 PM	**Posters Open**	**Convention Center, Exhibit Hall E**
10:30 AM - 12:30 PM	**Poster Session III**	**Convention Center, Exhibit Hall E**
10:30 AM - 1:30 PM	**Exhibits Open**	**Convention Center, Exhibit Hall E**
12:00 noon - 1:30 PM	**Curbstone Consults: Review and Discussion of Unique Cases - Especially for Clinicians and Counselors**	**Convention Center, Room 24B**
12:30 PM - 1:30 PM	A New Standard For High Throughput Genotyping Using Dynamic Array	San Diego Marriott Hotel and Marina, Marina D
*12:30 PM - 1:30 PM	ACMG Economics Committee Meeting	Convention Center, Room 22
*12:30 PM - 1:30 PM	ASHG Program Committee Meeting #2	Convention Center, Room 25A
12:30 PM - 1:30 PM	Association of Professors of Human and Medical Genetics (APHMG) Institutional Representatives Meeting	Convention Center, Room 32A
12:30 PM - 1:30 PM	Professional Women in Genetics Lunch	Convention Center, Room 32B
1:30 PM - 2:15 PM	**43. William Allan Award Presentation and Lecture**	**Convention Center, Hall H**
2:15 PM - 2:30 PM	**44. Leadership Award Presentation**	**Convention Center, Hall H**
2:30 PM - 3:30 PM	**45. ASHG Membership/Business Meeting**	**Convention Center, Hall H**

4:00 PM - 6:00 PM	**Concurrent Invited Sessions II:** **46. Human Brain Evolution: What Makes Us Unique?** **47. SNP Associations in Complex Disease, their Functional Consequences, and the Biological Meaning of Epistasis** **48. The Causes of Autism Spectrum Disorders: Clues, Views, and News** **49. Application of Recent Genetic Advances to Prenatal Diagnosis: Maternal Serum Screening, Molecular Diagnostic 'Power' and Ethical/ Cost Effective Use** **50. Thinking Outside the Colon: An Update on Non-colorectal Cancer Risk in Hereditary Polyposis Syndromes** **51. The Cohesinopathies: Developmental Disorders of Chromatin Function** **52. Submicroscopic Chromosomal Duplications and Deletions: Medical Consequences and Population Genetics**	**Convention Center,** **Hall H** **Room 20D** **Room 20A** **Room 28** **Room 30** **Room 29** **Room 20B/C**
6:00 PM - 8:00 PM	Canadian College of Medical Geneticists - Institute of Genetics, CIHR Mixer	San Diego Marriott Hotel and Marina, Santa Rosa
6:00 PM - 8:00 PM	PKU Therapy: A New Tool for Long-Term Success	San Diego Marriott Hotel and Marina, Marina D
8:00 PM - 9:30 PM	**53. Presidential Symposium: Genes and Identity**	**Convention Center, Hall H**
SATURDAY, October 27		
7:45 AM - 12:00 noon	**Registration Open**	**Convention Center, Hall D Lobby**
8:00 AM - 10:30 AM	**Concurrent Platform Sessions IV:** **54. Regulatory Element Discovery and Function** **55. Cancer Genetics** **56. Getting Under the Skin** **57. Evolution and Population Genetics** **58. Diabetes and Growth** **59. Therapy for Genetic Disorders** **60. Molecular Basis of Mendelian Disorders II**	**Convention Center,** **Hall H** **Room 20A** **Room 20B/C** **Room 20D** **Room 28** **Room 29** **Room 30**
11:00 AM - 12:30 PM	**61. Special Plenary Symposium: Evolution and Medicine**	**Convention Center, Hall H**
12:30 PM - 2:00 PM	**62. Mock Study Section** *By advance ticket purchase only. Arrive 15 minutes early to pick up lunch.*	**Convention Center, Room 33**
*12:30 PM - 2:00 PM	ASHG Awards Committee Lunch	Convention Center, Room 25A
2:00 PM - 2:15 PM	**63. ASHG Trainee Award Presentations**	**Convention Center, Hall H**

SATURDAY, October 27 *(continued)*		
2:15 PM - 2:30 PM	**64. C. W. Cotterman Award**	**Convention Center, Hall H**
2:30 PM - 2:50 PM	**65. Curt Stern Award Presentation**	**Convention Center, Hall H**
2:50 PM - 3:15 PM	**66. ASHG Award for Excellence in Human Genetics Education**	**Convention Center, Hall H**
3:15 PM - 5:15 PM	**67. Distinguished Speakers' Symposium: The Bridge Between Research and Practice**	**Convention Center, Hall H**
7:00 PM - 11:00 PM	**An Evening at San Diego's Natural History Museum Featuring the Dead Sea Scrolls** *By advance ticket purchase only. Further details will be provided via e-mail to all registered attendees.*	**San Diego Natural History Museum, 1788 El Prado, San Diego, CA 92101**

Wednesday, October 24 1:30 PM–3:30 PM

NOTES

SESSION 12 – Plenary Abstract Presentations
Hall H
Co-Moderators: Elizabeth R. Hauser, Duke University, Durham, NC; and Wylie Burke, University of Washington School of Medicine, Seattle

1/1:30 **Comparative sequence analysis of primate subtelomeres.** K. Rudd, R. Endicott, C. Friedman, M. Walker, J. Young, K. Osoegawa, R. Blakesley, P. de Jong, E. D. Green, B. Trask.

†**2**/1:50 **Large-scale evaluation of polymorphisms in predicted microRNA binding sites reveal effect on mRNA expression levels.** M. Jain, F. Pettersson, J. M. Taylor, J. L. Min, J. C. Barrett, J. Broxholme, M. I. McCarthy, K. T. Zondervan, L. R. Cardon, C. M. Lindgren.

†**3**/2:10 **Identification of a novel gene responsible for Charcot-Marie-Tooth disease (CMT4J).** C. Y. Chow, Y. Zhang, J. J. Dowling, N. Jin, M. Adamska, K. Shiga, K. Szigeti, M. E. Shy, J. Li, X. Zhang, J. R. Lupski, L. S. Weisman, M. H. Meisler.

4/2:30 **Bezafibrate cures clinical and metabolic symptoms of the muscular form of CPT2 deficiency.** F. Djouadi, J. Bastin, P. Laforet, F. Aubey, A. Mogenet, S. Romano, A. Vassault, S. Gobin, B. Eymard, J. L. Bresson, J. P. Bonnefont.

5/2:50 **Genetic and functional characterization of BRCA1 and BRCA2 variants of uncertain significance.** D. Goldgar, D. Easton, S. Tavtigian, C. Frye, M. Agarwal, D. Farrugia, F. Couch.

6/3:10 **Joint genome-wide analysis of 3200 Crohn disease patients documents more than 20 significant associations.** M. J. Daly on behalf of Crohn's Disease GWA Meta-analysis Working Group.

† Trainee Award Finalist

Thursday, October 25 8:00 AM–10:30 AM
Concurrent Platform Sessions I (15–21)

SESSION 15 – Fragile X: From Bench to Population
Hall H
Co-Moderators: Flora Tassone, University of California, Davis; and Elizabeth M. Berry-Kravis, Rush University Medical Center, Chicago, IL

7/8:00 **Fragile X mental retardation protein deficiency leads to spontaneous mGluR5-dependent internalization of AMPA receptors.** M. Nakamoto, V. Nalavadi, M. P. Epstein, U. Narayanan, G. J. Bassell, S. T. Warren.

8/8:15 **Genetic interaction between the fragile X mental retardation protein and Brachyury during mammalian embryonic development.** R. Alisch, P. Jin, M. Epstein, T. Caspary, S. Warren.

†**9**/8:30 ***FMR4*: a Novel Primate-Specific Transcript Silenced in Fragile X Syndrome.** A. Khalil, M. Faghihi, F. Modarresi, C. Wahlestedt.

10/8:45 **Penetrance of dementia in male carriers of the FMR1 premutation.** S. Jacquemont, M. Sevin, Z. Kutalik, P. Damier, M. Verceletto, P. Renou, P. Boisseau, S. Bergmann, J. M. Rival, J. S. Beckmann.

11/9:00 **Identification of novel small molecules suppressing rCGG-repeat-mediated neuronal toxicity.** A. Qurashi, H. Liu, P. Jin.

†**12**/9:15 **GABA agonists rescue morphological, biochemical and behavioral phenotypes of the Drosophila model of fragile X syndrome.** S. Chang, S. M. Bray, D. C. Zarnescu, P. Jin, S. T. Warren.

13/9:30 **Genetic mechanisms of trinucleotide repeat instability in Drosophila.** J. Jung, N. M. Bonini.

14/9:45 **Access to Credible Genetics Resources Network.** S. Terry, M. Weaver, K. Reed, H. Ferguson, C. Constantin, A. Vatave, C. Greene, A. Gepp, K. Clapp, P. Furlong, J. McInerney, M. Blitzer.

15/10:00 **Fragile X syndrome newborn detection: Pilot study.** R. Saul, M. Friez, K. Eaves, G. Stapleton, J. Collins, R. Stevenson.

16/10:15 **Offering carrier screening for fragile X syndrome to non-pregnant women.** S. Metcalfe, A. Archibald, J. Cohen, V. Collins, A. Henry, A. Jaques, K. McNamee, L. Sheffield, H. Slater, S. Wake.

Thursday, October 25 8:00 AM–10:30 AM
Concurrent Platform Sessions I (15–21)

SESSION 16 – Autoimmunity and Genetic Associations
Room 20A
Co-Moderators: Mark Daly, Massachusetts General Hospital, Boston; and Silke Schmidt, Duke University, Durham, NC

17/8:00 **Genome-wide association scan identifies new susceptibility loci for psoriatic arthritis and psoriasis.** P. Y. Liu, C. Helms, J. Gardner, A. Perlmutter, A. Miner, S. Duan, R. Donaldson, C. Wise, P. Kwok, W. Liao, N. L. Saccone, J. Worthington, A. Barton, A. Menter, A. M. Bowcock.

18/8:15 **A common copy number variant (CNV) associated with psoriasis.** R. Cid, L. Armengol, E. Ballana, M. Garcia, R. Pujol, X. Estivill.

19/8:30 **High density SNP screening of the major histocompatibility complex (MHC) in systemic lupus erythematosus (SLE) families demonstrates strong evidence for independent susceptibility regions.** L. F. Barcellos, S. L. Clark, P. P. Ramsay, H. Quach, M. F. Seldin, J. B. Harley, K. Moser, T. W. Behrens, P. Gaffney, L. A. Criswell.

20/8:45 **Mutations in the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus.** M. Lee-Kirsch, M. Gong, D. Choudhury, L. Senenko, K. Engel, Y. Lee, U. de Silva, T. Witte, T. J. Vyse, J. Kere, C. Pfeiffer, S. Harvey, S. Koskenmies, K. Rohde, A. F. Dominiczak, M. Gahr, T. Hollis, F. W. Perrino, J. Lieberman, N. Hubner.

21/9:00 **Whole Genome Association Study Identifies Novel Risk Alleles for Multiple Sclerosis.** J. L. Haines for The International Multiple Sclerosis Genetics Consortium.

22/9:15 **Fine mapping of a risk gene for multiple sclerosis.** D. Reich, N. Patterson, P. L. De Jager, A. Tandon, S. McCarroll, A. Waliszewska, J. Neubauer, C. Schirmer, R. R. Lincoln, S. Poduslo, O. Khan, S. L. Hauser, J. R. Oksenberg, D. A. Hafler.

23/9:30 **On the identification of causal genetic effects in family-based association studies.** C. Lange, S. Goetgeluk, I. Waldman, S. T. Weiss, S. VanSteelandt.

24/9:45 **Candidate Genes for Asthma and Atopy.** D. Daley, M. Lemire, P. D. Paré, A. J. Sanford, A. L. Kozyrskyj, C. Laprise, Y. Bosse, A. Motpetit, A. Becker, D. Zamar, B. Tripp, J. He, K. Tremblay, A. James, A. W. Musk, L. J. Palmer, T. J. Hudson.

25/10:00 **Genome-Wide Association Study (GWAS) Reveals a Novel Gene for Immunoglobulin E (IgE) Levels and Asthma.** Z. Tan, Y. Sun, L. Pan, R. Nicolae, S. Kudaravalli, A. Heinzmann, T. Kurz, J. E. Gern, R. F. Lemanske, Jr., K. A. Deichmann, J. K. Pritchard, D. Nicolae, A. I. Sperling, C. Ober.

26/10:15 **Large scale replication of a genome-wide association study in celiac disease.** K. A. Hunt, L. Franke, R. G. William, A. Zhernakova, M. Inouye, W. McLaren, R. McManus, R. McGinnis, L. R. Cardon, P. Deloukas, C. Wijmenga, D. A. van Heel.

† Trainee Award Finalist

Thursday, October 25 8:00 AM–10:30 AM
Concurrent Platform Sessions I (15–21)

SESSION 17 – Statistical Analysis of Genome-Wide Association Studies
Room 20B/C
Co-Moderators: Glen Satten, Centers for Disease Control and Prevention, Atlanta, GA; and Sanjay S. Shete, M. D. Anderson Cancer Center, Houston, TX

27/8:00 **A Bayesian multipoint allele sharing method for genome-wide studies.** Z. Su, P. Donnelly, J. Marchini.

28/8:15 **Analysis of Whole-Genome Data by Homozygosity Mapping and Adjustments for Relatedness.** B. F. Voight, D. Altshuler, M. J. Daly, representing the Diabetes Genetics Initiative.

29/8:30 **Simultaneous analysis of genome-wide SNP data and candidate region sequence data.** C. J. Hoggart, J. C. Whittaker, M. De Iorio, D. J. Balding.

30/8:45 **Efficient and Flexible Testing of Untyped Variants in Case-Control Studies.** M. P. Epstein, A. S. Allen, G. A. Satten.

31/9:00 **Genetic similarity matching for genome-wide association studies.** W. Guan, L. Liming, G. R. Abecasis, M. Boehnke.

32/9:15 **Powerful Bayesian gene-gene interaction analysis.** T. Ferreira, P. Donnelly, J. Marchini.

33/9:30 **Estimating significance thresholds for genomewide association scans.** F. Dudbridge, A. Gusnanto.

†**34**/9:45 **A general approach to combining genomewide association datasets.** J. C. Barrett, S. Purcell, M. J. Daly, L. R. Cardon.

35/10:00 **Fast and highly accurate haplotype inference for genome-wide datasets.** B. N. Howie, J. L. Marchini, P. Donnelly.

36/10:15 **Rapid and accurate haplotype phasing and missing data inference for whole genome association studies using localized haplotype clustering.** S. R. Browning, B. L. Browning.

Thursday, October 25 8:00 AM–10:30 AM
Concurrent Platform Sessions I (15–21)

SESSION 18 – Genomics
Room 20D
Co-Moderators: Stanley F. Nelson, Massachusetts General Hospital, Boston; and Michael E. Zwick, Emory University School of Medicine, Atlanta, GA

37/8:00 **EURExpress, a web-based transcriptome atlas of the developing mouse embryo.** G. Diez-Roux, The EURExpress Consortium.

38/8:15 **Nonsense-mediated mRNA decay modulates cellular fate in response to DNA damage.** D. Huang, F. Spencer, H. C. Dietz.

39/8:30 **Pooled heteronuclear RNA sequencing: a new tool for large-scale cis-acting regulatory haplotype discovery.** T. Pastinen, E. Grundberg, K. Lam, B. Ge, S. Gurd, N. Martin, E. Harmsen, T. Kwan, J. Majewski.

40/8:45 **Genome-wide analysis of transcript isoform variation in humans.** T. Kwan, D. Benovoy, C. Dias, S. Gurd, C. Provencher, T. J. Hudson, R. Sladek, J. Majewski.

41/9:00 **Copy number variations and gene expression in the mouse.** A. Reymond, C. Henrichsen, N. Vinckenbosch, E. Chaignat, S. Zoellner, H. Kaessmann.

42/9:15 **Genome-wide mapping and sequencing of Structural Variation using High-Resolution Paired-End Mapping (HR-PEM).** A. E. Urban, J. O. Korbel, J. Affourtit, F. Grubert, P. Kim, B. Taillon, D. Palejev, N. Carriero, L. Du, B. Godwin, J. Simons, J. Chi, F. Yang, M. Hurles, N. Carter, S. Weissman, T. Harkins, M. Gerstein, M. Egholm, M. Snyder.

43/9:30 **Advances in Sequencing Technology : enabling and Expanding Applications in Human and Cancer Genetics.** S. B. Gabriel, C. Russ, J. Baldwin, C. Sougnez, S. Fisher, P. Cahill, R. Onofrio, R. Nicol, T. Bloom, K. Cibulskis, W. Brockman, P. Alvarez, D. B. Jaffe, D. Altshuler, C. Nusbaum, E. S. Lander.

44/9:45 **Complete genome sequencing to high coverage of a single individual: James Watson.** D. A. Wheeler, M. E. Egholm, M. Srinivasan, A. L. McGuire, W. He, L. V. Nazareth, Y. Huan, Y. Liu, J. R. Lupski, D. M. Muzny, G. M. Weinstock, R. A. Gibbs.

45/10:00 **Microarray-based Direct Genomic Selection for High Throughput Resequencing.** M. E. Zwick, K. Meltz-Steinberg, C. Middle, T. Albert, D. Okou.

46/10:15 **The pattern of RET mutations and variants in Hirschsprung disease: a medical sequencing case study.** L. Hao, S. Arnold, J. Albertus, M. Dao, A. Rea, P. Cruz, J. Mullikin, A. Young, E. D. Green, A. Chakravarti.

† Trainee Award Finalist

Thursday, October 25 8:00 AM–10:30 AM
Concurrent Platform Sessions I (15–21)

SESSION 19 – Metabolic Disorders
Room 28
Co-Moderators: Joel Charrrow, Children's Memorial Hospital, Chicago, IL; and Nicola Longo, University of Utah, Salt Lake City

47/8:00 **Clinical and Molecular Analysis of Arylsulfatase E in Patients with Brachytelephalangic Chondrodysplasia Punctata.** N. Braverman, C. Matos, M. Maeda, L. Chen, J. Allanson, C. Armour, C. Greene, M. Kamaluddeen, D. Rita, L. Medne, E. Zackai, S. Mansour, A. Superti-Furga, A. Lewanda, M. Bober, K. Rosenbaum, M. Nino.

48/8:15 **A novel form of cerebellar ataxia with increased free sialic acid in cerebrospinal fluid.** F. Mochel, F. Sedel, U. F. H. Engelke, J. Barritault, E. Morava, M. Timmons, F. Seguin, A. Brice, R. Schiffmann, A. Durr, R. A. Wevers.

49/8:30 **Saposin B deficiency in mice leads to multiple glycosphingolipids accumulation and slowly developing neurological deficit.** Y. Sun, H. Ran, M. Zamzow, B. Quinn, M. T. Williams, C. V. Vorhees, D. P. Witte, H. Cheng, X. Han, G. A. Grabowski.

50/8:45 **Urinary globotriaosylceramide excretion correlates with the genotype in children and adults with Fabry disease.** C. Auray-Blais, D. Cyr, A. Ntwari, M. L. West, J. Cox-Brinkman, D. G. Bichet, D. P. Germain, R. Laframboise, S. B. Melançon, T. Stockley, J. T. R. Clarke, R. Drouin.

51/9:00 **In Chediak-Higashi syndrome melanocytes, giant melanosomes do not target to the dendritic tip's actin network.** W. Westbroek, A. Helip Wooley, H. Dorward, W. A. Gahl.

52/9:15 **Up-regulation of ARH1 in Galactose-stressed, Isogenic Human Fibroblasts deficient in Galactose-1-phosphate Uridyltransferase.** K. Lai, M. Tang, X. Yin, H. Klapper, K. Wierenga, L. J. Elsas.

53/9:30 **A silent substitution in the MCAD gene causes exon 2 skipping by disruption of a crucial SRp40 binding exonic splicing enhancer which is fundamental for MCAD gene expression.** B. S. Andresen, A. V. Jensen, L. D. Schroeder, E. Naylor, L. Halaby, C. A. Stanley, N. Gregersen.

54/9:45 **Deciphering Synergistic Heterozygosity in the Fatty Acid Oxidation Pathway.** K. M. Griffin, S. Ji, D. Matern, P. Rinaldo, J. D. Sharer, T. R. Schoeb, J. Vockley, P. A. Wood.

†**55**/10:00 **Apoptosis inducing effects of the signal sequence mutation in preproparathyroid hormone explains autosomal dominant familial isolated hypoparathyroidism and is corrected by a chemical chaperone.** R. Datta, A. Waheed, G. N. Shah, W. S. Sly.

56/10:15 **High frequency of uroporphyrinogen decarboxylase gene mutations in sporadic porphyria cutanea tarda patients.** K. H. Astrin, I. Nazarenko, E. Gehrie, K. E. Anderson, C. Lee, M. Yasuda, R. J. Desnick.

† Trainee Award Finalist

Thursday, October 25 8:00 AM–10:30 AM
Concurrent Platform Sessions I (15–21)

SESSION 20 – Perinatal and Reproductive Genetics
Room 29
Co-Moderators: Susan J. Gross, Albert Einstein School of Medicine, New York; and Deborah Krakow, Cedars-Sinai Medical Center, Los Angeles, CA

57/8:00 **Maternal Cigarette Smoking, Metabolic Gene Polymorphisms, and Preterm Delivery: new Insights on GxE Interactions and Pathogenic Pathways.** H.-J. Tsai, X. Liu, K. Mestan, X. Yu, S. Zhang, C. Pearson, K. Ortiz, B. Zuckerman, H. Bauchner, S. Cerda, P. Stubblefield, X. Xu, X. Wang.

58/8:15 **A Large Scale High-throughput Candidate Gene Association Study of Preterm Birth.** D. R. Velez, R. Menon, P. Thorsen, S. M. Williams, S. J. Fortunato.

59/8:30 **Racial differences in genetic association of cytokine concentrations in the presence and absence of bacterial vaginosis.** K. K. Ryckman, M. A. Krohn, H. N. Simhan, S. M. Williams.

60/8:45 **Whole genome analysis identifies a susceptibility locus to HIV-1.** S. Deutsch, C. Loeuillet, A. Ciuffi, D. Robyr, M. Munoz, P. Taffé, M. Rotger, J. S. Beckmann, S. E. Antonarakis, A. Telenti.

†**61**/9:00 **Insight on the role of maternal age and recombination in chromosome 21 nondisjunction.** T. Oliver, E. Feingold, K. Yu, S. Sherman.

62/9:15 **Testing models of human aneuploidy: age-related variation in recombination in trisomic meioses.** H. Hall, U. Surti, T. Hassold.

63/9:30 **Down Syndrome: genomic analyses link genes for GI malformation and leukemia.** T. Tirosh-Wagner, J. O. Korbel, A. E. Urban, X.-N. Chen, M. Snyder, J. R. Korenberg.

64/9:45 **An Intragenic Genomic Duplication Resulting in Loss of Function and other Novel Mutations in *NLRP7* in Women with Recurrent Biparental Hydatidiform Moles.** Y. Kou, L. Shao, R. Rosetta, D. del Gaudio, H. Peng, T. Al-Hussaini, I. Van den Veyver.

65/10:00 **Gastroschisis and Genitourinary Infections.** M. L. Feldkamp, L. D. Botto, J. Reefhuis, J. Kucik, S. Krikov, A. Wilson, C. Moore, J. C. Carey.

66/10:15 **Genetic Screening of Usher Syndrome in Children.** W. J. Kimberling, R. J. H. Smith, E. M. Stone, R. G. Weleber, C. Moller, C. Carney, M. Jensen, K. Trzupek.

† Trainee Award Finalist

Thursday, October 25 8:00 AM–10:30 AM

Concurrent Platform Sessions I (15–21)

SESSION 21 – Cancer Genetics and Cytogenetics
Room 30
Co-Moderators: Luca Roz, Instituto Nazionale Tumori, Milan, Italy; and Andrew Carroll, University of Alabama, Birmingham

67/8:00 **The human Y-encoded testis-specific protein (TSPY) interacts functionally with the eukaryotic translation elongation factor 1A (eEF1A), a putative oncoprotein.** Y. Lau, T. Kido.

68/8:15 **MicroRNA profiling in hypoxia identifies HSA-MIR-210 as an independent prognostic predictor in breast cancer.** C. Camps, F. Buffa, S. Colella, J. Moore, H. Sheldon, A. L. Harris, J. Gleadle, J. Ragoussis.

69/8:30 **Identification of a clinical and biological role for TGIF in leukemogenesis and hematopoiesis.** R. Hamid, J. Patterson, S. Brandt.

70/8:45 **Heritability of susceptibility to ionizing radiation induced apoptosis of human lymphocyte subpopulations.** A. Schmitz, J. Bayer, N. Dechamps, L. Goldin, G. Thomas.

71/9:00 **Molecular mapping of the 12q13-15 amplicon and identification of new target oncogenes in well-differentiated liposarcomas.** L. Bianchini, A. Italiano, F. Keslair, F. Pedeutour.

72/9:15 **Defective chromosome segregation and telomere dysfunction in aggressive Wilm tumors.** Y. Stewénius, Y. Jin, I. Øra, A. Frigyesi, J. Alumets, B. Sandstedt, A. K. Meeker, D. Gisselsson.

73/9:30 **The leukemogenic CALM/AF10 fusion protein alters the subcellular localization of the lymphoid regulator Ikaros.** P. A. Greif, B. Tizazu, A. Krause, E. Kremmer, S. K. Bohlander.

74/9:45 **Cryptic Xq duplications in *ETV6/RUNX1*-positive acute lymphoblastic leukemia.** H. Lilljebjörn, M. Heidenblad, B. Nilsson, C. Lassen, A. Horvat, J. Heldrup, M. Behrendtz, B. Johansson, A. Andersson, T. Fioretos.

75/10:00 **Detection of balanced translocations by DNA microarrays.** C. C. Lau, C. Davis, P. Rao, R. Selzer, P. Eis.

76/10:15 **Accelerating genome and tumor research with Single Cell Arrays.** H. Weier, J. F. Weier, S. Baehring, J. Laubenthal.

NOTES

Thursday, October 25 2:00 PM–4:30 PM
Concurrent Platform Sessions II (29–35)

SESSION 29 – Feeling Left Out: What Do Deletions Really Mean?
Hall H
Co-Moderators: Hope Northrup, University of Texas Medical School, Houston; and Eric J. Vilain, University of California, Los Angeles

77/2:00 **A novel high resolution genome-wide method identifies over 1, 500 very small deletion CNVs and triallelic SNPs.** L. Franke, C. G. F. de Kovel, Y. S. Aulchenko, D. A. van Heel, L. R. Cardon, P. Deloukas, R. A. Ophoff, L. H. van den Berg, C. Wijmenga.

78/2:15 ***ATM*** **gene deletion in children with *TEL-AML1*-positive acute lymphoblastic leukemia.** M. Shago, S. H. Hong, G. Nie, M. Abdelhaleem, I. Teshima, O. Abla.

79/2:30 **Dissecting clinical heterogeneity relevant to early development in autism by copy number variations.** P. I. Lin, S. Yoon, K. Ye, M. Wigler, J. Sebat.

80/2:45 **Novel deletion in chromosome 22 in schizophrenia patients from an internal isolate of Finland.** O. P. H. Pietilainen, T. Paunio, A. Loukola, A. Tuulio-Henriksson, J. Suvisaari, T. Varilo, J. Lönnqvist, H. Stefansson, L. Peltonen.

81/3:00 **Searching for genes contributing to autism in WAGR syndrome by oligo array CGH.** S. Xu, J. Han, A. Morales, C. Menzie, K. Williams, Y. Fan.

82/3:15 **Inferring carrier of copy number variation in Bipolar linkage region with novel Expectation-Maximization algorithm.** S. Zollner, Y. Chen, G. Su, M. G. McInnis, M. Burmeister.

83/3:30 **Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication.** B. Ballif, J. Coppinger, G. Gowans, J. Hersh, S. Madan-Khetarpal, K. Schmidt, R. Tervo, L. Escobar, C. Friedrich, M. McDonald, J. Ming, E. Zackai, B. A. Bejjani, L. G. Shaffer.

84/3:45 **Molecular delineation of the 9p deletion syndrome: phenotypic diversity of a common syndrome and the search for genes.** S. Schwartz, R. Anderson, S. Biton, M. Graf, H. K. Vance, D. J. Waggoner, C. A. Crowe.

85/4:00 **Recurrent genomic rearrangements of 17q12 are involved in a wide range of phenotypes: renal disease, diabetes and epilepsy.** H. Mefford, S. Clauin, A. Sharp, R. Moller, R. Ullmann, R. Kapur, D. Pinkel, G. Cooper, M. Ventura, H. Ropers, N. Tommerup, E. Eichler, C. Bellanne-Chantelot.

†**86**/4:15 **Cryptic deletions are a common finding in "balanced" reciprocal and complex chromosome rearrangements: a study of 43 cases.** M. De Gregori, R. Ciccone, F. Cifuentes, P. Magini, S. Gimelli, J. R. Vermeesch, J. Messa, O. Zuffardi.

Thursday, October 25 2:00 PM–4:30 PM
Concurrent Platform Sessions II (29–35)

SESSION 30 – Complex Disease Mechanisms
Room 20A
Co-Moderators: Nicola J. Camp, University of Utah, Salt Lake City; and Kevin Shianna, Duke University, Durham, NC

†**87**/2:00 **Disruption of an AP-2 binding site upstream of *IRF6* is commonly associated with nonsyndromic cleft lip and palate.** F. Rahimov, M. J. Hitchler, F. E. Domann, A. Jugessur, R. T. Lie, A. J. Wilcox, K. Christensen, E. D. Green, M. L. Marazita, B. C. Schutte, J. C. Murray.

88/2:15 **FAF1 a new gene for Cleft Palate and Pierre Robin Sequence.** M. Ghassibe, L. Desmyter, O. Boute, B. Bayet, P. Pellerin, N. Revencu, H. Poirel, J. Vermeesch, L. Backx, R. Vanwijck, M. Vikkula.

89/2:30 **A genome-wide association study of Kawasaki disease identifies multiple new loci validated in a family based follow-up study.** D. Burgner, S. Davila, T. W. Kuijpers, S. B. Ng, W. B. Breunis, M. Levin, J. C. Burns, V. J. Wright, M. L. Hibberd, US KD Genetics Consortium.

90/2:45 **A Whole Genome Scan For Polymorphisms Influencing Warfarin Dosing.** M. J. Rieder, G. M. Cooper, J. D. Smith, M. H. Wong, E. A. Johanson, D. L. Veenstra, A. E. Rettie.

91/3:00 **Biological and genetic interplay between the asthma susceptibility genes Neuropeptide S receptor 1 and Tenascin C.** C. Orsmark-Pietras, E. Melén, J. Vendelin, M. van Hage, F. Nyberg, G. Pershagen, A. Scheynius, M. Wickman, J. Kere, and the PARSIFAL Genetics Study Group.

92/3:15 **Genomic and genetic approaches identify IREB2 as a novel susceptibility gene for chronic obstructive pulmonary disease.** D. L. DeMeo, T. Mariani, C. Lange, S. S. Bhattacharya, S. Srisuma, S. Shapiro, R. Bueno, E. Silverman, J. Reilly.

93/3:30 **Genetic associations with ancestral differences in gene expression in the small airway epithelium in response to cigarette smoking.** T. P. O'Connor, B.-G. Harvey, W. Wang, A. Clark, J. Mezey, P. Schweitzer, J. Salit, I. Dolgalev, T. Raman, N. R. Hackett, R. G. Crystal.

94/3:45 **Assessing biological pathways using genome-wide association data reveals evidence for excess association of variants in the cell cycle, Wnt signaling and Adherens Junction pathways with type 2 diabetes.** J. R. B. Perry, H. Lango, N. J. Timpson, E. Zeggini, R. M. Freathy, C. M. Lindgren, K. S. Elliott, N. W. Rayner, B. Shields, C. J. Groves, A. T. Hattersley, M. I. McCarthy, T. M. Frayling, M. N. Weedon.

95/4:00 **Genetic and non-genetic sources of phenotypic variation in human lymphoblastoid cell-lines.** R. Yelensky, E. Choy, S. Bonakdar, R. M. Plenge, P. L. De Jager, R. Saxena, E. McFarland, C. Wolfish, E. Kieff, D. A. Hafler, M. Daly, D. Altshuler.

96/4:15 **Cis regulation of gene expression is an important target for selection in the human genome.** S. Kudaravalli, B. E. Stranger, E. T. Dermitzakis, J. K. Pritchard.

† *Trainee Award Finalist*

Thursday, October 25 2:00 PM–4:30 PM
Concurrent Platform Sessions II (29–35)

SESSION 31 – Neurogenetics
Room 20B/C
Co-Moderators: Gaofeng Wang, University of Miami, FL; and Dana C. Crawford, Vanderbilt University, Nashville, TN

97/2:00 **The mutational spectrum of the novel HSP gene REEP1 suggests haploinsufficiency and microRNA target site involvement.** S. Zuchner, C. Beetz, R. Schüle, T. Deconinck, J. Beats, K. N. Trans Viet, H. Zhu, N. Nagan, T. Deufel, C. Braastad, L. Schöls, P. de Jonghe, M. Pericak-Vance.

†**98**/2:15 **Genome-wide association study of sporadic amyotrophic lateral sclerosis identifies ITPR2 as a susceptibility gene.** M. A. van Es, P. W. van Vught, H. Blauw, L. Franke, C. G. J. Saris, P. M. Anderson, L. Vandenbosch, A. Birve, V. de Jong, F. Baas, H. J. Schelhaas, K. Sleegers, C. van Broeckhoven, J. H. J. Wokke, C. Wijmenga, W. Robberecht, J. H. Veldink, R. A. Ophoff, L. H. van den Berg.

99/2:30 **Interactions involving nitric oxide synthase genes and environmental risk factors in Parkinson disease.** D. B. Hancock, E. R. Martin, J. M. Vance, W. K. Scott.

100/2:45 **Characterization and replication of a novel locus for late-onset Parkinson disease detected in a genome-wide association study in an isolated population.** Z. Bochdanovits, P. Rizzu, K. Rak, L. Pardo-Cortes, P. Heutink.

101/3:00 **High-density linkage screen identifies potential dementia loci in the Amish.** L. Jiang, J. L. McCauley, P. J. Gallins, N. Schnetz-Boutaud, A. E. Crunk, L. L. McFarland, D. Fuzzell, C. Knebusch, M. Creason, L. Caywood, C. E. Jackson, W. K. Scott, M. A. Pericak-Vance, J. L. Haines.

102/3:15 **Genome-Wide Association for Late-Onset Alzheimer Disease (LOAD) Confirms Risk locus on Chromosome 12.** G. Beecham, E. Martin, Y.-J. Li, R. Carney, M. Slifer, J. Gilbert, J. Haines, M. Pericak-Vance.

103/3:30 **Nonallelic variants of neuronal sortilin-related receptor (SORL1) associated with distinct Alzheimer disease (AD) processes observed by magnetic resonance imaging (MRI).** K. T. Cuenco, K. L. Lunetta, L. A. Cupples, A. McKee, H. Chui, C. DeCarli, P. St. George-Hyslop, R. C. Green, C. Baldwin, L. A. Farrer, and MIRAGE Study Group.

104/3:45 **Investigation of genetic susceptibility to Late-onset Alzheimer disease through genomic convergence.** X. Liang, M. Slifer, E. R. Martin, N. Schnetz-Boutaud, J. Bartlett, B. M. Anderson, S. Zuchner, J. Gilbert, M. A. Pericak-Vance, J. H. Haines.

105/4:00 **A major genetic risk factor of periodic limb movements and restless legs syndrome.** H. Stefansson, D. Rye, A. Hicks, H. Petursson, A. Ingason, T. E. Thorgeirsson, S. Palsson, T. Sigmundsson, A. P. Sigurdsson, I. Eiriksdottir, L. M. Trotti, D. Bliwise, J. M. Beck, A. Rosen, S. Waddy, U. Thorsteinsdottir, A. Kong, J. Gulcher, D. Gudbjartsson, K. Stefansson.

106/4:15 **An enhancer in the intron 2 deletion regulates DCDC2 gene expression, and is associated with dyslexia.** H. Meng, J. R. Gruen, N. A. Cope, A. Citterio, G. Menozzi, M. L. Lorusso, M. Molteni, Y. Wang, J. J. LoTurco, C. Marino.

† Trainee Award Finalist

Thursday, October 25 2:00 PM–4:30 PM
Concurrent Platform Sessions II (29–35)

SESSION 32 – Statistical Genetics and Genetic Epidemiology
Room 20D
Co-Moderators: Michael Boehnke, University of Michigan, Ann Arbor; and Eleanor Feingold, University of Pittsburgh, PA

107/2:00 **Population structure in European American populations - Impact on the design and analysis of Genome-Wide Association Studies (GWAS).** K. Yu, Z. M. Wang, Q. Z. Li, S. Wacholder, R. Hoover, D. Hunter, S. Chanock, G. Thomas, CGEMS Project Team.

108/2:15 **A permutation test for estimating the number of subpopulations using whole-genome SNP data.** K. Bryc, H. Gao, C. D. Bustamante.

109/2:30 **Informative heterogeneity and failed replication: lessons from genome-wide association data for type 2 diabetes.** M. I. McCarthy, T. M. Frayling, N. J. Timpson, M. N. Weedon, C. M. Lindgren, H. Lango, K. S. Elliott, J. R. B. Perry, N. W. Rayner, R. M. Freathy, A. T. Hattersley, E. Zeggini, The Wellcome Trust Case Control and UK Type 2 Diabetes Genetics Consortia.

110/2:45 **Combining SNP genotype data and hybridization intensity to simultaneously detect and test deletions for disease association.** J. R. Kohler, D. J. Cutler.

†**111**/3:00 **Using LD to predict CNVs and test for disease associations.** N. Cardin, C. Barnes, V. Plagnol, D. Clayton, M. Hurles, P. Donnelly, J. Marchini on behalf of the WTCCC CNV Analysis Group.

112/3:15 **A robust statistical method for genome-wide Copy Number Variation association studies.** C. Barnes, V. Plagnol, N. Cardin, J. Marchini, D. Clayton, M. Hurles on behalf of the WTCCC CNV Analysis Group.

113/3:30 **Ordered Subset Analysis for Association Mapping.** R. H. Chung, S. Schmidt, X. Qin, X. Lou, E. R. Martin, E. R. Hauser.

114/3:45 **Population genomics of human gene expression.** E. T. Dermitzakis, B. E. Stranger, A. Nica, M. S. Forrest, A. Dimas, C. P. Bird, C. Beazley, C. Ingle, M. Dunning, P. Flicek, D. Koller, S. Montogomery, S. Tavare, M. E. Hurles, P. Deloukas.

115/4:00 **Searching for Master Regulatory Variants of Gene Expression.** J. Ding, G. R. Abecasis.

116/4:15 **Common Mitochondrial Haplogroups Do Not Predict Risk of Morbidity, Mortality, or Longevity in the General Population.** M. Benn, M. Schwartz, B. G. Nordestgaard, A. Tybjærg-Hansen.

† Trainee Award Finalist

Thursday, October 25 2:00 PM–4:30 PM
Concurrent Platform Sessions II (29–35)

SESSION 33 – Molecular Basis Mendelian Disorders I
Room 28
Co-Moderators: Andrew J. Griffith, National Institute on Deafness and Communication Disorders, National Institutes of Health, Bethesda, MD; and Deborah Krakow, Cedars-Sinai Medical Center, Los Angeles, CA

117/2:00 **Syne1 mutations cause a novel form of autosomal recessive pure cerebellar ataxia.** F. Gros-Louis, N. Dupré, P. Dion, M. Fox, S. Laurent, J. R. Sanes, J. P. Bouchard, G. A. Rouleau.

118/2:15 **Oligosaccharyltransferase subunits mutations in non-syndromic mental retardation.** F. Molinari, S. Romano, F. Foulquier, W. Morelle, P. de Lonlay, P. S. Tarpey, J. Teague, S. Edkins, P. A. Futreal, M. R. Stratton, M. Partington, G. Turner, G. Matthijs, J. Gecz, A. Munnich, L. Colleaux.

119/2:30 **Identification of mutations causing severe motoneuron disease.** H. O. Nousiainen, M. Kestilä, N. Pakkasjärvi, H. Honkala, S. Kuure, J. Tallila, K. Vuopala, J. Ignatius, R. Herva, L. Peltonen.

120/2:45 **Genotype-phenotype analysis in Retinal Vasculopathy with Cerebral Leukodystrophy with 3'-truncating mutations in human 3'-5' DNA Exonuclease TREX1.** A. M. J. M. van den Maagdenberg, A. Richards, J. C. Jen, D. Kavanagh, D. Spitzer, M. K. Liszewski, M. L. Barilla-LaBarca, G. M. Terwindt, Y. Kasai, M. G. Grand, K. R. J. Vanmolkot, P. T. V. M. de Jong, M. Dichgans, K. E. Kotschet, T. Hardy, S. F. Nelson, R. R. Frants, R. W. Baloh, M. D. Ferrari, J. P. Atkinson.

121/3:00 **Comprehensive analysis of aberrantly spliced exons in myotonic dystrophy type 1 using Affymetrix Exon Array.** Y. Yamashita, T. Matsuura, J. Shinmi, T. Ibi, M. Kinoshita, T. Kimura, O. Yahara, K. Sahashi, K. Ohno.

122/3:15 **Mutations in the Na^+/H^+ exchanger gene *SLC9A6* cause an X-linked variant of Angelman Syndrome.** K. K. Selmer, G. D. Gilfillan, C. E. Schwartz, R. E. Stevenson, A. L. Christianson, M. Kyllerman, T. Egeland, M. Kroken, M. Mattingsdal, K. Eiklid, D. E. Undlien, P. Strømme.

123/3:30 **Mutations in UPF3B, a member of the nonsense mediated mRNA decay surveillance complex, cause Lujan-Fryns and FG phenotypes and non-syndromic X-linked mental retardation.** J. Rodriguez, P. S. Tarpey, L. S. Nguyen, F. L. Raymond, A. Hackett, L. Vandeleur, R. Smith, C. Shoubridge, S. S. Bhat, M. Corbett, M. E. Porteous, G. Hoganson, D. Superneau, G. Turner, R. E. Stevenson, C. E. Schwartz, P. A. Futreal, M. R. Stratton, J. Gecz, A. K. Srivastava.

124/3:45 **Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with peripheral RPE atrophy.** C. Chakarova, M. Papaioannou, A. Shah, N. Waseem, I. Lopez, B. Wissinger, E. Zrenner, C. Ponting, R. Koenekoop, S. S. Bhattacharya.

125/4:00 **Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency amongst the Amish.** H. Cross, K. Kalidas, K. G. Zahka, J. Tumbush, B. B. Keller, C. Galambos, K. Gurtz, M. A. Patton, A. H. Crosby.

126/4:15 **A novel gene is disrupted in a patient with balanced translocation t(3;X)(q12.3-q22.3) associated with Cerebral Cavernous Malformations.** F. Gianfrancesco, T. Esposito, S. Penco, V. Maglione, F. Letizia, C. L. Liquori, M. C. Patrosso, O. Zuffardi, A. Ciccodicola, D. A. Marchuk, F. Squitieri.

Thursday, October 25 2:00 PM–4:30 PM
Concurrent Platform Sessions II (29–35)

SESSION 34 – Genetic Counseling and Clinical Services
Room 29
Co-Moderators: Cheryl A. Scacheri, Cleveland Clinic, Genomic Medicine Institute, Cleveland, OH; and David B. Flannery, Medical College of Georgia, Augusta

127/2:00 **Moral distress and burnout among clinical genetics service providers (GSPs).** B. A. Bernhardt, K. Kolodner, G. Geller.

128/2:15 **Transitioning to self-management (TSM): experience with Marfan syndrome (MFS).** R. E. Pyeritz, B. A. Bernhardt, E. Giarelli.

129/2:30 **Social support, communal coping and psychological status in sisters in Hereditary Breast and Ovarian Cancer (HBOC) families.** J. A. Peters, L. Koehly, L. Hoskins, N. Kuhn, A. Letocha, R. Kenen, J. Loud, M. H. Greene.

130/2:45 **Reimbursement for genetic counseling and related services.** J. Dungan, C. Yates, A. Trivedi, T. Bamlett Sherman, L. Shulman.

131/3:00 **Towards recommendations for genetic counseling.** H. Kaariainen, E. Rantanen, M. Hietala, U. Kristoffersson, I. Nippert, J. Schmidtke, J. Sequeiros.

132/3:15 **Telegenetic Use in the United States: results of 2007 NCC Telegenetics Workgroup Survey.** H. C. Andersson, B. Butler, J. Benkendorf, B. Bowdish, M. Watson.

133/3:30 **Predictive Testing for Multiple Genetic Variants in Common Diseases: a different ELSI landscape from testing for traditional genetic diseases.** A. C. J. W. Janssens, M. Gwinn, C. M. van Duijn, M. J. Khoury.

134/3:45 **High throughput testing for common and recurrent mutations responsible for Mendelian diseases.** J. W. Belmont, R. Chen, L. Nazareth, D. Stockton, W. Craigen, C. Shaw, A. L. Beaudet, J. Lupski, R. Gibbs.

135/4:00 **Model for disclosure of research genetic testing results.** S. Adam, D. Avard, P. Birch, P. Eydoux, B. Knoppers, S. Langlois, M. A. Marra, J. Samuel, J. M. Friedman.

136/4:15 **The challenge of counseling families with results of unclear clinical significance by array CGH as illustrated by duplications of the BCR gene region at 22q11.23.** J. Coppinger, D. McDonald-McGinn, E. Zackai, K. Shane, J. F. Atkin, R. Leland, K. Schmidt, H. Feldman, W. Cohen, J. Phalin, B. Powell, B. C. Ballif, B. A. Bejjani, T. Shaikh, S. Saitta, L. G. Shaffer.

Thursday, October 25 2:00 PM–4:30 PM

Concurrent Platform Sessions II (29–35)

SESSION 35 – Cardiovascular Genetics
Room 30
Co-Moderators: Paivi E. Pajukanta, University of California, Los Angeles; and John G. Seidman, Harvard Medical School, Boston, MA

137/2:00 **Identification of susceptibility genes for Myocardial Infarction following the combined analysis of two large genome scans in German and UK samples.** P. Deloukas on behalf of CARDIOGENICS.

138/2:15 **A Sequence Variant Adjacent to *CDKN2A* and *CDKN2B* Affects the Risk of Atherosclerosis in Several Vascular Beds.** A. Helgadottir, K. P. Magnusson, S. Gretarsdottir, G. Thorleifsson, A. Manolescu, K. Kostulas, R. Pola, B. Lindblad, G. Tromp, N. Sakalihasan, R. E. Ferrell, J. Hillert, J. Powell, H. Kuivaniemi, E. Valdimarsson, S. E. Matthiasson, G. Thorgeirsson, J. R. Gulcher, A. Kong, K. Stefansson.

139/2:30 **Genome-wide Scan for Coronary Artery Disease Genes using 500, 668 markers.** A. F. R. Stewart, R. McPherson, L. Chen, K. Williams, N. Kavaslar, J. Rutberg, H. Doelle, G. Ewart, G. A. Wells, R. Roberts.

140/2:45 ***VNN1*, A Novel Gene for Cardiovascular Disease Risk.** J. E. Curran, M. P. Johnson, H. H. H. Goring, T. D. Dyer, J. C. Charlesworth, S. A. Cole, J. B. Jowett, L. J. Abraham, D. L. Rainwater, M. C. Mahaney, L. Almasy, J. W. MacCluer, A. H. Kissebah, G. R. Collier, E. K. Moses, J. Blangero.

141/3:00 **Identification and replication of FAM5C polymorphisms associated with myocardial infarction.** J. J. Connelly, A. B. Hale, S. Gadson, J. F. Doss, X. Lou, D. R. Crosslin, S. H. Shah, D. C. Crossman, C. B. Granger, V. Mooser, C. J. H. Jones, J. M. Vance, P. J. Goldschmidt-Clermont, W. E. Kraus, E. R. Hauser, S. G. Gregory.

142/3:15 **ACTA2 mutations cause diverse and diffuse vascular diseases, including aortic aneurysms, premature coronary artery disease and Moyamoya disease.** D. Guo, H. Pannu, V. Tran-fadulu, C. Papke, N. Avidan, S. Bourgeois, R. Yu, A. Estrera, H. Safi, P. Tung, L. Buja, S. Scherer, C. Raman, S. Shete, D. Milewicz.

143/3:30 **Variants on 4q25 confer risk of atrial fibrillation.** D. Gudbjartsson, D. Arnar, A. Helgadottir, S. Gretarsdottir, G. Thorleifsson, G. Thorgeirsson, K. Kostulas, J. Hillert, R. Ma, M. C. Y. Ng, J. Rosand, P. Ellinor, H. Holm, J. Gulcher, U. Thorsteinsdottir, A. Kong, K. Stefansson.

144/3:45 **Association mapping of the five quantitative ECG traits RR, P, PQ, QRS AND QT in a 500K genome-wide scan: confirmation of the NOS1AP association to QT and identification of a spectrum of additional QTLs.** A. Pfeufer, M. Akyol, M. F. Sinner, S. Perz, C. Gieger, B. M. Beckmann, T. Illig, H. E. Wichmann, S. Kaab, T. Meitinger.

145/4:00 **Genetic association of the epithelial sodium channel γ-subunit with 25-year follow-up blood pressures in Utah pedigrees - a replication study.** C. J. Büsst, K. J. Scurrah, J. A. Ellis, Y. Xin, E. A. Brinton, P. N. Hopkins, S. C. Hunt, S. B. Harrap.

146/4:15 **Whole-genome association study in the Old Order Amish identifies *STK39* as a novel hypertension susceptibility gene.** Y. Wang, P. F. McArdle, E. Rampersaud, H. Shen, X. Shi, N. I. Steinle, B. D. Mitchell, A. R. Shuldiner, Y.-P. C. Chang.

NOTES

Friday, October 26 8:00 AM–10:30 AM
Concurrent Platform Sessions III (36–42)

SESSION 36 – Noncoding RNAs
Hall H
Co-Moderators: Nicholas Katsanis, The Johns Hopkins University, Baltimore, MD; and Christopher E. Pearson, Hospital for Sick Children, Toronto, Canada

147/8:00 **Functional characterization of a sensory organ-specific miRNA cluster.** P. D. Witmer, S. Xu, J. T. Mendell, S. Fisher, D. Valle.

148/8:15 **Misregulation of small noncoding regulatory RNAs by the loss of MeCP2 in a mouse model of Rett Syndrome.** K. Szulwach, X. Li, S. Mathias, X. Zhao, P. Jin.

149/8:30 **Prader-Willi syndrome is caused by paternal deficiency for the HBII-85 C/D box snoRNA cluster.** T. Sahoo, D. del Gaudio, J. R. German, M. Shinawi, S. U. Peters, R. Person, A. Garnica, S. W. Cheung, A. L. Beaudet.

150/8:45 **SnoRNA Pwcr1/MBII-85 deletion mouse model for Prader-Willi syndrome shows growth retardation, hyperphagia and altered metabolism.** F. Ding, H. H. Li, S. Zhang, N. Solomon, S. Camper, E. Mignot, U. Francke.

151/9:00 **MicroRNAs influence gene expression phenotypes of ataxia telangiectasia carriers.** V. G. Cheung, D. A. Smirnov.

152/9:15 **Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of α-synuclein.** G. Wang, J. van der Walt, G. Mayhew, Y. Li, S. Züchner, W. K. Scott, E. Martin, J. M. Vance.

153/9:30 **Enrichment and variability of PIWI-interacting RNAs (piRNAs) in segmental duplications and copy number variants (CNVs) suggest a functional role in the integrity of the genome.** L. Armengol, M. Caceres, A. Brunet, X. Estivill.

154/9:45 **Exhaustive analysis of non-coding DNA around *phox2b* reveals most biologically important sequences are not detected by sequence conservation.** D. M. McGaughey, R. M. Vinton, J. Huynh, A. Al-Saif, M. A. Beer, A. S. McCallion.

155/10:00 **Polysome fractionation suggests that a fraction of Txfrags is translated.** S. Nikolaev, S. Deutsch, R. Genolet, L. Parand, B. Conne, P. Descombes, J.-D. Vassalli, J. Curran, S. E. Antonarakis.

156/10:15 **Insights into the noncoding RNA transcriptome in the mammalian brain.** S. Sunkin, T. Mercer, M. Dinger, M. Mehler, A. Jones, J. Mattick.

Friday, October 26 8:00 AM–10:30 AM
Concurrent Platform Sessions III (36–42)

SESSION 37 – Animal Models
Room 20A
Co-Moderators: David R. Beier, Brigham and Women's Hospital, Boston, MA; and Donna M. Martin, University of Michigan Medical Center, Ann Arbor

157/8:00 **Genome-wide association and platelet system biology studies to unravel the genetic architecture of coronary artery disease.** A. H. Goodall, The Bloodomics and WTCCC Consortia.

158/8:15 **AAV mediated expression of myotubularin in muscle corrects the myotubular myopathy phenotype in a mouse model and suggests a function in membrane remodeling at the sarcolemma.** A. Buj-Bello, F. Fougerousse, Y. Schwab, N. Messaddeq, D. Spehner, P. Schultz, O. Danos, J. Laporte, A.-M. Douar, J.-L. Mandel.

159/8:30 **The first report of a de novo heterozygous missense *DISP1* mutation in a patient with congenital diaphragmatic hernia (CDH) and additional malformations.** S. Kantarci, F. O'Neill, M. K. Russell, K. M. Noonan, R. Pieretti-Vanmarcke, L. Mitova, J. Wilson, P. Dickman, K. Yboa, P. K. Donahoe, B. R. Pober.

†**160**/8:45 **Genetic interaction of Bardet-Biedl syndrome genes and implication for polydactyly utilizing zebrafish model system.** M. Tayeh, H.-S. Yen, J. Beck, C. Searby, H. Griesbach, E. Stone, D. Slusarski, V. Sheffield.

161/9:00 **Growth retardation, hyperactivity, abnormal anxiety-related responses, and impaired neuromuscular and sensorineural coordination in a mouse model overexpressing *Rai1*.** S. Girirajan, N. Patel, R. E. Slager, M. E. Tokarz, M. Bucan, J. L. Wiley, S. H. Elsea.

162/9:15 **Hypomorphic mutations in the syndromic encephalocoele gene MKS1 perturb gastrulation movements and cause Bardet-Biedl syndrome.** C. Leitch, J. L. Badano, N. A. Zaghloul, C. Stotzel, B. Drehman, M. Al-Fadhel, R. A. Lewis, W. Eyaid, H. Dollfus, P. L. Beales, N. Katsanis.

163/9:30 **Mutations in insulin-like factor 3 receptor are associated with osteoporosis.** A. Ferlin, A. Pepe, L. Gianesello, A. Garolla, S. Feng, R. Morello, A. I. Agoulnik, C. Foresta.

164/9:45 **Mutations in NIMA-related kinase NEK8 causes nephronophthisis in humans and affects ciliary and centrosomal localization.** E. Otto, M. Trapp, U. Schultheiss, L. Quarmby, F. Hildebrandt.

165/10:00 **Bioinformatics approach to identification of genes involved in multiple pituitary hormone deficiency: homeotic selector *Ash1l*.** S. Camper, N. Solomon, A. Mortensen, M. Brinkmeier, J. MacDonald, D. Ghosh, P. Carninci, Y. Hayashizaki, R. Lyons.

166/10:15 **Loss of function of the *ACTN3* gene alters muscle metabolism and has been selectively favored during recent human evolution.** D. G. MacArthur, J. M. Raftery, G. A. Huttley, J. T. Seto, K. G. R. Quinlan, S. Easteal, N. Yang, K. N. North.

† Trainee Award Finalist

Friday, October 26 8:00 AM–10:30 AM
Concurrent Platform Sessions III (36–42)

SESSION 38 – Psychiatric Genetics
Room 20B/C
Co-Moderators: Michael S. Philips, University of Montreal, Canada; and Lisa J. Martin, Cinncinnati Children's Hospital, OH

167/8:00 **A genome-wide autism association study identifies a common variant with sex-dependent effects at the neurexin-superfamily member *CNTNAP2*.** D. E. Arking, D. J. Cutler, C. W. Brune, T. M. Teslovich, K. West, M. Ikeda, A. Rea, M. Guy, S. Lin, E. H. Cook, Jr., A. Chakravarti.

168/8:15 **Identification of OXTR and MAFF deletions within independent autism families by whole genome tilepath microarray analysis.** S. G. Gregory, J. J. Connelly, S. Donnelly, R. Abramson, H. Wright, M. Cuccaro, J. P. Hussman, J. R. Gilbert, M. A. Pericak-Vance.

169/8:30 **A comprehensive association study of 106 candidate genes for Attention Deficit Hyperactivity Disorder.** B. S. Maher, B. Devlin, R. E. Ferrell, G. P. Kirillova, H. Chilcoat, E. L. Murrelle, R. E. Tarter, M. M. Vanyukov.

170/8:45 **Integration of novel statistical and biological methods identifies a causal SNP for schizophrenia in NOS1AP.** L. Brzustowicz, N. S. Wratten, H. Memoli, Y. Huang, M. A. Azaro, J. Messenger, J. E. Hayter, E. W. C. Chow, A. S. Bassett, S. Buyske, V. J. Vieland.

171/9:00 **High Risk Cohort Specific Variants in DISC1 are Identified and Associated with Schizophrenia with an Estimated Attributable Risk of 2%.** W. Song, J. Feng, W. Li, J. Longmate, L. Heston, S. Sommer.

172/9:15 **Genome-scan with a quantitative phenotype identifies new genes for the susceptibility to schizophrenia.** F. Macciardi, J. Turner, D. Keator, L. Geronazzo, J. Fallon, S. G. Potkin.

173/9:30 **Replicated analyses suggest a network of dopaminergic genes confer risk for schizophrenia.** M. E. Talkowski, M. Bamne, H. Mansour, K. Chowdari, J. Wood, L. McClain, G. Kirov, M. C. O'Donovan, M. Owen, B. Devlin, V. L. Nimgaonkar.

174/9:45 **Polymorphisms in the SNAP25 gene are associated with early-onset bipolar affective disorder.** S. Jamain, B. Etain, A. Dumaine, F. Mathieu, F. Chevalier, J. Deshommes, C. Henry, J. P. Kahn, F. Bellivier, M. Leboyer.

175/10:00 **Psychotherapeutic mechanisms of change: the role of genes in depression treatment outcome.** A. Kotte, J. R. McQuaid, J. R. Kelsoe.

176/10:15 **The Development of a Broad-Based ADME Panel for use in Pharmacogenomic Studies and Drug Development.** A. M. K. Brown, Y. Renaud, I. Mongrain, N. Gaudreault, C. Ross, C. Taylor Lawley, R. Shen, C. H. Lin, J.-C. Tardif, M. S. Phillips.

Friday, October 26 8:00 AM–10:30 AM
Concurrent Platform Sessions III (36–42)

SESSION 39 – Letting the Genie Out of the Bottle: Genotype/Phenotype Correlations
Room 20D
Co-Moderators: J. Edward Spence, Carolinas Medical Center, Charlotte, NC; and Lisa Schimmenti, University of Minnesota, Minneapolis

177/8:00 **Refining the molecular and clinical definitions for JP-HHT syndrome.** C. J. Gallione, C. L. Clericuzio, T. P. Leedom, J. C. Fahl, J. M. Drautz, J. D. Waldman, K. Henderson, M. J. Beis, M. Ludman, T. Berk, M. K. Maisenbacher, C. A. Williams, Z. Fan, A. S. Aylsworth, J. Garvie, M. E. Faughnan, R. I. White, D. A. Marchuk.

178/8:15 **Sporadic Venous Malformation is Caused by Somatic Mutations in TIE2.** V. Wouters, N. Limaye, M. Uebelhoer, J. B. Mulliken, L. M. Boon, M. Vikkula.

179/8:30 **Phenotypic features associated with TGFBR1 and TGFBR2 mutations in familial thoracic aortic aneurysms and dissections.** H. Pannu, V. Tran-Fadulu, M. C. Willing, A. Muilenberg, C. Ahn, D. M. Milewicz.

180/8:45 **Hutchinson-Gilford Progeria Syndrome (HGPS): comprehensive characterization of 15 children.** M. A. Merideth, W. J. Introne, L. B. Gordon, M. B. Perry, S. B. Clauss, V. Sachdev, C. K. Zalewski, C. C. Brewer, J. Kim, J. C. Graf, A. C. M. Smith, L. H. Gerber, J. A. Yanovski, D. L. Domingo, T. C. Hart, F. S. Collins, E. G. Nabel, R. O. Cannon, W. A. Gahl.

181/9:00 **Phenotypic subclassification amongst individuals with cohesin-related Cornelia de Lange Syndrome: *SMC1A*, *SMC3* and *NIPBL* specific features.** D. Yaeger, M. A. Deardorff, M. Kaur, L. G. Jackson, I. D. Krantz.

182/9:15 **It's in your hands: a combined clinical, molecular and developmental approach to the diagnosis of radial ray defects.** R. A. Newbury-Ecob, A. Sharif, M. Logan.

183/9:30 **Delineation of star syndrome (syndactyly, telecanthus, anogenital, and renal anomalies).** S. Unger, D. Böhm, W. Borozdin, B. Steiner, T. Schmitt Mechelke, K. Borowski, K. Keppler-Noreuil, G. Mortier, R. Sandford, B. Zabel, A. Superti-Furga, J. Kohlhase.

184/9:45 ***SPECC1L*, a Novel Cytoskeletal Protein, is Haploinsufficient in a Patient with Bilateral Oblique Facial Clefts, Ocular Hypoplasia and Club Feet.** I. Saadi, F. S. Alkuraya, J. J. Lund, A. Turbe-Doan, T. W. Glover, R. Erickson, R. L. Maas.

185/10:00 **Severe mutations of ARX are associated with an abnormal phenotype in most heterozygous females but not in mothers of affected children.** J. Sudi, M. Kato, G. Mancini, A. Toutain, S. Das, S. Christian, W. Dobyns.

186/10:15 **Clinical, cellular and neuropathological consequences of *AP1S2* mutations: delineation of a novel recognizable X-linked mental retardation syndrome, MESCH-X.** G. Borck, A. Molla Herman, N. Boddaert, F. Encha-Razavi, A. Philippe, L. Robel, F. Brunelle, A. Benmerah, A. Munnich, L. Colleaux.

Friday, October 26 8:00 AM–10:30 AM
Concurrent Platform Sessions III (36–42)

SESSION 40 – Mitochondria and Disease
Room 28
Co-Moderators: Fernando Scaglia, Baylor College of Medicine, Houston, TX; and Marni T. Falk, Children's Hospital of Philidelphia, PA

187/8:00 **Mutant mitochondrial genes in Drosophila: a model for mitochondrial dysfunction and disease.** B. H. Graham, Z. Li, E. P. Alesii, C. V. Ly, P. Verstreken, H. J. Bellen, W. J. Craigen.

188/8:15 **Drosophila NnaD mutant flies model mouse purkinje cell degeneration (pcd) and implicate mitochondrial dysfunction in Nna recessive phenotypes.** S. M. Jackson, G. Dunn, S. L. Baccam, L. J. Pallanck, A. R. La Spada.

189/8:30 **Mitochondrial *ADCK3*, an ancestral prokaryotic kinase involved in Coenzyme Q biosynthesis, is mutant in a new form of recessive ataxia.** C. Lagier-Tourenne, M. Tazir, C. Quinzii, L. López, C. Busso, N. Drouot, M. Assoum, S. Makri, L. Pacha, T. Benhassine, M. Anheim, S. Schmucker, D. Lynch, F. Plewniak, C. Tranchant, O. Poch, J. L. Mandel, M. Barros, M. Hirano, M. Koenig.

190/8:45 **Mitochondrial Dysfunction and Glutathione Depletion in a Murine Model of muto Methylmalonic Acidemia.** C. Venditti, R. Chandler, S. Shanske, P. Zerfas, T. Cowan, G. Enns, V. Hoffman, S. DiMauro.

†**191**/9:00 **Mitochondrial dysfunction caused by germline mutations in succinate dehydrogenase subunit genes in Cowden and Cowden-like syndromes.** K. Zbuk, A. Patocs, G. Lobo, T. Sadler, J. Stein, K. Waite, C. Eng.

192/9:15 **Global transcript profiles of adipose tissue in weight-discordant MZ twin pairs: pathways behind acquired obesity.** J. Naukkarinen, K. H. Pietiläinen, A. Rissanen, J. Saharinen, P. Ellonen, H. Yki-Järvinen, M. Oresic, J. Kaprio, L. Peltonen.

193/9:30 **Prenyldiphosphate synthase (PDSS1) and OH-benzoate prenyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders.** J. Mollet, I. Giurgea, D. Schlemmer, G. Dallner, D. Chretien, A. Delahodde, D. Bacq, P. de Lonlay, A. Munnich, A. Rötig.

194/9:45 **Cell biology, genetics and genomics; a powerful liaison to match genetic to phenotypic variation: the example of Reactive Oxygen Species.** H. Attar, K. Bedard, H. Prokisch, T. Meitinger, D. Mehta, E. Wichmann, E. T. Dermitzakis, K. H. Krause, S. E. Antonarakis.

195/10:00 **Mutation of *RRM2B*, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion.** A. Bourdon, L. Minai, V. Serre, J.-P. Jais, E. Sarzi, S. Aubert, D. Chrétien, P. de Lonlay, V. Paquis-Flucklinger, H. Arakawa, Y. Nakamura, A. Munnich, A. Rötig.

196/10:15 **Preimplantation diagnosis for mitochondrial DNA disorders: contribution to understanding mitochondrial DNA segregation during early human embryonic development.** J. Steffann, N. Gigarel, N. Frydman, P. Burlet, V. Kerbrat, G. Tachdjian, J. P. Bonnefont, R. Frydman, A. Munnich.

Friday, October 26 8:00 AM–10:30 AM
Concurrent Platform Sessions III (36–42)

SESSION 41 – Cytogenetics
Room 29
Co-Moderators: Gail H. Vance, Indiana University, Indianapolis; and Warren G. Sanger, Munroe-Meyer Institute, Omaha, NE

197/8:00 **SNP array mapping of 20p deletions: genotypes, phenotypes and copy number variation.** N. B. Spinner, A. J. Greco, B. T. Thiel, J. Glessner, P. Munoz, X. Gai, D. A. Piccoli, S. F. A. Grant, H. Hakonarson, I. D. Krantz, B. M. Kamath.

198/8:15 **Somatic partial chromosome 11 duplication in patients with Proteus Syndrome.** K. Duffy, D. Bick, P. vanTuinen, S. Dugan, A. Yilmaz, C. Schwartz, W. Foulkes, M. Olivier.

199/8:30 **The 1q41q42 Microdeletion Syndrome: characterization of a New Genomic Disorder.** T. H. Shaikh, S. Saitta, D. Kostiner, M. MacDonald, J. W. Ellison, A. S. Aylsworth, L. G. Shaffer.

200/8:45 **Complex Segmental Duplication Superstructure found on Human Chromosome 17q.** D. Chen, V. Leppä, T. Miettinen, A. Palotie, L. Peltonen, J. Saarela.

201/9:00 **Nonrecurrent MECP2 duplications in neurodevelopmentally delayed males reveal a prone rearrangement region in Xq28.** C. Carvalho, A. Patel, T. Sahoo, C. Bacino, S. Peacock, A. Pursley, S. W. Cheung, J. R. Lupski.

202/9:15 **BubR1 deficiency causes centrosome amplification in PCS (MVA) syndrome.** S. Matsuura, H. Izumi, Y. Matsumoto, T. Ikeuchi, H. Saya, T. Kajii.

203/9:30 **The effect of chromosomal rearrangements on gene expression.** L. A. J. Harewood, F. Schütz, M. Delorenzi, A. Reymond.

204/9:45 **Cytogenetic approaches for identifying novel genes and regulatory elements associated with hearing loss.** K. Kocher, R. Williamson, K. Arnos, K. Crow, J. Reiss, C. C. Morton.

205/10:00 **A Gene Dosage Map of the Human Genome: a Map with Clinical Utility.** J. D. Cody, P. L. Heard, A. C. Crandall, E. M. Carter, D. E. Hale.

206/10:15 **Mitotic reduction divisions in adult murine hepatocytes.** A. W. Duncan, N. K. Paulk, M. J. Finegold, M. Grompe.

† Trainee Award Finalist

Friday, October 26 8:00 AM–10:30 AM

Concurrent Platform Sessions III (36–42)

SESSION 42 – Methods in Gene Mapping
Room 30
Co-Moderators: Richard W. Morris, Duke University, Durham, NC; and Alkes Price, Harvard Medical School, Boston, MA

207/8:00 **Haplotype-Sharing Test as a tool to map genes for familial cardiomyopathy.** F. Gerbens, J. P. van Tintelen, P. A. van der Zwaag, L. G. Boven, J. J. van der Smagt, R. N. Hauer, R. M. W. Hofstra, G. J. te Meerman.

208/8:15 **LDOrbits: Feature Definition in Genome Wide Association Studies.** P. Croteau, J. Segal, Q. Nguyen-Huu, T. Keith, J. Raelson, P. Van Eerdewegh.

209/8:30 **Whole Genome Linkage Disequilibrium Association Mapping of Binary Traits.** P. Scheet, M. Stephens, G. R. Abecasis.

210/8:45 **BIMBAM: Bayesian IMputation Based Association Mapping.** Y. Guan, M. Stephens.

211/9:00 **Bayesian approaches for detecting association in case-control studies.** D. Vukcevic, P. Donnelly.

212/9:15 **Coverage and power for genetic association studies using near-complete variation data from candidate genes.** T. R. Bhangale, M. J. Rieder, D. A. Nickerson.

213/9:30 **A comprehensive analysis of the HapMap for trans- and long-range cis- associated SNPs: potential inference errors for genome-wide association studies.** R. W. Lawrence, L. R. Cardon, E. Zeggini.

214/9:45 **Highly cost efficient genome-wide association studies using DNA pools and dense SNP arrays.** S. Macgregor, Z. Z. Zhao, A. Henders, N. G. Martin, G. W. Montgomery, P. M. Visscher.

†**215**/10:00 **Broad and fine scale recombination rate variation in humans.** G. Coop, W. Wen, C. Ober, J. K. Pritchard, M. Przeworski.

216/10:15 **Post genome-wide association challenges at the complex-disease associated locus *CD25* on chromosome 10p15.** C. E. Lowe, J. D. Cooper, J. A. Todd.

NOTES

† Trainee Award Finalist

Saturday, October 27 8:00 AM–10:30 AM
Concurrent Platform Sessions IV (54–60)

SESSION 54 – Regulatory Element Discovery and Function
Hall H
Co-Moderators: Len A. Pennacchio, Lawrence Berkeley National Laboratory, Berkeley, CA; and Bing Ren, University of California-San Diego, La Jolla

217/8:00 **Genome-wide Mapping of Allele-specific Protein-DNA Interactions in Human Cells.** N. D. Maynard, T. H. Kim, J. Chen, J. B. Fan, B. Ren.

218/8:15 **CTCF binding in *cis* regulates CAG/CTG instability at the spinocerebellar ataxia type 7 (SCA7) locus.** K. A. Hagerman, R. T. Libby, V. V. Pineda, R. Lau, J. D. Cleary, B. L. Sopher, D. H. Cho, S. Baccam, S. J. Tapscott, G. N. Filippova, C. E. Pearson, A. R. La Spada.

219/8:30 **Genome-wide analysis of RUNX/AML target genes.** L. Cao, Y. Liu, J. Paschal, A. M. Bowcock.

220/8:45 **A multi-lineage, whole-genome map of human DNaseI hypersensitive sites: identification of candidate functional elements underlying multiple common diseases.** P. Sabo, M. Kuehn, R. Thurman, J. Goldy, A. Haydock, M. Weaver, K. Lee, R. Sandstrom, S. Neph, W. Noble, M. Dorschner, J. Stamatoyannopoulos.

221/9:00 **Identification and characterization of cell type-specific and ubiquitous chromatin regulatory elements.** G. E. Crawford, H. Xi, H. P. Shulha, J. M. Lin, T. R. Vales, Y. Fu, D. M. Bodine, R. D. G. McKay, J. G. Chenoweth, P. J. Tesar, T. S. Furey, B. Ren, Z. Weng.

222/9:15 **Chromatin accessibility is associated with recombination hotspots of genomic rearrangements.** M. O. Dorschner, M. A. Weaver, A. Haydock, J. Goldy, K. Lee, S. Vong, F. Neri, A. Shafer, P. Sabo, J. A. Stamatoyannopoulos.

223/9:30 **Combinatorial Potential of Human Enhancers.** L. A. Pennacchio, A. Visel, E. M. Rubin.

†**224**/9:45 **A whole-genome association study of global gene expression.** L. Liang, A. L. Dixon, M. F. Moffatt, W. Chen, S. Heath, K. C. C. Wong, J. Taylor, I. Gut, M. Farrall, G. M. Lathrop, G. R. Abecasis, W. O. C. Cookson.

225/10:00 **Functional interactions of conserved non-coding (CNCs) sequences with other CNCs using circular chromosome conformation capture (4C).** D. Robyr, G. Duriaux-Sail, S. Polti, C. Wyss, S. Deutsch, S. E. Antonarakis.

226/10:15 **Ultraconserved Knockout Mice are Viable.** N. Ahituv, A. Visel, Y. Zhu, L. A. Pennacchio, E. M. Rubin.

Saturday, October 27 8:00 AM–10:30 AM
Concurrent Platform Sessions IV (54–60)

SESSION 55 – Cancer Genetics
Room 20A
Co-Moderators: James D. Fackenthal, University of Chicago, IL; and Sharon Plon, Baylor College of Medicine, Houston, TX

227/8:00 **Constitutional telomere shortening may be a predisposition to young onset microsatellite stable colorectal cancer.** L. A. Boardman, D. L. Riegert-Johnson, R. A. Johnson, S. L. Slager, S. J. Achenbach, S. N. Thibodeau, G. M. Petersen.

228/8:15 **TopoIIIα, a Member of the BRAFT Complex, functions in the Fanconi anemia pathway.** A. Hemphill, S. Philip, M. Al-Dhalimy, S. Olson, R. Moses.

229/8:30 **The Fanconi Anemia pathway plays a critical role in recombinational telomere maintenance in ALT-immortalized human cells.** H. Root, M. S. Meyn.

230/8:45 **The telomeric protein TRF2 and Nijmegen Breakage syndrome protein NBS1 modulate the association of the ataxia-telangiectasia protein ATM with DNA damage.** P. Bradshaw, W. Wang, D. J. Stavropoulos, M. S. Meyn.

231/9:00 **The role of hMSH5 in DNA double-strand break repair.** J. D. Tompkins, N. Zhao, C. Her.

232/9:15 **A randomised controlled trial of aspirin and resistant starch to prevent colorectal neoplasia in Lynch Syndrome: the CAPP2 Study.** J. Burn, D. T. Bishop, J.-P. Mecklin, F. Macrae, G. Moeslein, S. Olschwang, M. L. S. Bisgaard, R. Ramesar, F. Elliott, G. Barker, J. Jass, H. T. Lynch, J. Mathers.

233/9:30 **Molecular basis of the Li-Fraumeni syndrome (LFS): an update from the French LFS cohort.** G. Bougeard, S. Baert-Desurmont, C. Martin, S. Vasseur, L. Brugières, A. Chompret, D. Stoppa-Lyonnet, C. Bonaïti-Pellié, T. Frebourg, the French LFS Network.

234/9:45 **Identification of modifiers of BRCA1/2: results from combined analysis from the Consortium of Investigators of Modifiers of BRCA1/2.** A. Antoniou, J. Beesley, D. F. Easton, G. Chenevix-Trench, Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA).

235/10:00 **Multiple ADH genes are associated with head and neck cancers in three large independent studies.** P. Brennan, M. Hashibe, V. Gaborieau, J. McKay On behalf of the Central Europe, ARCAGE and the Latin America Head and Neck Cancer Study.

236/10:15 **Confirmation study of prostate cancer risk variants at 8q24 in African Americans identifies a novel risk locus.** R. Kittles, C. Robbins, J. Benn Torres, S. Hooker, C. Bonilla, W. Hernandez, A. Candereva, C. Ahaghotu, J. Carpten.

† Trainee Award Finalist

Saturday, October 27 8:00 AM–10:30 AM
Concurrent Platform Sessions IV (54–60)

SESSION 56 – Getting Under the Skin
Room 20B/C
Co-Moderators: Ophir D. Klein, University of California, San Francisco; and Michael J. Gambello, University of Texas, Houston

237/8:00 **Translational re-initiation of DNp63 protein causes Rapp-Hodgkin syndrome.** T. Rinne, K. Krahn, E. Lamme, J. C. Murray, B. van den Heuvel, J. Schalkwijk, H. G. Brunner, J. Zhou, H. van Bokhoven.

238/8:15 **The Debré type of autosomal recessive cutis laxa is associated with brain dysgenesis or neurodegeneration and defective N-glycosylation.** L. Van Maldergem, M. Yuksel-Apak, H. Kayserili, E. Seemanova, S. Giurgea, J. Vigneron, M. Greally, E. Leao-Teles, L. Basel-Vanagaite, J. Jaeken, S. Mundlos, W. B. Dobyns.

239/8:30 **Connective Tissue Conundrum: The EDS IV Clinical Spectrum.** B. D. Rink, C. L. Blout, M. E. Nunes.

240/8:45 **Hereditary disorders of connective tissue may present with Chiari I malformation, occipitoatlantoaxial hypermobility, and functional cranial settling.** C. A. Francomano, T. Milhorat, P. Bolognese, M. Nishikawa, N. McDonnell.

241/9:00 **Generalized Arterial Calcification of Infancy (GACI): clinical Course and Prevalence of *ENPP1* mutations.** F. Rutsch, P. Böyer, Y. Nitschke, N. Ruf, G. Weissen-Plenz, P. Nürnberg, R. Terkeltaub.

242/9:15 **Evidence for T(Brachyury) as a Candidate Gene for Vertebral Malformations.** P. Giampietro, C. Raggio, J. Staubli, E. McPherson, L. Ivacic, K. Rasmussen, F. S. Jacobsen, F. Faciszewski, R. M. Pauli, J. Burmester, I. Glurich, O. Boachie-Adjei, R. Blank.

243/9:30 **Molecular mechanisms underlying congenital scoliosis.** K. Staehling-Hampton, A. S. Cornier, K. M. Delventhal, J. F. Caubet, J. B. Emans, H. Welsh, P. Turnpenny, O. Pourquie.

244/9:45 **Molecular analysis of the SHOX gene in 409 children with short stature.** C. Huber, M. Rosilio, A. Munnich, V. Cormier-Daire, The French SHOX Genesis Module.

245/10:00 **Carrier Frequency of Recurring Mutation Causing Severe/Lethal Recessive Type VIII Osteogenesis Imperfecta in African-Americans.** W. A. Cabral, A. M. Barnes, F. D. Porter, J. C. Marini.

246/10:15 **Genotype and phenotype correlation of *CRTAP* or *P3H1* mutations with recessive osteogenesis imperfecta.** D. Baldridge, R. Morello, J. Lennington, T. K. Bertin, M. Weis, D. R. Eyre, A. Green, J. Walsh, D. Lambert, D. Krakow, D. L. Rimoin, D. H. Cohn, U. Schwarze, P. H. Byers, B. Lee.

Saturday, October 27 8:00 AM–10:30 AM
Concurrent Platform Sessions IV (54–60)

SESSION 57 – Evolution and Population Genetics
Room 20D
Co-Moderators: Rick A. Kittles, University of Chicago, IL; and Joshua Akey, University of Washington, Seattle

247/8:00 **High-resolution genetic characterization of 51 unique human populations from the Human Genome Diversity Project.** D. Absher, J. Li, H. Tang, S. Ramachandran, A. Southwick, G. Barsh, M. W. Feldman, L. Cavalli-Sforza, R. M. Myers.

248/8:15 **Linkage disequilibrium and haplotype variation in Sub-Saharan Africa.** M. Jakobsson, F. A. Reed, T. J. Pemberton, G. Coop, D. F. Conrad, J. D. Wall, J. K. Pritchard, S. A. Tishkoff, N. A. Rosenberg.

249/8:30 **Ancestral reconstruction of segmental duplications reveals punctuated cores of human genome evolution.** Z. Jiang, H. Tang, M. Ventura, M. F. Cardone, R. Hubley, A. Smit, X. She, P. A. Pevzner, E. E. Eichler.

250/8:45 **Impact of diet on the evolution of human amylase gene copy number.** G. H. Perry, N. J. Dominy, K. G. Claw, A. S. Lee, H. Fiegler, R. Redon, J. Werner, F. A. Villanea, J. L. Mountain, R. Misra, N. P. Carter, A. C. Stone, C. Lee.

251/9:00 ***SGK* expression is increased by an ancestral allele showing a latitudinal cline in human populations.** F. Luca, M. Zou, S. Kashyap, S. Conzen, A. Di Rienzo.

252/9:15 **A scan for genetic determinants of human hair morphology: EDAR is associated with Asian hair thickness.** A. Fujimoto, R. Kimura, J. Ohashi, U. Samakkarn, W. Settheetham-Ishida, T. Ishida, Y. Morishita, T. Furusawa, M. Nakazawa, R. Ohtsuka, R. Yuliwulandari, L. Batubara, M. S. Mustofa, K. Tokunaga.

253/9:30 **Genetic determinants of hair, eye and skin pigmentation in Europeans.** P. Sulem, D. Gudbjartsson, S. Stacey, A. Helgason, T. Rafnar, K. P. Magnusson, F. Jonasson, B. Sigurgeirsson, K. Thorisdottir, R. Ragnarsson, K. R. Benediktsson, K. K. Aben, L. A. Kiemeney, J. H. Olafsson, J. Gulcher, A. Kong, U. Thorsteinsdottir, K. Stefansson.

254/9:45 **Patterns of microsatellite variation within the KITLG and TYRP1 genes: implications for the evolutionary history of skin pigmentation in human populations.** S. Beleza, C. Martinho, I. Alves, E. Parra, M. Shriver, J. Rocha.

255/10:00 **Inference of the peopling of the world under sequential bottlenecks with admixture.** G. Hellenthal, D. Falush.

256/10:15 **Waves of expansion? Interpreting principal components analyses of human genetic variation.** J. Novembre, M. Stephens.

Saturday, October 27 8:00 AM–10:30 AM
Concurrent Platform Sessions IV (54–60)

SESSION 58 – Diabetes and Growth
Room 28
Co-Moderators: Jerome I. Rotter, Cedars-Sinai Medical Center, Los Angeles, CA; and Joann E. Curran, Southwest Foundation for Biological Research, San Antonio, TX

257/8:00 **Overlap between genome-wide linkage and association scan signals: insights from type 2 diabetes.** E. Zeggini, N. J. Timpson, T. M. Frayling, M. N. Weedon, K. S. Elliott, C. M. Lindgren, H. Lango, J. R. B. Perry, N. W. Rayner, R. M. Freathy, A. T. Hattersley, M. I. McCarthy, UK Type 2 Diabetes Genetics Consortium, The Wellcome Trust Case Control Consortium.

258/8:15 **Meta-analysis of 4552 type 2 diabetes (T2D) cases and 5576 controls on ~1.9 million genotyped and imputed SNPs spanning the human genome.** L. J. Scott, B. F. Voight, J. L. Marchini, R. Saxena, C. J. Ding, N. P. Burtt, G. Abecasis, E. Zeggini for the FUSION, DGI, and WTCCC/UKT2D Studies.

259/8:30 **Genome-wide association scans in cohorts from Sardinia and Finland identify a locus for fasting glucose levels.** W. M. Chen, A. U. Jackson, A. Scuteri, M. R. Erdos, M. Uda, W. L. Duren, S. Sanna, H. M. Stringham, A. Mulas, H. Shen, L. J. Scott, S. Najjar, A. R. Shuldiner, J. Tuomilehto, E. Lakatta, R. N. Bergman, D. Schlessinger, M. Boehnke, G. R. Abecasis, R. M. Watanabe.

260/8:45 **Type 2 diabetes whole genome association study in four populations: the DiaGen Consortium.** J. T. Salonen, P. Uimari, J.-M. Aalto, M. Pirskanen, B. Todorova, T.-P. Tuomainen, J. Luedemann, M. Nauck, W. Kerner, R. H. Stephens, J. M. Gibson, B. Ollier, N. Pendleton, W. Mahoney, D. Meyre, J. Delplanque, P. Froguel, O. Luzzatto, B. Yakir, A. Darvasi.

261/9:00 **A two-stage genome-wide association study for type 2 diabetes.** R. Sladek, L. Shen, D. Meyre, G. Rocheleau, C. Dina, J. Rung, L. Shen, A. Mazur, C. Polychronakos, D. J. Balding, P. Froguel.

262/9:15 **Glucagon is a Thrifty Gene in Mexican Americans.** C. S. Carlson, M. O. Goodarzi, A. Reiner, X. Guo, L. J. Raffel, A. Xiang, T. A. Buchanan, W. A. Hsueh, D. Siskovick, J. I. Rotter, M. J. Rieder, D. A. Nickerson.

263/9:30 **A functional common polymorphism in the Vitamin D-Responsive Element (VDRE) of the GH1 promoter contributes to Isolated Growth Hormone Deficiency (IGHD) susceptibility.** P. Momigliano-Richiardi, M. Godi, S. Mellone, L. Tiradani, Y. Carlomagno, A. Petri, G. Corneli, D. Vivenza, S. Bellone, C. Santoro, G. Bona, M. Giordano.

264/9:45 **A genome-wide association study in 5, 402 individuals identifies several susceptibility variants for body mass index.** R. J. F. Loos, S. Li, J. H. Zhao, E. Wheeler, S. Debbenham, D. Strachan, D. Hadley, K. Papadakis, W. McArdle, P. Deloukas, M. Inouye, R. McGinnis, M. Sandhu, I. Barroso, N. J. Wareham.

265/10:00 **Analysis of 16784 individuals shows that BMI-altering *FTO* genotypes are associated with obesity-related quantitative traits in the general population.** R. M. Freathy, N. J. Timpson, D. A. Lawlor, P. Elliott, A. Pouta, A. Ruokonen, S. Ebrahim, B. Shields, Y. Ben-Shlomo, L. Ferrucci, G. Paolisso, M. J. Neville, F. Karpe, C. N. A. Palmer, A. D. Morris, M.-R. Jarvelin, G. Davey Smith, M. I. McCarthy, A. T. Hattersley, T. M. Frayling.

266/10:15 **The yin and yang of T2D and cancer risk: evidence of pleiotropy from genome-wide association studies.** K. S. Elliott, E. Zeggini, N. W. Rayner, M. N. Weedon, C. M. Lindgren, N. J. Timpson, T. M. Frayling, C. J. Groves, R. M. Freathy, J. R. B. Perry, H. Lango, B. Shields, A. T. Hattersley, M. I. McCarthy.

Saturday, October 27 8:00 AM–10:30 AM
Concurrent Platform Sessions IV (54–60)

SESSION 59 – Therapy for Genetic Disorders
Room 29
Co-Moderators: Jess G. Thoene, University of Michigan, Ann Arbor; and Thomas C. Markello, Children's National Medical Center, Washington, DC

267/8:00 **Long-term oral cysteamine therapy attenuates the morbidity and mortality of nephropathic cystinosis in adults.** W. A. Gahl, J. Z. Balog, K. O'Brien, G. Golas, R. Kleta, I. Bernardini.

268/8:15 **Phase 3 extension 96-week study data for Naglazyme (galsulfase) enzyme replacement therapy in MPS VI patients.** P. Harmatz, R. Giugliani, I. Schwartz, N. Guffon, C. S. Miranda, E. Teles, J. E. Wraith, M. Beck, M. Scarpa, Z. F. Yu, J. Rhorer, S. Swiedler, S. Turbeville, H. Nicely, J. White, C. Decker.

269/8:30 **Sapropterin dihydrochloride (sapropterin) increases phenylalanine (Phe) tolerance in children with phenylketonuria (PKU) maintained on a Phe-restricted diet.** D. Gruskin, A. Dorenbaum, J. Bebchuk, N. Longo.

270/8:45 **Antisense-mediated exon 51 skipping restores local dystrophin expression in muscle of Duchenne muscular dystrophy patients.** A. Aartsma-Rus, J. J. G. M. Verschuuren, A. A. M. Janson, G. Platenburg, G.-J. B. van Ommen, J. C. T. van Deutekom.

271/9:00 **Translational read-through of a nonsense mutation in ATP7A is associated with treatment responsiveness in Menkes disease.** A. Donsante, J. Tang, A. Yergey, P. Backlund, S. G. Kaler.

272/9:15 **Gene expression profiling of rheumatoid arthritis patients treated with anti-tumor necrosis factor.** E. J. M. Toonen, P. Barrera, H. Scheffer, T. R. D. J. Radstake, P. L. C. M. van Riel, B. Franke, M. J. H. Coenen.

273/9:30 **Clinical practice protocols for 3-methylcrotonyl CoA carboxylase (3-MCC) deficiency.** G. L. Arnold, D. D. Koeberl, B. A. Barshop, B. K. Burton, S. Cederbaum, A. Feigenbaum, C. O. Harding, D. Kronn, D. Matern, J. B. Gibson, C. L. Garganta, N. Braverman, N. Longo, S. G. Kahler, the 3-MCC working group.

274/9:45 **Bronchoscope-guided, targeted lobar aersolization of HDAd into nonhuman primate lungs results in uniform, high level pulmonary transduction, long-term transgene expression and negligible toxicity.** A. L. Beaudet, P. Hiatt, N. Brunetti-Pierri, R. McConnell, D. Palmer, R. Zuo, F. Vetrini, M. Finegold, P. Ng.

275/10:00 **A farnesyltransferase inhibitor prevents cardiovascular disease in a progeria mouse model.** B. C. Capell, M. Olive, M. R. Erdos, K. Cao, D. A. Faddah, K. N. Conneely, H. San, X. Qu, H. Avallone, F. Kolodgie, R. Virmani, E. G. Nabel, F. S. Collins.

†**276**/10:15 **Small molecule correction of an inherited learning defect in *Neto1* mutant mice.** D. Ng, M. Kanisek, G. M. Pitcher, R. K. Szilard, A. Sertie, S. J. Clapcote, J. C. Roder, M. W. Salter, R. R. McInnes.

† Trainee Award Finalist

Saturday, October 27 8:00 AM–10:30 AM

Concurrent Platform Sessions IV (54–60)

SESSION 60 – Molecular Basis of Mendelian Disorders II
Room 30
Co-Moderators: Brendan Lee, Baylor College of Medicine, Houston, TX; and David Ng, Genetic Epidemiology Branch, National Institutes of Health, Rockville, MD

277/8:00 **Deficiency of PORCN, a regulator of Wnt signaling, causes focal dermal hypoplasia.** K.-H. Grzeschik, D. Bornholdt, F. Oeffner, A. Koenig, M. Boente, H. Enders, B. Fritz, M. Hertl, U. Grasshoff, K. Hoefling, V. Oji, M. Paradisi, C. Schuchardt, Z. Szalai, G. Tadini, H. Traupe, R. Happle.

278/8:15 **Spectrum of *PORCN* mutations in Focal Dermal Hypoplasia.** V. R. Sutton, X. Wang, J. O. Peraza-Llanes, Z. Yu, R. Rosetta, Y. C. Kou, T. N. Eble, A. Patel, C. Thaller, P. Fang, P. H. Fernandes, I. B. Van den Veyver.

†**279**/8:30 **Mutation of FAM20C leads to lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development.** M. A. Simpson, R. Hsu, L. S. Keir, J. Hao, G. Sivapalan, L. M. Ernst, E. H. Zackai, L. I. Al Gazali, G. Hulskamp, H. M. Kingston, T. E. Prescott, A. Ion, M. A. Patton, V. Murday, A. George, A. H. Crosby.

280/8:45 **Molecular genetics of Meckel syndrome.** T. Attié-Bitach, L. Baala, S. Saunier, S. Audollent, M. Delous, R. Khaddour, C. Ozilou, J. Martinovic, A. Munnich, F. MacDonald, M.-C. Gubler, S. Schneider-Maunoury, F. Encha-Razavi, C. Johnson, M. Vekemans.

281/9:00 **Mutations in the gene encoding the basal body protein RPGRIP1L, a novel nephrocystin-4 interactor, cause Joubert syndrome.** D. Doherty, H. Arts, S. E. C. van Beersum, M. A. Parisi, S. J. F. Letteboer, N. T. Gorden, T. A. Peters, T. Märker, K. Voesenek, A. Kartono, H. Ozyurek, F. M. Farin, H. Y. Kroes, U. Wolfrum, H. G. Brunner, F. P. M. Cremers, I. A. Glass, N. V. A. M. Knoers, R. Roepman.

282/9:15 **Domain-specific mutations in FBN1 cause a congenital form of scleroderma: Stiff Skin Syndrome.** B. Loeys, D. Riegert-Johnson, P. Whiteman, V. McDonnell, P. J. Coucke, A. De Paepe, D. Judge, P. Handford, L. Sakai, H. C. Dietz.

283/9:30 **Complex genetic approaches to monogenic disease: cystinosis as an example.** E. K. Moses, J. E. Curran, M. P. Johnson, J. Charlesworth, T. D. Dyer, S. A. Cole, H. H. H. Goring, J. Blangero.

284/9:45 **Synergistic heterozygosity for functional TGFβ1 SNPs and BMPR2 mutations modulate age of diagnosis and penetrance of Familial Pulmonary Arterial Hypertension (FPAH).** J. A. Phillips III, J. S. Poling, C. A. Phillips, K. C. Stanton, E. D. Austin, J. D. Cogan, L. A. Wheeler, J. E. Loyd.

285/10:00 ***PDE8B*, encoding a high affinity cAMP phosphodiesterase, is mutant in Micronodular Adrenocortical Hyperplasia.** A. Horvath, C. Giatzakis, E. Levine, P. Osorio, A. Robinson-White, K. Tzang, S. Boikos, M. Nesterova, C. A. Stratakis.

286/10:15 **Importance of functional studies for diagnosing effects of rare disease-causing missense mutations.** K. V. Krasnov, M. Tzetis, J. Cheng, G. G. Germino, W. B. Guggino, G. R. Cutting.

NOTES

† Trainee Award Finalist

ABSTRACTS

Abstracts are divided into two sections: platform and poster presentations. In each section, the number located above the title identifies the abstract in all other listings in this book.

■ Platform Presentations

Abstracts for platform presentations appear first and are organized chronologically by day of presentation. These abstracts are numbered consecutively beginning with **1** and ending with **286**.

Day/Date	*Time*	*Session Number and Type*	*Abstract Numbers*
Wednesday, October 24	1:30 PM – 3:30 PM	12: Plenary Platform Session	1 – 6
Thursday, October 25	8:00 AM – 10:30 AM	15–21: Concurrent Platform Sessions I	7 – 76
	2:00 PM – 4:30 PM	29–35: Concurrent Platform Sessions II	77 – 146
Friday, October 26	8:00 AM – 10:30 AM	36–42: Concurrent Platform Sessions III	147 – 216
Saturday, October 27	8:00 AM – 10:30 AM	54–60: Concurrent Platform Sessions IV	217 – 286

For session date, time of day and location, and for each platform presentation's start time, check the Platform Session Listings in the front of this book.

■ Poster Presentations

All posters will be displayed from Wednesday, October 24, through Friday, October 26, in Exhibit Hall E of the San Diego Convention Center. The Poster Presentation Listings in this book include W, T, F, to indicate the day on which each poster is presented: W = Wednesday, T = Thursday, F = Friday. Following is the schedule for open viewing and for sessions (when authors are present at their boards).

Wednesday, October 24

10:30 AM – 12:30 PM	Poster authors place posters on boards
10:30 AM – 6:30 PM	Posters open
4:30 PM – 6:30 PM	**Poster Session I (W)**

Thursday, October 25

8:00 AM – 6:30 PM	Posters open
4:30 PM – 6:30 PM	**Poster Session II (T)**

Friday, October 26

8:00 AM – 1:30 PM	Posters open
10:30 AM – 12:30 PM	**Poster Session III (F)**
12:30 PM – 1:30 PM	All authors remove posters from boards

New this year: To encourage discussion and to facilitate the exchange of information, posters are sorted by related topics, keyword (selected by the first author at the time of abstract submission and used to group poster presentations with a related theme), and then in alphabetical order by the last name of the first author.

Abstracts are consecutively numbered from **287** through **2814** for poster presentation and appear in chronological order in Exhibit Hall E of the San Diego Convention Center. On the following pages, the number above each abstract's title corresponds to the poster board number in Exhibit Hall E. Refer to the listing below for poster groupings by topic:

Abstract/ Poster Board	*Topic*
287 – 345	Cancer Cytogenetics
346 – 491	Cancer Genetics
492 – 683	Clinical Genetics, Malformations and Dysmorphology
684 – 741	Epigenetics
742 – 786	Clinical Genetics, Malformations and Dysmorphology
787 – 816	Genetic Counseling and Clinical Testing
817 – 839	Genetics Education
840 – 917	Molecular Basis of Mendelian Disorders
918 – 949	Development
950 – 968	Psychiatric Genetics and Neurogenetics
969 – 1024	Molecular Basis of Mendelian Disorders
1025 – 1032	Molecular Basis of Disorders with Complex Inheritance
1033 – 1071	Pharmacogenetics
1072 – 1137	Molecular Basis of Mendelian Disorders
1138 – 1231	Mapping, Linkage and Linkage Disequilibrium
1232 – 1273	Molecular Basis of Mendelian Disorders
1274 – 1379	Evolution and Population Genetics

Abstract/ Poster Board	*Topic*
1380 – 1435	Mapping, Linkage and Linkage Disequilibrium
1436 – 1553	Metabolic Disorders
1554 – 1697	Cytogenetics
1698 – 1808	Cardiovascular Genetics
1809 – 1978	Psychiatric Genetics and Neurogenetics
1979 – 2190	Statistical Genetics and Genetic Epidemiology
2191 – 2225	Ethical, Legal and Social Issues in Genetics
2226 – 2228	Public Policy
2229 – 2298	Therapy for Genetic Disorders
2299 – 2327	Reproductive Genetics
2328 – 2386	Molecular Basis of Disorders with Complex Inheritance
2387 – 2428	Prenatal and Perinatal Genetics
2429 – 2506	Molecular Basis of Disorders with Complex Inheritance
2507 – 2533	Genomics
2534 – 2618	Molecular Basis of Disorders with Complex Inheritance
2619 – 2758	Genomics
2759 – 2814	Gene Structure and Function

1

Comparative sequence analysis of primate subtelomeres. *K. Rudd[1], R. Endicott[1], C. Friedman[1], M. Walker[1], J. Young[1], K. Osoegawa[2], R. Blakesley[3], P. de Jong[2], E.D. Green[3], B. Trask[1].* 1) Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Children's Hospital of Oakland Res Inst, Oakland, CA; 3) NHGRI, NIH, Bethesda, MD.

Subtelomeric regions are among the most structurally complex, variable, and dynamic areas of the genome. Subtelomeres are the transition zones between chromosome-specific sequences and the arrays of telomere repeats at the end of chromosomes. The identity, arrangement, and polymorphism of the blocks of subtelomeric sequence shared among multiple chromosomes suggest that subtelomeric duplications spread recently. We traced the evolutionary history of the chromosome-15 subtelomere in the genomes of human, chimpanzee, gorilla, orangutan and macaque using FISH, PCR, and sequencing of genomic clones. The ancestral locus lies internally on macaque chromosome 7; however, a chromosome fission event gave rise to two acrocentric chromosomes in the common ancestor of the great apes. Sequence originating at this fission site now resides at the terminus of 15q and the pericentromere of 14q in great apes. Subsequent exchanges have added and removed subtelomeric material on chromosome 15q, as well as transferred large subtelomeric regions to other chromosomes. At least 250 kb from the fission site region transferred to the end of chromosome 4 in the ancestor of chimpanzee and gorilla. This hybrid subtelomere contains sequences orthologous to the human 4q and 15q. Interestingly, the proximal 4q-like subtelomeric region is associated with facioscapulohumeral muscular dystrophy in humans. Eight olfactory receptor (OR) genes encompassing 125 kb have been lost from the end of the 15q subtelomere in the human and chimpanzee genomes. A terminal subtelomeric region containing a highly conserved gene has been affixed to the 15q subtelomere in the human lineage only. The orangutan chromosome 15 subtelomere is very similar to the ancestral locus, and the gorilla 15q subtelomere has lost a subset of ORs. Our detailed analysis of the chromosome 15 subtelomere has shown significant structural changes in each lineage, demonstrating that subtelomeres are one of the most rapidly evolving regions of the genome.

2

Large-scale evaluation of polymorphisms in predicted microRNA binding sites reveal effect on mRNA expression levels. *M. Jain[1,3], F. Pettersson[1], J.M. Taylor[1], J.L. Min[1], J.C. Barrett[1], J. Broxholme[1], M.I. McCarthy[1,2], K.T. Zondervan[1], L.R. Cardon[1], C.M. Lindgren[1,2].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Oxford Centre for Diabetes, Endocrinology and Medicine, University of Oxford, Oxford, UK; 3) Medical Genetics Branch, NHGRI, Bethesda, MD.

MicroRNAs (miRNAs) are non-coding small RNAs that regulate mRNA by binding to *cis*-regulatory sites in 3' untranslated regions (UTR). They wield their influence by either targeting mRNA for cleavage or by repressing translation. Mutations in mRNA binding sites are known to result in a handful of diverse phenotypes emphasizing their functional importance. The effect of more common polymorphisms, however, remains unknown. We examined the role of miRNA on mRNA levels by performing an association analysis between SNPs in miRNA binding sites of *cis*-acting expression quantitative trait loci (eQTL) and mRNA expression data from the 4 HAPMAP populations. Results from 5,424 SNPs matched to 3,802 different transcripts in the HAPMAP CEU population indicate that 37 SNPs in different binding sites within 34 transcripts are associated with transcript level (empirical significance: $p<1x10^{-5}$). We substantiated the presence of seven miRNAs using probes for host mRNAs in the expression dataset. For all studied transcripts permutations validate an over-representation of associated miRNA binding SNPs compared to exon, intron, 5'UTR and non-miRNA binding 3'UTR SNPs (empirical significance: $p<1x10^{-4}$). Of the 37 associated SNPs in CEU, 22 replicate in CHB and JPT populations and 14 in the YRI population ($p<1x10^{-3}$). Nine eQTLs replicate in all three populations with seven leading to down-regulation of mRNA level. Of these, two, *ZNF230* and *ZNF584*, are purported transcription factors, one, *CRIPT*, is potentially functionally important in excitatory synapses and one, *AXIN1*, has been implicated in a variety of cancers. Our analyses show for the first time that miRNA binding site polymorphisms are associated with mRNA expression differences and suggest functional relevance for these variants and potential involvement in common, complex traits.

3

Identification of a novel gene responsible for Charcot-Marie-Tooth disease (CMT4J). *C.Y. Chow[1], Y. Zhang[2], J.J. Dowling[3], N. Jin[2], M. Adamska[1], K. Shiga[4], K. Szigeti[4,6], M.E. Shy[8], J. Li[8,9], X. Zhang[8], J.R. Lupski[4,5,7], L.S. Weisman[2], M.H. Meisler[1].* 1) Human Genetics; 2) Life Sciences Institute; 3) Department of Neurology, University of Michigan, Ann Arbor MI; 4) Departments of Molecular and Human Genetics; 5) Pediatrics; 6) Neurology, Baylor College of Medicine, Houston TX; 7) Texas Children's Hospital, Houston TX; 8) Department of Neurology, Wayne State University School of Medicine, Detroit MI; 9) John D. Dingle VA Medical Center, Detroit MI.

The spontaneous mouse mutant *pale tremor* displays a severe movement disorder with extensive loss of neurons from sensory and autonomic ganglia during the neonatal period. There is also neuron loss from specific brain regions by 3 weeks of age. Sciatic nerves exhibit reduction in the number of large myelinated axons and reduced nerve conduction velocity and amplitude of the compound action potential. LAMP2-positive vacuoles accumulate in mutant cells demonstrating involvement of the late endo-lysosomal pathway. We generated a large F2 cross with CAST/Ei and mapped the *pale tremor* gene to a 2 Mb nonrecombinant region of mouse Chr10. Analysis of genes in this region identified insertion of an Etn2β transposon in the intron of a novel gene, resulting in loss of the normal transcript. The disrupted gene is homologous to a yeast gene that regulates the levels of $PI(3,5)P_2$. To evaluate the role of the *pale tremor* gene in human peripheral neuropathy, we screened DNA from 95 patients with CMT disorder who were negative for mutations in known CMT genes. Mutations were identified in four unrelated Caucasian patients with severe disease. All four individuals were compound heterozygotes carrying a distinct protein truncation mutation on one chromosome. All four also carried the same missense mutation, I41T, on a common 15 kb haplotype. This variant was not present in 300 neurological normal controls. Pedigree analysis shows autosomal recessive transmission from heterozygous, unaffected parents. The corresponding mutation in the yeast homolog resulted in partial loss of function. The sensitivity of neurons to $PI(3,5)P_2$ levels reveals an unappreciated role of this low-abundance signalling lipid.

4

Bezafibrate cures clinical and metabolic symptoms of the muscular form of CPT2 deficiency. *F. DJOUADI[1], J. BASTIN[1], P. LAFORET[3], F. AUBEY[1], A. MOGENET[4], S. ROMANO[5], A. VASSAULT[5], S. GOBIN[2], B. EYMARD[3], JL. BRESSON[5], JP. BONNEFONT[2].* 1) CNRS UPR 9078 and; 2) INSERM U781,Université Paris-Descartes; 3) Myology Institute, Hopital de la Pitié; 4) CIC Necker and; 5) Metabolism and Biochemistry Department, Hopital Necker; Paris, France.

Carnitine Palmitoyltransferase 2 (CPT2) is a key-enzyme of mitochondrial fatty acid beta-oxidation (FAO), involved in the control of long-chain fatty acids (LCFA) entry into mitochondria. CPT2 deficiency has 2 clinical presentations: a neonatal form with fatal hepatocardiac failure, or an adult myopathic form with myalgia, exercise intolerance, and recurrent attacks of rhabdomyolysis triggered by exercise, fever, starvation.... The phenotypic variability correlates with residual CPT2 activity, degree of impairment of long-chain FAO (LCFAO), and CPT2 gene mutations. Management of patients based on reduced lipid intake or exercise limitation has little effect on clinical condition, and there is no established therapy for this disorder We recently showed that exposure of CPT2-deficient fibroblasts or myoblasts to bezafibrate, a widely prescribed hypolipidemic drug acting as PPAR agonist, could up-regulate CPT2 gene expression and residual enzyme activity, and possibly led to correct LCFAO flux in the deficient cells. This led us to set up a clinical trial in 6 patients with the muscular form of CPT2-deficiency, who received a daily 600-mg dose of bezafibrate for 6 months. Clinical tolerance of the treatment was excellent. Clinical condition dramatically improved in 5/6 patients, with a marked decrease in myalgia intensity and frequency, and a strong improvement in exercise tolerance and life quality. In vitro analyses were carried out on lymphocytes and skeletal muscle, sampled prior to- and at the end of the trial. LCFAO in isolated muscle mitochondria was strongly induced in 6/6 patients, and this effect was shown to result from drug-induced up-regulation of CPT2 mRNA and protein levels. For the first time, a pharmacological approach impacting the cause of the disease and not only its consequences has proven to be efficient in treating a mitochondrial FAO disorder.

5

Genetic and functional characterization of BRCA1 and BRCA2 variants of uncertain significance. *D. Goldgar[1], D. Easton[2], S. Tavtigian[3], C. Frye[4], M. Agarwal[5], D. Farrugia[5], F. Couch[5].* 1) University of Utah, Salt Lake City, UT; 2) Strangeways Research Laboratories, University of Cambridge, Cambridge, UK; 3) International Agency for Research on Cancer, Lyon France; 4) Myriad Genetic Laboratories, Inc. Salt Lake City UT; 5) Mayo Clinic, Rochester MN.

Mutation screening of the breast and ovarian cancer predisposition genes BRCA1 and BRCA2 is becoming an increasingly important part of clinical practice. Classification of rare non-truncating sequence variants in these genes is problematic because it is not known whether these subtle changes alter function sufficiently to predispose cells to cancer development. Using data from the Myriad Genetic Laboratories database of nearly 70,000 full-sequence tests, we have assessed the clinical significance of 1433 sequence variants of uncertain significance (VUS) in the BRCA genes. Three independent measures were employed in the assessment: co-occurrence in trans of a VUS with known deleterious mutations; detailed analysis of personal and family history of cancer in VUS-carrying probands by logistic regression; and in a subset of probands, an analysis of co-segregation with disease in pedigrees. For each of these factors a likelihood ratio was computed under the hypothesis that the VUS were equivalent to an "average" deleterious mutation compared to neutral with respect to risk. The likelihood ratios derived from each component were combined to provide an overall assessment for each VUS. Logistic regression based on family history was shown to be a powerful discriminator of neutral vs. deleterious variants. Statistical analysis of heterogeneity within classes of VUSs showed that deleterious variants were those that were predicted to affect splicing, fell at positions that are highly conserved among BRCA orthologs, and were more likely to be located in specific domains of the proteins. We characterized a subset of the BRCA2 missense mutations using functional assays that measure the ability of wildtype and mutant forms of BRCA2 to repair DNA damage by homologous recombination and to control centriole amplification. Overall, both assays displayed strong correlations with the results of the genetic studies.

6

Joint genome-wide analysis of 3200 Crohn's disease patients documents more than 20 significant associations. *M.J. Daly on behalf of Crohn's Disease GWA Meta-analysis Working Group.*

Genome-wide association studies (GWAS) in Crohn's disease (CD) published as of June 2007 have defined unequivocal evidence of 9 novel, replicating loci, increasing the total number of confirmed risk factors from 2 to 11. It is clear from the published data, however, that the loci identified to date constitute only a minority of the overall heritability, and that power in the individual studies was quite low to identify even loci that were later confirmed. Thus, to further gene discovery from these efforts, we have embarked on a joint analysis and coordinated replication study of top results. The combined study begins with a meta-analysis of the three published scans: 1748 cases/2938 controls (Wellcome Trust Case Control Consortium - UK) - Affymetrix 500K, 946 cases/977 controls (NIDDK IBD Genetics Consortium - North America) - Illumina HumanHap 300K, 547 CD cases/928 controls (Belgium/France) - Illumina HumanHap 300K. We have combined the existing genotype data with "imputed" genotypes predicted via statistical models of known haplotypes from the HapMap project to enable a joint analysis of roughly 3200 cases and 4800 controls on the superset of ~750,000 SNPs contained on one or both genotyping platforms. This combined data set offers substantially increased power to detect genes of modest effect - a 20% risk allele with an OR of 1.2 has only a 6% chance of achieving a p<.0001 in a "typical" 1000 case/1000 control GWAS, but a 78% chance of doing so in the combined study. The initial meta-analysis convincingly confirms all replicated published loci (including established older associations at NOD2 and IBD5 and all recently reported hits such as IL23R, ATG16L1, IRGM, NKX2-3), 9 of which have $p < 10^{-8}$. Importantly, the meta-analysis has revealed more than 30 additional loci with $p < 10^{-5}$. Very few such results are expected by chance and, as the bulk of the distribution indicates no systematic inflation ($\lambda_{GC}<1.1$), the majority of these likely constitute novel risk factors. The results of a coordinated replication effort in independent samples will be reported, providing a dramatically augmented picture of the genetic architecture of Crohn's disease.

7

Fragile X mental retardation protein deficiency leads to spontaneous mGluR5-dependent internalization of AMPA receptors. *M. Nakamoto[1], V. Nalavadi[2], M.P. Epstein[1], U. Narayanan[1], G.J. Bassell[2], S.T. Warren[1, 3, 4].* 1) Dept of Human Genetics; 2) Dept of Cell Biology; 3) Dept of Biochemistry; 4) Dept of Pediatrics, Emory Univ. School of Medicine, Atlanta, GA.

Fragile X syndrome (FXS), a common inherited form of mental retardation, is due to the functional absence of the fragile X mental retardation protein (FMRP), an RNA-binding protein that regulates the translation of specific mRNAs at synapses. Altered synaptic plasticity has been described in a mouse FXS model. However, the mechanism by which the loss of FMRP alters synaptic function, and subsequently causes the mental impairment, is unknown. Here, in cultured hippocampal neurons, we used siRNAs against *Fmr1* to demonstrate that a reduction of FMRP in dendrites leads to an increase in internalization of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) subunit, GluR1, in dendrites. This abnormal AMPAR trafficking was observed spontaneously at basal level, without synaptic stimulation by exogenous agonist and was rescued by MPEP, an mGluR5-specific inverse agonist. Since AMPAR internalization is dependent upon local protein synthesis following mGluR5 stimulation, FMRP, a negative regulator of translation, may be viewed as counter balancing signal, wherein the absence of FMRP leads to an apparent excess of mGluR5 signaling in dendrites. Because AMPAR trafficking is a driving process for synaptic plasticity underlying learning and memory, our data suggest that hypersensitive AMPAR internalization in response to excess mGluR signaling may represent the principal cellular defect in FXS, which may be corrected using mGluR antagonists.

8

Genetic interaction between the fragile X mental retardation protein and Brachyury during mammalian embryonic development. *R. Alisch, P. Jin, M. Epstein, T. Caspary, S. Warren.* Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA.

Fragile X syndrome is a common form of mental retardation, generally resulting from the absent expression of the *FMR1* gene. FMRP, the encoded protein, is an RNA-binding protein that associates with translating ribosomes and is believed to regulate translation of target mRNAs. We have previously demonstrated a genetic interaction between the *FMR1* ortholog in *Drosophila* and AGO1, a key component of the RNA-induced silencing complex (RISC) associated with the microRNA pathway. Since microRNAs also regulate translation, these data together suggest that FMRP may regulate translation in conjunction with the microRNA pathway. In order to more fully evaluate this possibility in mammals, we disrupted the AGO1 mammalian ortholog, *Argonaute2* (*Ago2*), in mice. The loss of *Ago2* results in gastrulation arrest, mesoderm expansion and ectopic expression of *Brachyury* (*T*). Previous work demonstrated two quantitative trait loci that modify the classic shortened tail phenotype in heterozygous *T* (T+) mice that map within 2 cM of both *Ago2* and *Fmr1*. We have demonstrated that heterozygosity for the *Ago2* knockout modifies the *T*/+ tail phenotype, suggesting *Ago2* is indeed a modifier of *T*. Here we show similar evidence that *Fmr1* may be the other *T* modifier. *Fmr1* knockout (ko) mice were crossed with *T*/+ mice, and *T*/+ *Fmr1* ko offspring were analyzed for tail length. While the tail to body length ratio (TBR) in wild-type and *Fmr1* ko mice is ~0.8 and the TBR in *T*/+ is ~0.23, the TBR in *T*/+ *Fmr1* ko mice is ~0.4. This partial rescue of the shortened tail phenotype in the *T*/+ *Fmr1* ko mice (P value <0.03) reveals a genetic interaction between *Fmr1* and *T*. These studies are the first to suggest a role for FMRP during embryonic development in mammals. Since mammals have three *Fmr1* paralogs, a role for FMRP in early development may have been missed in *Fmr1* ko mice due to partial complementation by the *Fmr1* paralogs *FXR1* and *FXR2*. The elucidation of these interactions may provide new insight into the molecular pathogenesis of fragile X syndrome.

9

***FMR4*: A Novel Primate-Specific Transcript Silenced in Fragile X Syndrome.** *A. Khalil, M. Faghihi, F. Modarresi, C. Wahlestedt.* Biochemistry, The Scripps Research Institute, Jupiter, FL.

Fragile X syndrome (FXS) is the most common cause of inherited mental retardation. It is caused by a CGG expansion in the 5' UTR of *FMR1* which leads to the absence of the fragile X mental retardation protein (FMRP). However, *Fmr1* knockout and CGG repeat expansion knock-in mouse models for FXS did not fully recapitulate all of the phenotypes observed in human patients. Furthermore, longitudinal clinical observations of fragile X patients have shown that the severity of the cognitive, behavioral and morphological symptoms of FXS is highly variable. Therefore we postulated that there could be other genetic elements in addition to *FMR1* that could be responsible for the fragile X syndrome phenotype.

Using genomic approaches we discovered a new transcript upstream of *FMR1* which we refer to as *FMR4*. We found *FMR4*, similar to *FMR1*, to be silenced in fragile X patients and up-regulated in pre-mutation carriers in untransformed leukocytes. Northern blot analysis shows that *FMR4* is expressed in several human tissues including brain, liver, placenta, small intestine, colon and spleen. Knockdown of *FMR1* by several siRNAs did not affect *FMR4* and vice versa suggesting that *FMR4* is not a regulatory transcript for *FMR1*. Interestingly, however, the knockdown of *FMR4*, but not *FMR1*, is important for human cell viability *in vitro*; knockdown of *FMR4* resulted in cell cycle defects and apoptosis.

These findings are potentially significant since: 1) like *FMR1*, the newly discovered *FMR4* transcript is silenced in fragile X patients and could therefore relate directly to fragile X syndrome symptomatology; 2) *FMR4* is a primate-specific transcript which could help explain the failure of animal models to fully recapitulate all of the human phenotypes in fragile X syndrome; and 3) our results also demonstrate a potential role for a non-coding RNA transcript in an inherited human disorder.

10

Penetrance of dementia in male carriers of the FMR1 premutation. *S. Jacquemont[1], M. Sevin[2], Z. Kutalik[3,4], P. Damier[2], M. Verceletto[2], P. Renou[2], P. Boisseau[5], S. Bergmann[3,4], J.M. Rival[5], J.S. Beckmann[1,3].* 1) Service de génétique médicale, CHUV, Lausanne, Switzerland; 2) Centre d'Investigation Clinique, CHU de Nantes, France; 3) Departement de Génétique Médicale, UNIL, Lausanne, Switzerland; 4) Swiss Institute of Bioinformatics; 5) Service de Génétique Médicale, CHU de Nantes, France.

The Fragile X-associated tremor ataxia syndrome (FXTAS) is a newly recognized neurodegenerative disorder found among carriers of the FMR1 premutation. Core clinical features are progressive cerebellar ataxia and intention tremor. Several studies have also reported cognitive decline/dementia in these patients, however, there is no data on the penetrance of this debilitating symptom. 67 males aged 50 years or older were recruited from fragile X families, regardless of their medical history or genetic status. The Mattis Dementia Rating Scale (MDRS) was used to quantify global cognitive capacities. Other tests assessed executive functioning, verbal and non-verbal working memory, visuospatial skills, and reasoning capacities. The evaluator was blinded as genetic status was obtained after evaluation. Molecular testing revealed 30 premutation carriers and 37 intrafamilial controls. Based on a cut-off score of 129 for the MDRS and an education level corrected at 9.5 years, the frequency of dementia at age 70 among individuals with large premutation (90 CGG), small premutation (60 CGG) and controls was 80%, 50%, and 4%, respectively. At age 60 these frequencies were 42%, 1%, and 0%, respectively. Multiregression analysis accounting for age and education found a significant correlation between CGG repeat length and the following: MDRS, tests of executive functions, visuospatial performances, and reasoning capacities ($p<0.001$). The penetrance of dementia among carriers of large premutation is particularly high. There is also a sizeable effect for smaller alleles which is of importance due to their higher prevalence in the general population. These data will greatly contribute to genetic counseling as this multiregression model is now able to provide estimates for the penetrance of dementia for any given allele size, age and education level.

11

Identification of novel small molecules suppressing rCGG-repeat-mediated neuronal toxicity. *A. Qurashi, H. Liu, P. Jin.* Department of Human Molecular Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurodegenerative disorder recognized in fragile X premutation carriers. Using Fruit fly, we have previously demonstrated that elongated noncoding CGG repeats in FMR1 allele as the pathogenic cause of FXTAS. Here we are utilizing this FXTAS fly model to identify small molecules that can ameliorate rCGG-mediated neuronal toxicity. We have found that neuronal overexpression of rCGG repeats could lead to lethality during early stages of development. Using this lethal phenotype, we have screened a collection of 2,000 FDA-approved, biologically active and structurally diverse compounds. We identified 20 compounds that could reverse the lethality caused by rCGG repeats, with several of them having the potential to target glutamatergic pathways. Of particular interest among them are 5-fluoroindole-2-carboxylic acid and 6, 7-dichloro-3-hydroxy-2-quinoxalinecarboxylic acid, known NMDA receptor antagonists, suggesting that over-activation of NMDA receptor could be involved in rCGG-mediated neurodegeneration observed in FXTAS. Candidate drugs are being further evaluated in rescuing locomotor and brain morphological anomalies observed in FXTAS fly model. Our results demonstrate the utility of a Drosophila model for screening small molecule libraries. This approach may identify potential therapies, and reveal the cellular and molecular pathways involved in FXTAS.

12

GABA agonists rescue morphological, biochemical and behavioral phenotypes of the *Drosophila* model of fragile X syndrome. *S. Chang[1], S.M. Bray[1], D.C. Zarnescu[2], P. Jin[1], S.T. Warren[1].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Molecular & Cellular Biology, University of Arizona, Tucson, AZ.

Fragile X syndrome (FXS) is caused by the functional loss of the fragile X mental retardation 1 (FMR1) gene. Deletion of the FMR1 ortholog in *Drosophila*, *dFmr1*, produce phenotypes useful as a FXS model system. We have discovered that dFmr1-deficient *Drosophila* die when reared on food containing increased levels of glutamate, consistent with the theory that loss of FMRP disrupts the regulation of glutamate signaling. Based on this observation, we have previously conducted a small molecule screen and identified several compounds that could rescue *dFmrp*-mediated lethality. In this study, we focused on three of them that have been implicated in the GABAergic inhibitory pathway. We found that treatments of GABA agonists, including GABA, could rescue *dFmrp*-deficiency lethality on glutamate supplemented food and that this rescue was blocked by a selective GABA(B) receptor antagonist. GABA agonists also rescued known *dFmr1*-deficient phenotypes such as mushroom bodies defects, excess Futsch translation and abnormal male courtship behavior. Our results show that GABA agonists, whether compensating for reduced GABA receptor expression or tempering excess excitatory glutamate stimulation, are capable of rescuing numerous *dFmrp*-deficient phenotypes *in vivo*. Since GABA agonists are already medically available, these data may accelerate pharmaceutical intervention in FXS.

13

Genetic mechanisms of trinucleotide repeat instability in Drosophila. *J. Jung[1], N.M. Bonini[1, 2].* 1) Department of Biology, University of Pennsylvania, Philadelphia, PA; 2) Howard Hughes Medical Institute.

Expansion of trinucleotide repeat sequences is responsible for over 20 human diseases, including polyglutamine (polyQ) diseases caused by CAG repeat expansions and Fragile X syndromes or Myotonic Dystrophy caused by CGG or CTG repeat expansions in the non-coding part of respective genes. Disease phenotype and the age of disease onset are strongly affected by repeat expansions. However, mechanisms of repeat expansions in vivo remain poorly understood. Previously, we have reported the development of a Drosophila model of germline trinucleotide repeat instability, which recapitulates key features of human repeat instability. Utilizing the model, we uncovered two novel modifiers of repeat instability, nucleotide excision repair (NER) and CREB-binding protein (CBP). Moreover, through genetic or pharmacological treatments targeting NER or CBP, we were able to suppress trinucleotide repeat expansions, presenting a potential therapeutic opportunity to suppress repeat instability. Currently, We are conducting a genome-wide genetic screen to identify additional modifiers of repeat instability. We will report the progress in our modifier screen during the meeting.

14

Access to Credible Genetics Resources Network. *S. Terry[1], M. Weaver[2], K. Reed[3], H. Ferguson[1], C. Constantin[7], A. Vatave[7], C. Greene[2], A. Gepp[4], K. Clapp[5], P. Furlong[6], J. McInerney[3], M. Blitzer[2].* 1) Genetic Alliance, Washington, DC; 2) Univ. of MD, Baltimore, MD; 3) NCHPEG, Lutherville, MD; 4) National Council of La Raza, Washington, DC; 5) FRAXA Research Foundation, Newburyport, MA; 6) Parent Project Muscular Dystrophy, Middletown, Ohio; 7) CDC, Atlanta, GA.

Quality information on single gene disorders is limited. Individuals and their families need accurate information to make informed decisions about management, while healthcare providers need quality information to offer appropriate care. To that end, Access to Credible Genetics Resources Network (ATCGRN) has created tools: a metric, toolkit and quality presentation document, for developing, assessing, and disseminating quality information about single gene disorders. These tools are valuable to both patients and clinicians to provide a means to improve the overall quality of information on rare genetic conditions. We used a model that maximizes input from content specialists and end users and is heavily skewed toward formative evaluation by the intended audience. This process provides corrective feedback to the developers at points along the way. To facilitate these processes, the partners participate in monthly conference calls to update each other on the progress of their particular part of the project. The tools were applied to Fragile X Syndrome and Duchenne Becker Muscular Dystrophy. The metric evaluates quality information and was pilot tested by end-users. The toolkit assesses the accuracy and completeness of topics parents and clinicians need to make informed decisions. The quality presentation document has also been vetted with end-users: educational material developers and support group leaders, in particular. This tool has evolved from a simple checklist to a document that includes information about the process of developing an educational material as well as an evaluation of the content of an educational material. These three distinct tools have been refined through the cooperation of the ATCGRN collaborators and should be applicable to all rare, single gene disorders for the production of high quality information.

15

Fragile X syndrome newborn detection--Pilot study. *R. Saul, M. Friez, K. Eaves, G. Stapleton, J. Collins, R. Stevenson.* Greenwood Genetic Ctr, Greenwood, SC.

Fragile X syndrome, an X-linked disorder, is the most common form of hereditary mental retardation. Phenotypic detection in the prepubertal period is very difficult, and unfortunately often occurs after families have had a second affected child. Early detection in the newborn period could allow for appropriate developmental intervention and reproductive counseling for the immediate and extended family, and such detection will become increasingly important if specific therapeutic interventions become available. Counseling for potential adult complications in premutation carriers could also be offered. A pilot study was conducted to establish the feasibility of newborn screening for Fragile X syndrome. Over the course of two years, a total of 1458 newborn males at two hospitals in upstate South Carolina (out of a potential pool of 6562 newborn males) were studied after appropriate consent procedures. The blood specimen was obtained via heelstick at the time of the standard newborn metabolic screening sample. Analyses were performed by PCR and questionable or abnormal results were confirmed by Southern blot analysis on a retained cord blood sample or a second sample from the infant. Five (5) of the newborn males had abnormal results2 with full mutations for Fragile X, 2 with premutations for Fragile X, and 1 with sex chromosome aneuploidy (47, XXY). Genetic consultations were provided for all the patients and their families. Our preliminary results suggest a much higher prevalence of Fragile X syndrome (1:729) than that reported (about 1:4,000) in previous population studies. Our study was limited by testing of a relatively small number of males only yet was the first study to test newborns in the United States prospectively over a given period of time. Further studies are suggested in a larger population using automated, high-throughput technologies capable of detecting full mutations and premutations in both sexes to establish the feasibility of adding this analysis to the standard newborn screening panel. In addition, potential benefits and risks should be studied before universal application of this test.

16

Offering carrier screening for fragile X syndrome to non-pregnant women. *S. Metcalfe[1,2], A. Archibald[1,2], J. Cohen[3], V. Collins[1], A. Henry[1], A. Jaques[1], K. McNamee[4], L. Sheffield[1,5], H. Slater[1,6], S. Wake[5].* 1) Genetics Education & Health, MCRI, Royal Children's Hosp, Melbourne, Australia; 2) Dept Paediatrics, University of Melbourne, Melbourne, Australia; 3) Fragile X Alliance, Melbourne, Australia; 4) Family Planning Victoria, Melbourne, Australia; 5) Genetic Health Services Victoria, Melbourne, Australia; 6) Victorian Clinical Genetics Services Pathology, Melbourne, Australia.

Population-based carrier screening for fragile X syndrome (FXS) remains controversial despite fulfilling many criteria. Concerns surround perceived difficulties communicating complexities of FXS. This three-phase study assessed acceptability and feasibility of offering FXS carrier screening to non-pregnant women attending a family planning clinic. Phase 1: staff and female patients participated in focus groups to discuss their views, understanding, interest and concerns about offering FXS carrier screening. Overall, women and staff were positive towards screening. These data informed production of a brochure, two questionnaires (Q1/Q2) and testing protocols. Validated questionnaires included demographics, awareness, knowledge of FXS, attitudes towards carrier screening, decision-making, and anxiety. Phase 2: women were recruited, completed Q1, and offered FXS screening. Q2 was completed one month later. Phase 3: a sample of women completing both questionnaires took part in follow-up interviews discussing their experiences in participation. Of 338 women recruited, 94% completed Q1, 59% completed Q2, to date, and 31 have been interviewed. Of the women tested (n=65; 20%), three grey-zones and one pre-mutation were found. Women's understanding of FXS was reasonably good (45% scored 8/10 or greater). They were overwhelmingly in favour of FXS screening being available to all women, although fewer had screening for a variety of reasons. Women need time to deliberate to make a decision about testing. Offering testing in this type of health setting is feasible and acceptable, and raises awareness, so that women who choose to wait for the appropriate life-stage (eg when planning a pregnancy) are already informed to consider being tested.

17

Genome-wide association scan identifies new susceptibility loci for psoriatic arthritis and psoriasis. *P.Y. Liu[1], C. Helms[1], J. Gardner[1], A. Perlmutter[2], A. Miner[2], S. Duan[1], R. Donaldson[1], C. Wise[3], P. Kwok[4], W. Liao[5], N.L. Saccone[1], J. Worthington[6], A. Barton[6], A. Menter[2], A.M. Bowcock[1].* 1) Dept Genetics, Washington Univ, St Louis, MO, USA; 2) Dept Dermatology, University of Texas Southwestern Medical Center at Dallas, TX, USA; 3) Texas Scottish Rite Hospital, TX, USA; 4) Cardiovascular Research Institute and Center for Human Genetics, UCSF, CA, USA; 5) Dept of Dermatology, UCSF, CA, USA; 6) Univ of Manchester, UK.

Psoriasis (PS) affects approximatley 2% of the European population. Psoriatic arthritis (PsA) is an inflammatory arthritis that occurs in up to one-third of patients with PS. The genetic basis of both PSA is poorly understood. A genome-wide association study (using the Illumina HumanHap300 genotyping beadchip) was performed to identify genetic factors involved in PsA susceptibility among the Caucasian population. A case-control study design was used for the initial gene discovery and for replication. We genotyped 142 patients with PsA, 132 patients with PS and 223 healthy controls from the New York Health Project for 310,000 SNPs. Case-control comparisons identified 7 regions where P < 6E-6 within the PsA group. The top-ranking SNPs associated with PsA included a novel locus within the MHC (multiple histocompatibility locus antigen cluster) that is distinct from HLA-C. This trend was not observed in the PS analysis where the top ranking SNPs lay within the class I region of the MHC. Replication studies with an independent UK PsA cohort (576 cases, 480 controls) were performed to validate the above findings. They confirmed strong evidence for association with a gene (P < 10-33) activated by nitric oxide - a strong inflammatory mediator and regulator of inflammatory responses, a protease inhibitor (P < 10-21) and a suppressor of TNF alpha induced cell death (P < 10-7). These pathways play important roles in the development of inflammation, and their role in the development of psoriasis or psoriatic arthritis start to provide a framework for the development of therapeutic interventions.

18

A common copy number variant (CNV) associated with psoriasis. *R. Cid[1], L. Armengol[1], E. Ballana[1], M. Garcia[1], R. Pujol[2], X. Estivill[1,3].* 1) Genes and Disease Program, CeGen and CIBERESP, Center for Genomic Regulation (CRG-UPF), Barcelona, Catalonia, Spain; 2) Dermatology Service, IMIM-Hospital del Mar, Barcelona, Catalonia, Spain; 3) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Psoriasis is a chronic skin disorder characterized by the presence of immune, red, scaly and patch skin lesions. Psoriasis affects most ethnic groups with the highest prevalence (3%) in northern European populations. The biology of psoriasis is poorly understood, and it is characterized by recruitment of inflammatory cells to the skin and hyperproliferation of keratinocytes. Nowadays psoriasis is regarded as a systemic immune-mediated, genetic disease of unknown cause. It is believed that psoriasis is the result of genetic predisposition and environmental factors, which involve skin lesions, infections, stress or medications. Despite the strong genetic basis for psoriasis, the genes responsible for the disorder have not yet been fully identified. We present here evidences of copy number variation (CNV) for a gene expressed in the epidermis in a significant subset of patients with psoriasis. Through comparative genome hybridization and pooling DNA of psoriatic patient samples we have identified that several chromosome regions contain consecutive sequences that vary in signal intensity. One such region spans a gene specifically expressed in the skin and is present in a lower copy number in patients than controls. The link between this region and psoriasis was further confirmed by single nucleotide polymorphisms (SNPs) tagging this region and the gene that it contains. Analysis of gene dosage in a larger number of subjects with psoriasis confirmed that a large proportion of psoriatic patients carry lower copy numbers of this gene. This CNV, which also shows ethnic differences in allele distribution, and the variations it contains, could be used to evaluate genetic predisposition to psoriasis and could be a target for pharmacological and biological treatment and prevention of psoriasis. Supported by Catalan Government (Generalitat de Catalunya).

19

High density SNP screening of the major histocompatibility complex (MHC) in systemic lupus erythematosus (SLE) families demonstrates strong evidence for independent susceptibility regions. *L.F. Barcellos[1], S.L. Clark[1], P.P. Ramsay[1], H. Quach[1], M.F. Seldin[2], J.B. Harley[3], K. Moser[3], T.W. Behrens[4, 5], P. Gaffney[3], L.A. Criswell[6].* 1) Univ of CA, Berkeley, CA; 2) Univ of CA, Davis, CA; 3) Oklahoma Medical Research Foundation, Oklahoma City, OK; 4) Univ of Minnesota, Minneapolis, MN; 5) Genentech, South San Francisco, CA; 6) Univ of CA, San Francisco, CA.

A substantial genetic contribution to SLE risk is conferred by MHC gene(s) on chr. 6p21; the most consistent associations are with class I and II genes, including HLA-A*01, -B*08, -DRB1*0301, -DRB1*1501, and -DRB1*08. Genes within class III and extended MHC regions have also been implicated. Previous studies of MHC variation in SLE have lacked statistical power and genetic resolution to fully characterize MHC influences. We recently completed state-of-the-art MHC SNP genotyping in 446 Caucasian SLE trio families (N=1,338) and 546 additional SLE cases. A total of 2,360 MHC SNPs spanning 4.9 Mb (~1 SNP/2 kb) were investigated, including variants from 159 MHC region genes (~10.7 SNPs/gene). Analyses of LD and haplotype diversity using HAPLOVIEW (v.3.31) revealed a complex architecture; 203 distinct haplotype blocks were identified. Preliminary results from TDT analyses of single SNPs were evaluated. Strong signals emerged from three MHC regions; in particular, significant associations (p<10-5) were observed for TCF19 (rs7750641) near HLA-C in class I and loci in class III regions: MICB (rs2516408), complement component 2 (rs497309), and factor B (rs537160). In addition, class II loci (BTLN2, DRA, and DQA1 on the extended DRB1*1501 haplotype) demonstrated strong evidence for association (p<10-5). Our results suggest that centromeric and telomeric boundaries of the DRB1*1501 haplotype are marked by BTLN2 and DQA1 loci (320 kb region), and that class I and III associations are independent of DRB1. Full characterization of candidate loci in class I and III regions is underway. Our large family-based study of MHC and HLA variation in 800 families (Total N=2,400) will identify all MHC gene(s) contributing to SLE risk and related phenotypes, such as lupus nephritis.

20

Mutations in the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *M. Lee-Kirsch[1], M. Gong[2], D. Choudhury[3], L. Senenko[1], K. Engel[1], Y. Lee[2,4], U. de Silva[5], T. Witte[6], T.J. Vyse[7], J. Kere[8], C. Pfeiffer[9], S. Harvey[5], S. Koskenmies[10], K. Rohde[2], A.F. Dominiczak[11], M. Gahr[1], T. Hollis[5], F.W. Perrino[5], J. Lieberman[3], N. Hubner[2].* 1) Klinik für Kinder- und Jugendmedizin, Technische Universität Dresden, Dresden, Germany; 2) Max-Delbrück-Centre for Molecular Medicine, Berlin-Buch, Germany; 3) CBR Institute for Biomedical Research, Harvard Medical School, Boston, MAä; 4) Charité, Department of Pediatrics, Berlin, Germany; 5) Department of Biochemistry, Wake Forest University Health Sciences, Winston-Salem, NC; 6) Medizinische Hochschule Hannover, Klinische Immunologie, Hannover, Germany; 7) Imperial College, Section of Rheumatology and Molecular Genetics, London, UK; 8) Karolinska Institute, Department of Biosciences and Nutrition, and Clinical Research Centre, Huddinge, Sweden; 9) Klinik für Dermatologie, Technische Universität Dresden, Germany; 10) University of Helsinki, Department of Medical Genetics and Department of Dermatology, Helsinki, Finland; 11) Department of Medicine and Therapeutics, Glasgow University, Glasgow, UK.

The hallmark of systemic lupus erythematosus (SLE), a complex autoimmune disease, is the elaboration of autoantibodies to nuclear antigens including DNA. Although several genes, which function in processing DNA or immune complexes or lowering the threshold for T cell activation, have been implicated, the genetic and molecular basis of SLE remains ill defined. We show that a mutation in the gene encoding 3'-5'DNA exonuclease (TREX1) leads to familial chilblain lupus, an autosomal dominant monogenic form of cutaneous lupus erythematosus that presents in the first years of life. We extended our findings to SLE by resequencing the entire coding region of TREX1 in SLE patients and controls. We observed monoallelic frameshift or missense mutations and one 3' UTR variant of TREX1 in 9/417 individuals with SLE that were not found in 1712 controls ($P=4.1x10^{-6}$). We functionally tested 3 mutant TREX1 alleles and show impaired enzyme activity, granzyme A-mediated apoptosis or subcellular targeting. Our findings implicate TREX1 in the pathogenesis of SLE.

21

Whole Genome Association Study Identifies Novel Risk Alleles for Multiple Sclerosis. *J.L. Haines for The International Multiple Sclerosis Genetics Consortium.* Ctr Human Genetics Research, Vanderbilt Univ Medical Ctr, Nashville, TN.

Background: Multiple sclerosis (MS) is an autoimmune disease with significant heritability. We present the first large scale, replicated whole genome association scan to identify risk alleles associated with MS.

Methods: The Affymetrix GeneChip Human Mapping 500K array was used to examine common genetic variation in ~1,000 MS affected offspring, their ~2,000 parents and 2,431 independent controls. A strict quality control analysis in which SNPs and DNA samples with low genotyping rates, excessive Mendelian errors or low minor allele frequencies were removed from further analysis resulted in a final screening data set of 931 trios genotyped for 334,923 SNPs. In the second stage, 110 SNPs were genotyped in an additional 609 trios, 2,322 cases, and 2,987 controls. The overall combined sample sizes were 1,540 trios, 2,322 independent cases and 5,418 controls (total 12,360 individuals) and final P values were calculated.

Results: After extensive quality control, a transmission disequilibrium test analysis of the initial data revealed 49 SNPs with P values <1 x 10-4, 38 of which were selected for the second stage analysis. The case-control analysis identified an additional (non-overlapping) 32 SNPs with P values <1 x 10-3. A further 40 SNPs with less stringent P-values (at least <0.01) but supported from other, a priori sources were also selected. The strongest replicated results for MS susceptibility came from two SNPs within the CD25 gene encoding the IL-R2α chain (P = 2.96 x 10-8). A non-synonymous SNP in the IL-7Rα chain gene was also strongly associated with MS susceptibility (P = 2.94 x 10-7). As expected, the HLA-DR locus was unequivocally associated with disease susceptibility (P = 8.94 x 10-81).

Conclusions: The combined results from this first whole genome association scan in MS have unequivocally confirmed non-MHC genetic variants associated with MS susceptibility and strongly implicate cytokine pathways.

22

Fine mapping of a risk gene for multiple sclerosis. *D. Reich[1,2], N. Patterson[2], P.L. De Jager[2,3,4], A. Tandon[1,2], S. McCarroll[2,5], A. Waliszewska[1,2,3], J. Neubauer[1,2], C. Schirmer[1,2], R.R. Lincoln[4], S. Poduslo[6], O. Khan[7], S.L. Hauser[4], J.R. Oksenberg[4], D.A. Hafler[1,2,3].* 1) Harvard Med School, Boston MA; 2) Broad Institute, Cambridge MA; 3) Brigham & Women's Hospital, Boston MA; 4) UCSF, San Francisco CA; 5) Mass General Hospital, Boston MA; 6) Med College of Georgia, Atlanta GA; 7) Wayne State School of Med, Detroit MI.

We recently reported a whole genome admixture scan in African Americans with multiple sclerosis (MS), demonstrating a risk locus in a 28 Mb region of chromosome 1. We have since increased the sample size to 882 cases and 1,056 controls, and the evidence for association is overwhelming (LOD=9.0). We estimate that the rate of MS on average in African Americans is ~48% lower than in those who inherit entirely European ancestry at the locus. This is sufficient to explain the lower incidence of MS in Africans Americans compared with European Americans.

Here we report a saturation fine-mapping study of the peak of MS association, using the same strategy we used in the last year to successfully identify risk variants underlying a prostate cancer admixture peak. We genotyped the African American MS cases and controls at a panel of ~2,350 single nucleotide polymorphisms (SNPs), tagging >95% of common variants in the Human Haplotype Map (HapMap) in both European Americans and West Africans. Among the 1,399 SNPs analyzed so far, none predicts MS risk beyond the admixture association. A whole genome scan in European-derived populations also failed to find a variant in the region explaining the association, suggesting that HapMap may not include the variant(s) responsible for MS risk.

A striking feature of the peak is that it includes the q-arm side of the centromere, which is incompletely assembled because of copy number polymorphisms and repetitive sequence. The coverage of SNPs in HapMap across this region is also thin, perhaps explaining why screens of HapMap SNPs have not identified a causative variant. We describe the discovery and genotyping of copy number polymorphisms in this region, and testing them for association to MS.

23

On the identification of causal genetic effects in family-based association studies. *C. Lange[1], S. Goetgeluk[1], I. Waldman[2], S.T. Weiss[3], S. VanSteelandt[2,3].* 1) Department of Applied Mathematics and Computer Sciences, Ghent University, Ghent, Belgium; 2) Dept Biostatistics, Harvard Sch Public Health, Boston, MA; 3) Harvard Medical School, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA} altaffiltext{2}{Harvard Medical School, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA.

In genetic association analysis of complex traits, endophenotypes for different diseases are often associated with the same candidate gene. To understand the biological disease pathways, statistical methodology is needed that is able to distinguish whether such an association with two endophenotypes is purely attributable to environmental factors or whether it is has genetic causes. Here we propose a weighted family-based tests to infer whether a candidate gene has a direct biological influence on a given quantitative trait other than through its influence on another endo-phenotype. The proposed tests allow for incomplete parental mating types and are robust against unmeasured confounding due to population admixture and stratification. We illustrate the practical relevance of our approach by an application to an asthma study in which SNPs in the IL10 gene are associated with both BMI and FEV. Simulation studies show that the proposed methodology performs well with realistic sample sizes and in the presence of admixture and stratification.

24

Candidate Genes for Asthma and Atopy. *D. Daley[1], M. Lemire[2], P.D. P.D. Paré[1], A.J. Sanford[1], A.L. Kozyrskyj[3], C. Laprise[4], Y. Bosse[2], A. Motpetit[2], A. Becker[3], D. Zamar[1], B. Tripp[1], J. He[1], K. Tremblay[4], A. James[5], A.W. Musk[5], L.J. Palmer[6], T.J. Hudson[2].* 1) University of British Columbia; 2) McGill University and Genome Quebec Innovation Centre; 3) University of Manitoba; 4) University of Quebec at Chicoutimi; 5) Sir Charles Gairdner Hospital; 6) University of Western Australia.

To better understand the development of asthma and allergic diseases, we conducted a genetic association study combining the power and resources of 4 study populations: 1) a high risk birth cohort the Canadian Asthma Primary Prevention Study(CAPPS), 2) a population-based birth cohort of children from the Study of Asthma Genes and Environment (SAGE), 3) a French Canadian founder population the Saguenay-Lac St. Jean Quebec family based sample (SLSJ) and 4) a population based sample from the town of Busselton Australia the Busselton Health Study population. We examined candidate genes with the same set of 1536 single-nucleotide polymorphisms (SNPs), a common genotyping platform, and stringent standardized phenotypes. Our panel comprised candidate genes associated with asthma and allergic phenotypes (asthma, atopy, atopic asthma, and airway hyperresponsiveness) with strong biologic plausibility and/or prior evidence for association. For a full list of genes and SNPs see *http://genapha.icapture.ubc.ca/.* For each gene a maximally informative set of SNPs was selected and genotyped using the Illumina GoldenGate assay. Genetic analysis was carried out using Family Based Tests of Association (TDT for the family based samples CAPPS (549 families), SAGE (723 families), and SLSJ (260 families)) and logistic/linear regression as appropriate for the Busselton Health Study (800 cases and 800 controls) with correction for the number of SNPs and phenotypes tested. Preliminary population genetic information including LD plots and allele frequencies by cohort and ethnicity can be found at *http://genapha.icapture.ubc.ca/.* Preliminary findings have identified associations with IL13 (asthma, atopy, and atopic asthma), IL18, and IFNGR2 (atopy) in the combined analysis of the CAPPS, SAGE and SLSJ cohorts. Analysis is ongoing and updated results are forthcoming.

25

Genome-Wide Association Study (GWAS) Reveals a Novel Gene for Immunoglobulin E (IgE) Levels and Asthma. *Z. Tan[1], Y. Sun[1], L. Pan[1], R. Nicolae[1], S. Kudaravalli[1], A. Heinzmann[2], T. Kurz[2], J.E. Gern[3], R.F. Lemanske, Jr.[3], K.A. Deichmann[2], J.K. Pritchard[1], D. Nicolae[4], A.I. Sperling[4], C. Ober[1].* 1) Dept. Human Genetics, U Chicago, Chicago, IL; 2) Dept. Pediatrics, U Freiburg, Germany; 3) Dept. Pediatrics, U Wisconsin, Madison; 4) Dept. Medicine, U Chicago, Chicago, IL.

IgE is a major immune mediator of atopic disorders, such as asthma, atopic dermatitis, and allergic rhinitis. We performed a GWAS of total IgE levels in 693 Hutterites using the Affymetrix 500k Array. High quality genotypes for 295,307 SNPs with minor allele frequencies ≥0.05 were analyzed using the general 2-allele model, developed for association studies of quantitative traits in complex pedigrees (Abney et al. AJHG 2002; 70:920). 10 SNPs with p-values <10-5 in the Hutterites were genotyped in 202 children in a birth cohort study from Madison, Wisconsin; 3 SNPs were associated with IgE levels in those children (p=0.006 to 0.034). We then typed those 3 SNPs in 3 additional replication samples: 215 Caucasian asthma cases and controls from Chicago, 264 African American asthma cases and controls from Chicago, and 707 asthma or atopy cases and controls from Freiburg, Germany. rs4733142, located in an intron of a predicted gene BC034319 on 8p12, was associated with IgE in the Hutterites and the 2 Chicago samples (p-values ranging from 0.01 to 4.42 x 10-6) and with asthma in the Hutterites (p=0.032) and in the Chicago Caucasian samples (p=0.02). We used 5'-RACE and RT-PCR to obtain the whole cDNA sequence of the gene, which is either a non-coding RNA or a gene with a small (45 amino acid) open reading frame. We detected BC034319 mRNA in activated T cells and transformed B cells, as well as in spleen, thymus and lung. Moreover, using publicly available expression data in LBLs from CEPH Caucasians, we showed that rs4733142 is a trans eQTL for IL21R (p= 0.003), which is involved in regulating IgE synthesis. Our study demonstrates the power of GWAS combined with expression studies to detect novel genes and pathways involved in asthma pathogenesis. Supported by HL56399, HL66533, HL72414, HL70831, HL85197 to C.O. and RR00055 to the U of Chicago GCRC.

26

Large scale replication of a genome-wide association study in celiac disease. *K.A. Hunt[1], L. Franke[2], R. Gwilliam[3], A. Zhernakova[2], M. Inouye[3], W. McLaren[3], R. McManus[4], R. McGinnis[3], L.R. Cardon[5], P. Deloukas[3], C. Wijmenga[6], D.A. van Heel[1].* 1) Queen Mary University of London, UK; 2) University Medical Center Utrecht, The Netherlands; 3) Wellcome Trust Sanger Institute, Cambridge; 4) Trinity College Dublin, Ireland; 5) Wellcome Trust Centre for Human Genetics, UK; 6) University Medical Center Groningen, The Netherlands.

INTRODUCTION: Celiac disease is a common (1% prevalence) chronic inflammatory small bowel disease with strong heritability. An immune response against dietary wheat, rye and barley occurs. We performed (Nature Genetics June 2007) a genome wide association (GWA) study using Illumina Hap300/550 BeadChips in 778 UK celiac individuals and 1422 population controls. AIMS: 1. to confirm GWA findings in further independent collections 2. test optimal strategies for SNP selection for replication studies METHODS: 1536 SNPs from the UK celiac GWA study were selected for genotyping in Dutch, further UK, and Irish celiac case-control collections (total ~8000 samples). SNP selection was based on the most significant findings from the following analyses: single SNP association (criteria P<0.0025); sliding window and CEU HapMap based two SNP haplotype association (criteria haplotype P<0.001 and no SNP showing single SNP association); nsSNP association (criteria P<0.01); SNPs over-represented in specific biological pathways (pathway P<0.01, selected from Reactome, KEGG, HPRD, BIND, IntAct and BioGrid). RESULTS: Outside the HLA, the most significant finding (UK GWA P=2.0 x 10-7) was in a linkage disequilibrium block containing the IL2/IL21 cytokine genes. Association was confirmed using further SNPs and using two further independent collections (meta-analysis of 4600 samples P=10-10 to 10-14, OR 0.6). Replication data of further regions will be presented. CONCLUSIONS: We have identified and confirmed, using a genome wide association study and replication approach, novel risk variants in the IL2/IL21 region predisposing to celiac disease. We are confirming further regions and finding optimal strategies for replication.

27

A Bayesian multipoint allele sharing method for genome-wide studies. *Z. Su, P. Donnelly, J. Marchini.* Department of Statistics, University of Oxford, Oxford, Oxfordshire, United Kingdom.

Genome-wide association studies are set to become the method of choice for uncovering the genetic basis of human diseases. For complex traits, we expect the effect sizes of the underlying risk variants to be relatively small. Environmental effects, allelic heterogeneity and failure to type the causal locus will introduce substantial noise into the relationship between phenotype and genotype. It is therefore important to develop statistical methods that can extract as much information from the data as possible to detect the causal locus. The literature in this area is large and complex; there is no consensus on the best method and often powerful methods are computational intractable for large-scale association studies. We present a novel approach to association testing that is applicable to large-scale genotype data and is more powerful than popular methods currently available to detect disease causing variants.

Our approach is based on the idea of measuring allele sharing between individuals in the study and is analogous to the IBD methods used in linkage analysis. We calculate the extent of allele sharing at all typed and untyped variants across a region and use novel Bayesian methods of testing for association that are robust to allelic heterogeneity. A novel aspect of our method is that we condition upon a fine-scale recombination map so that allele sharing at a given locus is measured using information from all markers, but in a way that decreases with genetic distance from the locus. This avoids the decision faced by some other methods as to how many markers to use, or how to use them, or over what physical distance to define haplotypes for haplotype analyses.

We have found that our approach provides a marked boost in power (5-20% increase) over single-SNP and multi-marker prediction based approaches for a single-SNP model of disease risk and can be even more powerful under a model of allelic heterogeneity. We illustrate our method using the genome-wide association studies carried out as part of the Wellcome Trust Case-Control Consortium (WTCCC).

28

Analysis of Whole-Genome Data by Homozygosity Mapping and Adjustments for Relatedness. *B.F. Voight[1,2], D. Altshuler[1,2], M.J. Daly[1,2], representing the Diabetes Genetics Initiative.* 1) Broad Institute of Harvard and MIT, Cambridge, MA; 2) Massachusetts General Hospital, Boston MA.

Whole-genome association data is well suited to detect unusual tracts of homozygosity over many genetic markers, as well as detecting familial relationships present in the ascertained sample. Typically, this information is obtained from the marker data *in silico* by estimating the number of alleles shared identical by descent (IBD). To date, how empirical estimates of IBD and homozygosity can be utilized in the context of whole genome association studies has not been fully explored.

We describe two approaches with take advantage of this information. First, we conjectured that individuals with extreme quantitative trait values would be enriched for homozygosity at specific genomic locale relative to the background population, and that low-frequency recessive mutations of strong effect might be found in those homozygous regions. We computed tracts of homozygosity in a sample of ~3000 individuals collected from Finland and Sweden as part of the Diabetes Genetics Initiative (DGI) and have genotyped them on the 500K Affymetrix platform. These samples have been characterized for measures of glucose metabolism, lipids, obesity, blood pressure, as well as Type 2 diabetes. We demonstrate statistical support ($P < 10^{-5}$) for regions enriched for homozygosity in trait extremes relative to the background population. Second, we propose a modification to the standard allele-based association testing that, in the variance term, corrects for the estimated IBD state for each pair of individuals at each marker, including both individuals with known and unknown relatedness. We illustrate the features of this testing framework via simulation, and highlight results of a re-analysis of the original DGI scan for Type 2 diabetes.

29

Simultaneous analysis of genome-wide SNP data and candidate region sequence data. *C.J. Hoggart[1], J.C. Whittaker[2], M. De Iorio[1], D.J. Balding[1].* 1) Department of Epidemiology & Public Health, Imperial College, Norfolk Place, London W2 1PG; 2) Non-communicable Disease Epidemiology Unit, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT.

The ideal analysis of a genome-wide association study for a complex disease would involve analyzing all the SNP genotypes simultaneously to find a set of SNPs most associated with disease risk. The computational challenge of handling up to one million SNPs simultaneously is daunting, but it could greatly improve performance over single-SNP analyses, since a weak effect may be stronger and a false signal weakened when other causal effects are accounted for. Our algorithm estimates regression coefficients for each SNP by maximizing the likelihood subject to a penalty that strongly favors zero values, corresponding to no association. For each causal variant our algorithm typically reports one SNP that best captures the association and not other SNPs in strong LD with it. By default the algorithm searches for additive effects but can also search for recessive and dominant effects. We consider two forms for the penalty corresponding to the Laplace and normal-exponential-gamma prior distributions. The Laplace prior improves SNP selection in comparison with single-SNP tests, and the normal-exponential-gamma prior improves selection further. We demonstrate the performance of the algorithm using simulated and real genome-wide datasets and simulated sequence data of up to 500K SNPs. These analyses require only a few hours on a desktop workstation, exploiting an approximate calibration of the type-I error that avoids the need for permutation analyses.

30

Efficient and Flexible Testing of Untyped Variants in Case-Control Studies. *M.P. Epstein[1], A.S. Allen[2], G.A. Satten[3].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept of Biostatistics and Bioinformatics, Duke University, Durham, NC; 3) Centers for Disease Control and Prevention, Atlanta, GA.

Candidate-gene and genomewide association studies of disease typically avoid the examination of all existing polymorphisms (for economical reasons) and instead focus inference on a reduced set of tag single-nucleotide polymorphisms (SNPs) that efficiently capture all relevant genetic variation. While investigators subsequently consider only such tagSNPs in association analyses, recent literature has proposed novel statistical methods for testing untyped variants using the sample tagSNP data coupled with external information from databases describing linkage-disequilibrium (LD) patterns across the genome. Here, we consider a flexible likelihood-based approach for testing untyped variants in case-control studies of disease using supplemental LD data from the International HapMap Project. Compared to existing approaches, our method is novel in that it permits estimation of the effects of the untyped variants and further allows for covariates and interactions (between different untyped variants, as well as between untyped variants and environmental covariates). Using both simulated and real data, we demonstrate our approach provides an attractive test to gain additional knowledge in association studies without incurring any additional genotyping cost.

31

Genetic similarity matching for genome-wide association studies. *W. Guan, L. Liming, G.R. Abecasis, M. Boehnke.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Recently, genome-wide association studies have been used to great effect to dissect complex diseases such as diabetes, obesity and multiple sclerosis. These studies require particular care in dealing with population stratification, which can lead to spurious association or mask true signals. We have developed a similarity score matching method for efficient matching of cases and controls after genotyping a large number of genetic markers in a genome-wide association study or large-scale candidate gene association study. Our method is comprised of three steps: 1) calculating similarity scores using the genotype data; 2) conducting optimal matching based on the scores so that matched cases and controls have similar genetic background; 3) using conditional logistic regression to perform association tests.

Here we present an evaluation of our method using simulated data and genome-wide data from the Finland-United States Investigation of NIDDM Genetics (FUSION) study of type 2 diabetes. We evaluate the effectiveness of our matching strategy by evaluating the proportion of matched FUSION samples that originate from the same province in Finland. We also illustrate how our approach affects conclusions of the study by comparing association signals using our method to those using a standard chi-square test and examining the ranks of true association signals. Our results show that our approach provides a simple and effective way to guard against population stratification when GWA data are available.

32

Powerful Bayesian gene-gene interaction analysis. *T. Ferreira[1,2], P. Donnelly[1], J. Marchini[1].* 1) Department of Statistics, University of Oxford, Oxford, United Kingdom; 2) Department of Mathematics, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal.

Most statistical methods that are used to detect associations for complex traits proceed by looking for marginal effects at each locus or set of loci in each small region and effectively ignore the possibility of interaction between genes. Our previous work has shown that when the underlying genetic model involves biologically plausible interactions between different genes this may not be the most powerful strategy.

We have developed a Bayesian method that assesses the probability that each locus acts to increase disease risk by averaging over a small set of plausible genetic models (both single and 2-locus models). Using simulated data, we show that this approach has more power to detect the disease loci involved in the disease for a large class of genetic models in which there is gene-gene interaction and has no loss of power when no gene-gene interactions exist. This approach is computationally tractable for genome-wide studies but we also show that two-stage approaches and MCMC-based approaches for our model can offer computational advantages in some situations. We illustrate the approach using the genome-wide association data from the Wellcome Trust Case-Control Consortium.

33

Estimating significance thresholds for genomewide association scans. *F. Dudbridge, A. Gusnanto.* Biostatistics Unit, Medical Research Council, Cambridge, United Kingdom.

The question of what significance level is appropriate for genomewide association studies is somewhat unresolved. Permutation testing is advocated, but does not resolve the difference between the genomewide multiplicity of the experiment, and the subset of markers actually tested. A standard significance level would facilitate reporting of results and reduce the need for permutation tests. We used genotypes from the Wellcome Trust Case-Control Consortium to estimate a genomewide significance level. We sub-sampled the genotypes at increasing densities, using permutation to estimate the nomimal p-value for 5% family-wise error. By extrapolating to infinite density, we estimated the genomewide significance level to be about 6.39E-8. We compared this to two estimators of the effective number of tests. The first fits a beta distribution to permutation replicates, and the second is based on an eigenvector decomposition of the genotype data. The beta distribution is not exact, but we found that it provides a workable approximation for calculating genomewide significance. Patterson's eigenvalue estimator requires less computation but was found to be an order of magnitude too low, leading to increased type-1 errors. We found that this estimator is only accurate when the effective number is closer to the actual number of tests. We conclude that permutation is still needed to obtain genomewide significance levels, but with subsampling, extrapolation and estimation of an effective number of tests, the significance level can be standardized for all studies of the same population.

34

A general approach to combining genomewide association datasets. *J.C. Barrett[1], S. Purcell[2], M.J. Daly[2], L.R. Cardon[1].* 1) WTCHG, Oxford University, Oxford, United Kingdom; 2) CHGR, Massachusetts General Hospital, Boston.

Genome-wide association (GWA) studies in 2007 have yielded an unprecedented number of novel genotype-phenotype associations to complex diseases such as diabetes, breast cancer, Crohn's disease and heart disease. In addition to these primary discoveries, many studies are making their data publicly available for other researchers to use, allowing for at least two exciting possibilities: (1) Meta-analysis of several scans of the same phenotype with excellent power to detect modest effects missed in the individual datasets. (2) Inexpensive future GWA scans genotyping only new cases and comparing them to already genotyped control samples. The potential benefit of such approaches must be considered in light of the possible risks, however. We examine results from the control samples of the Wellcome Trust Case Control Consortium (3000 UK samples) and the controls from the NIMH Center for Collaborative Genetic Studies on Mental Disorders (1700 caucasian US samples). Initial analysis reveals that, despite having broadly similar ethnic ancestry, these samples cannot be naively merged: the two groups have a genomewide inflation of differences in allele frequencies (λ=1.5) and hundreds of loci show dramatic frequency shifts (individual p values < 10^{-6}). We present analyses to address these issues and unlock the full potential of these datasets. First we discuss the necessity to update the standard for distribution of genotype data to include detailed strand and allele information as well as raw intensity (pre genotype calling) data. Next we present a series of genotype quality control and matching techniques to detect and remove the large number of spurious associations. Finally, we use multidimensional scaling and identity by state (IBS) clustering to correct the genomewide inflation (likely due to a blend of population structure and technical artifacts). We also evaluate the effect on 'replication' vis a vis meta-analysis of shared samples. We believe this approach has widespread and general applicability to the problem of reusing and combining genome scans performed on different populations, in different laboratories, using different genotype calling algorithms.

35

Fast and highly accurate haplotype inference for genome-wide datasets. *B.N. Howie, J.L. Marchini, P. Donnelly.* Department of Statistics, University of Oxford, Oxford, United Kingdom.

A number of genome-wide association studies are currently being planned or underway, and it will be essential to develop fast and accurate haplotype phasing methods if we are to realize the full potential of these massive datasets. Some existing methods can handle datasets of this size, but their speed often comes at the expense of accuracy; conversely, the most accurate methods are far too slow for genome-wide analyses. One largely untapped source of information is the population genetic variation catalogued in the HapMap: the highly accurate haplotypes and recombination rates curated there provide strong prior knowledge about the patterns of linkage disequilibrium in human populations. We have designed a novel phasing method that aggressively exploits this information to infer highly accurate haplotypes in a fraction of the time needed for other comparable methods. On multiple simulated and real datasets, our algorithm consistently generates solutions as good as or better than those provided by fastPHASE (a leading method in the field) and runs ~20 times faster. Our method can process at least 50,000 SNP genotypes per minute on a standard desktop computer, with computation scaling linearly in the number of markers and the number of individuals, and yields error rates only slightly worse than PHASE, the gold standard in the field. We have used this approach to phase 17,000 individuals at ~500,000 SNPs as part of the Wellcome Trust Case Control Consortium; this is the largest set of phased human haplotypes ever created, and we envisage that it will be an invaluable resource for population genetic studies.

36

Rapid and accurate haplotype phasing and missing data inference for whole genome association studies using localized haplotype clustering. *S.R. Browning, B.L. Browning.* Department of Statistics, The University of Auckland, Auckland, New Zealand.

Whole genome association studies present many new statistical and computational challenges due to the large quantity of data obtained. One of these challenges is haplotype inference: methods for haplotype inference designed for small data sets from candidate gene studies do not scale well to the large number of individuals genotyped in whole genome association studies. We present a new method and software for inference of haplotype phase and missing data that can accurately phase data from whole genome association studies. Our method is based on fitting a localized haplotype cluster model[1,2], to initial estimates of haplotype phase. The localized haplotype cluster model is extended to give a hidden Markov model, from which revised estimates of haplotype phase can be sampled. This process is iterated, with most likely haplotype phase inferred at the last iteration. Our method is compared with existing haplotype inference methods, including fastPHASE and HaploRec, on real and simulated data sets with thousands of genotyped individuals. We find that our method outperforms existing methods in both speed and accuracy for large data sets with thousands of individuals and densely spaced genetic markers, and we use our method to phase a real data set of 3002 individuals genotyped for 490,032 markers in 3.1 days computing time, with 99% of masked alleles imputed correctly. Our method is implemented in the Beagle software package which is available at http://www.stat.auckland.ac.nz/~browning/beagle/beagle.html

[1] Browning, B.L. & Browning, S.R. 2007. Efficient multilocus association mapping for whole genome association studies using localized haplotype clustering. Genetic Epidemiology, in press.

[2] Browning, S.R. 2006. Multilocus association mapping using variable-length Markov chains. Am J Hum Genet 78, 903-13.

37

EURExpress, a web-based transcriptome atlas of the developing mouse embryo. *G. Diez-Roux, The EURExpress consortium.* Telethon Institute of Genetics & Medicine, Naples, Italy.

Genome-wide expression analyses have a crucial role in functional genomics. RNA in situ hybridization (ISH) provides an accurate spatio-temporal description of the distribution of transcripts at cellular resolution. The EU-funded EURExpress consortium is generating a transcriptome-wide acquisition of expression patterns by means of ISH with non-radioactive probes and using this data to establish a web-linked, interactive digital transcriptome atlas (www.eurexpress.org). The goal of EURExpress is to generate the expression data of > 20,000 genes on sagittal sections from E14.5 wild type murine embryos. To date we generated over 9000 expression patterns, which have been thoroughly annotated using a special interface for high-throughput annotation. This interface includes 1420 anatomical structures and correlative trees regarding ontological (embryological) and topological relations allowing advanced queries. The analysis of the data produced so far has determined that 45-50% of genes show a specific/restricted pattern of expression at E14.5. Over 20% of these are unknown genes of which a large percentage show restricted expression patterns in organs such as the central nervous system, ear, eye, skin, liver, skeletal muscle, mesenchyme and salivary glands, indicating that this database represents a unique resource to identify novel molecular markers. The potential impact on these data on the study of human development and disease is enormous allowing to identify tissue specific markers to characterize disease phenotype, to evaluate disease prognosis, to measure therapeutic benefits and to help identifying genes whose mutations lead to disease phenotypes. In addition, the data has allowed also performing detail molecular characterization of the CNS and has identified, for example, novel molecular regionalization of the thalamus, diencephalon and the telencephalic pallium. Analysis are being performed to find relationships between gene expression and gene ontology and to characterize the mouse genome based on the expression patterns of genes.

38

Nonsense-mediated mRNA decay modulates cellular fate in response to DNA damage. *D. Huang, F. Spencer, H.C. Dietz.* Inst Genetic Med, Johns Hopkins Univ Sch of Med, Baltimore, MD.

All eukaryotes degrade transcripts harboring PTCs through the action of the nonsense-mediated mRNA decay (NMD) pathway. NMD protects the organism from the deleterious effects of truncated peptides that would be expressed from nonsense alleles if the transcripts were stable. The rare occurrence of nonsense mutations could not plausibly account for complete evolutionary maintenance of this function. Recent evidence suggests that NMD can coordinate cell survival pathways in response to environmental stress such as starvation. In yeast, as in higher eukaryotes, the core NMD machinery is composed of three gene products termed UPF1, UPF2 and UPF3. In an attempt to further define physiologic functions of NMD, we performed a synthetic lethal screen in S. cerevisiae with a *upf1*Δ strain. This screen identified known and novel factors that genetically interact with UPF1. The only known function of one of these factors (ESC2) is a weak contribution to mating type locus silencing. Here we show that *esc2*Δ*upf1*Δ cells show a severe synthetic-sick phenotype and demonstrate a dramatic increase in sensitivity to DNA-damaging agents including bleomycin, hydroxyurea and MMS. This phenotype was also observed upon targeting of the other UPF genes in *esc2*Δ cells, documenting that the genetic interaction was with the NMD pathway *per se*. Untreated *esc2*Δ*upf1*Δ cells showed an increase in spontaneous double-stranded DNA breaks, as evidenced by increased YAC telomeric marker loss that was resolved by de novo telomere addition. Exponentially growing *esc2*Δ*upf1*Δ cells revealed accumulation of large-budded cells with one nucleus and also showed increased loss of large YACs, indicative of unequal chromosome segregation. These data infer a role for NMD in determining cellular susceptibility and/or response to DNA damage, expanding the repertoire of survival responses coordinated by this pathway. Parallels between the DNA damage susceptibility and aneuploidy predisposition in *esc2*Δ*upf1*Δ cells and early phases of tumorigenesis warrant further scrutiny.

39

Pooled heteronuclear RNA sequencing: a new tool for large-scale cis-acting regulatory haplotype discovery. *T. Pastinen[1,2], E. Grundberg[1,2], K. Lam[2], B. Ge[2], S. Gurd[2], N. Martin[2], E. Harmsen[2], T. Kwan[2], J. Majewski[1,2].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada.

We have developed a sequencing-based approach for quantitation of differences in allele frequencies between DNA and RNA pools derived from same population of individuals. This allows identification of polymorphisms that are in LD with regulatory variants. We have focused our screening to unspliced hnRNA allowing specific interrogation of common haplotypes in the vicinity of human genes and focus on transcriptional cis-regulatory effects. Our results in over 500 genes indicate that the method has high sensitivity for cis-acting effects based on comparison to results from three expression profiling studies using different microarray platforms in the same population of HapMap CEU lymphoblastoid cell lines (LCLs).The pooled hnRNA sequencing approach led to identification many additional cis-acting effects, such as a strong association of INSIG2 allelic expression to a regulatory haplotype -undetectable by traditional approaches. Other examples of disease associated genes in which regulatory haplotypes were detected are SLC22A5, PTGER4 and IL23R. A comparison of CEU LCL data to a pool of RNA / DNA derived from a panel of human primary cells (osteoblasts) reveals shared cis-acting associations in approximately 50% cases with the remainder of being tissue restricted. We have further validated the associations by allelic expression mapping studies in an independent YRI and/or Caucasian LCL panels as well as in primary cells. Comparison to exon array data has revealed that the hnRNA targeting assays may also pick up allelic isoform differences. Our results demonstrate that heritable cis-acting variation is common in human genome and allows insight to functional variation potentially altering risk for complex diseases. Finally, we suggest that focusing on functional variants in population based cell panels derived from donors of different ethnic backgrounds may provide a shortcut to fine mapping of functional variants underlying disease phenotypes. This work is supported by Genome Quebec and Genome Canada.

40

Genome-wide analysis of transcript isoform variation in humans. *T. Kwan, D. Benovoy, C. Dias, S. Gurd, C. Provencher, T.J. Hudson, R. Sladek, J. Majewski.* Human Genetics, McGill University, Montreal, Quebec, Canada.

We conducted a genome-wide association analysis of variation in transcript isoform structures among individuals from the CEU HapMap population using a comprehensive exon-tiling microarray, the Affymetrix Human Exon 1.0 ST Array. Significant genetic associations of cis-acting SNPs with splicing variants (cassette exon skipping, alternate splice site usage, intron retention), differential 5' UTR (initiation of transcription) and 3' UTR (alternative polyadenylation) usage, and differential transcript expression levels were observed. Many of the confirmed splicing events within coding exons are predicted to affect protein structure, while variations in the UTRs are predicted to have transcriptional and translational effects through the addition or removal of important regulatory sequences. Our method has identified the putative causative SNP responsible for the variations in isoform structure in several cases, such as *CAST*, where the associated SNP disrupts a consensus splice site. Several of the transcript isoform variants, such as *OAS1* (cryptic splice site usage) and *IRF5* (differential polyadenylation), have been associated with disease phenotypes, indicating the potential of our analysis method for discovering disease-associated isoform variants. Previous genome-wide association studies have linked genetic variations with changes in gene expression levels, however we now find that a large number of these are in fact transcript isoform variations with differences mainly at the 3' end. Our results show that the variation of gene expression in humans is qualitatively different than previously believed, and illustrates the value and importance of using exon-tiling microarrays for identifying variations in overall gene structure and shedding new light on the detailed effects of cis-acting genetic variants. Under this new paradigm, the functional consequences of future and many previously identified changes need to be re-evaluated.

41

Copy number variations and gene expression in the mouse. *A. Reymond[1], C. Henrichsen[1], N. Vinckenbosch[1], E. Chaignat[1], S. Zoellner[2], H. Kaessmann[1].* 1) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 2) Department of Biostatistics, University of Michigan, Ann Arbor, Michigan.

Copy number variations (CNVs), defined as large stretches of DNA that vary in number of copies among phenotypically normal individuals, have recently gained considerable interest as a possible source of phenotypic variation. Previous studies using BAC arrays to identify CNVs have produced datasets that only partially overlap, suggesting that copy number variation vastly remains uncatalogued To obtain a finer resolution, we used whole-genome oligonucleotide array comparative genome hybridization (CGH), with a median probe spacing of 6 kb, to identify CNVs in 13 inbred mouse strains and in 21 wild mice caught throughout the European, Middle-Eastern and Northern African range of Mus musculus domesticus subspecies. Using a hidden Markov model, we identified some 700 CNV candidate regions, which we subsequently validated using a custom-made array with probe density increased to one every 550 bp, thus multiplying by more than eight-fold the number of copy-number variable regions reported in the mouse genome. We identified functional categories of genes that were enriched within CNVs using the Gene Ontology (GO) database. Significantly enriched GO categories include host defense and immunity, as well as neurotransmission. To address whether CNVs affect gene expression, we assessed the expression levels of 45'037 transcript units in liver, kidney, brain, heart, lung and testis of three individuals for each of six commonly used laboratory inbred mouse strains. We found that the variance of the expression levels for each of the recorded tissues is significantly larger for genes mapping inside than for genes mapping outside of CNVs, suggesting that copy number variation affects the variability of gene expression and must be taken into account when considering phenotypic differences between strains. A more detailed analysis of the effect of CNVs on gene expression will be presented.

42

Genome-wide mapping and sequencing of Structural Variation using High-Resolution Paired-End Mapping (HR-PEM). *A.E. Urban[1], J.O. Korbel[1], J. Affourtit[2], F. Grubert[1], P. Kim[1], B. Taillon[2], D. Palejev[1], N. Carriero[1], L. Du[2], B. Godwin[2], J. Simons[2], J. Chi[3], F. Yang[3], M. Hurles[3], N. Carter[3], S. Weissman[1], T. Harkins[4], M. Gerstein[1], M. Egholm[2], M. Snyder[1].* 1) Yale University, New Haven, CT; 2) 454 Life Sciences, Branford, CT; 3) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 4) Roche Applied Science, Indianapolis, IN.

Structural variation (SV), i.e. deletions, duplications, insertions and inversions, kbp to Mbp of genomic sequence in size, is being found to be a pervasive architectural feature of the human genome, expected to have a phenotypic effect in health and disease. Most methods for genome-wide identification of SV, predominantly microarray based, cannot normally detect variation smaller than 50 kb or breakpoint-sequences and also fail to identify copy-number neutral variation events such as inversions and balanced translocations. We present a novel approach, High-Resolution Paired-End Mapping (HR-PEM) [Korbel, Urban, Affourtit et al., in preparation], using 454/Roche-FLX next-generation sequencing technology to rapidly identify SVs, and sequence the associated breakpoints. The approach involves sequencing the ends of, intermittently circularized, but not cloned, 3 kb genomic DNA fragments and mapping them onto the human genome reference sequence. The resolution of breakpoint assignments is ≤3 kb and is thus well suited for PCR validation. We have used HR-PEM to study SVs of all classes genome-wide in two subjects from different ethnic backgrounds. Based on 21 and 10 million, respectively, end-pair sequence reads (with a read-length of typically >200nt) from each individual, several hundred SVs have been predicted so far, ranging in size from 2 kbp to several Mbp. The junction sequence of approximately 60% of predicted SVs does immediately produce a PCR amplicon, multiples of which are then pooled and 'shotgun'- sequenced with 454/Roche-FLX. This has yielded already well over 100 breakpoint-sequences, most of them novel. Predicted SVs are also validated by comparison with known variants, by array CGH and FISH, and reveal as yet unexplored aspects of structural variation in the human genome, such as insights into mechanisms by which SV arises.

43

Advances in Sequencing Technology : Enabling and Expanding Applications in Human and Cancer Genetics. *S.B. Gabriel[1], C. Russ[1], J. Baldwin[1], C. Sougnez[1], S. Fisher[1], P. Cahill[1], R. Onofrio[1], R. Nicol[1], T. Bloom[1], K. Cibulskis[1], W. Brockman[1], P. Alvarez[1], D.B. Jaffe[1], D. Altshuler[1,2], C. Nusbaum[1], E.S Lander[1].* 1) Broad Institute of MIT and Harvard, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA.

Targeted re-sequencing is critical for identifying genetic variation contributing to common human disease and for discovering mutations driving the development of cancer cells. Recently genome-wide association studies have pointed to new regions harboring heritable variation that impacts the risk of various common disease. Re-sequencing is the only way to characterize the full allelic spectrum in these regions to determine the full role of genetic variation in risk. Similarly genomic studies in cancer are identifying important regions. However, large-scale re-sequencing in many samples has not been practical due to high cost and technical limitations (e.g.,the requirement for high sample purity).

New single-molecule-based sequencing can dramatically impact the scope, feasibility and quality of re-sequencing projects through decreased cost and increased sensitivity. Lower cost allows for deep sampling. The ability to read out individual DNA strands obviates calling heterozygous sequence and thus has the potential to deliver high sensitivity and specificity. In addition, the technologies have the potential to detect variants present at lower molarity, such as in a mixed sample of tumor and stroma.

We describe various applications of new sequencing technologies. In a pilot study, we re-sequenced germline DNA in a 500kb ENCODE region sequenced as part of the HapMap project. We observe near-complete sensitivity and specificity in 7 HapMap samples. Results for somatic mutation detection are equally encouraging. We sequenced 60 genes in tumor DNAs and matched controls for which conventional sequencing data is available. We identified all mutations previously found, as well as additional mutations. Studies underway are aimed at complete characterization of several disease associated regions (~5 Mb) and sequencing of ~1000 genes in hundreds of tumor samples.

44

Complete genome sequencing to high coverage of a single individual: James Watson. *D.A. Wheeler[1], M.E. Egholm[5], M. Srinivasan[5], A.L McGuire[3], W. He[5], L.V. Nazareth[1], Y. Huan[1], Y. Liu[1], J.R. Lupski[2,4], D.M. Muzny[1], G.M. Weinstock[1,2], R.A. Gibbs[1,2].* 1) Human Genome Sequencing Center, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030; 2) Department of Molecular and Human Genetics, One Baylor Plaza, Baylor College of Medicine, Houston, TX, 77030; 3) Center for Ethics and Health Policy, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 4) Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston TX 77030; 5) 454 Life Sciences, Roche Diagnostics, 20 Commercial St., Bradford, CT 06405.

Recent advances in DNA sequencing using a combination of genomic DNA shearing, limited dilution, and single molecule-primed amplification by emulsion PCR to eliminate plasmid libraries and bacterial cloning, and a massively-parallel method of sequencing in picoliter size reaction vessels, provide the reduced cost and increased speed to enable the generation of data for 'personalized genome sequencing'. With this technology, we produced a 6X coverage sequence of the genome of James D. Watson. Comparison to the reference genome yielded 1.8 million single base variants present in dbSNP plus approximately 230,000 novel SNPs, affording the first genome-wide compendium of SNPs from a single individual. Over 6,500 SNPs were classified as non-synonymous amino acid changes. 23 heterozygous alleles were found in the Human Gene Mutation Database. A large number of insertion/deletion polymorphisms were also readily observed; over 70 lay within exons and were validated by independent methods. Structural variation events 30-400 kb in size are also evident. A key aim of personal genome sequencing is to detect alleles that may be associated with disease, predictive of response to medication, or else prognostic indicators. Notable among the alleles identified in Watson's genome are mutations in both BRCA1 and Fanconi anemia 1,and two genes involved in DNA repair, which may suggest an increased risk of cancer. The identification of alleles with subtle implications for current health but potential to influence later decisions are at the heart of both the excitement and the dilemma of the new era of genomic medicine.

45

Microarray-based Direct Genomic Selection for High Throughput Resequencing. *M.E. Zwick[1], K. Meltz-Steinberg[1], C. Middle[2], T. Albert[2], D. Okou[1].* 1) Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) 2Nimblegen Systems, Inc. Madison, WI.

Comprehensive resequencing can identify all genetic variants, whether they be rare or common, that contribute to disease susceptibility. This vision has fueled the development of a number of highly efficient sequencing technologies (Resequencing Arrays - RAs, 454, Solexa). Similar progress, however, has not been forthcoming in methods of target DNA preparation. Template DNA preparation in complex eukaryotic genomes is currently based upon multiple PCR fragments or other clone-based methodologies that remain arduous, inefficient and expensive. Here we report our results using a novel strategy, Microarray-based Direct Genome Selection (MGS) that offers revolutionary improvements in speed, efficiency and cost of template DNA preparation for resequencing. MGS isolates user-specified genomic fragments that can be generically amplified and sequenced by any of the next-generation sequencing technologies.

Using MGS and RAs, we have resequenced in replicate 300kb of unique sequence in 10 HapMap samples (5 CEPH/5 Yoruban) and a sample with a known mutation (TR91) from a 1.3 MB sized region on the human X chromosome that contains the FMR1/FM2 genes. The observed replication rate is 99.99%; in the 4.8MB of genomic sequence. An accuracy rate of 99.8%; (10 discrepancies / 4812 identical) is observed as compared to HapMap Genotype calls. Using a single MGS array design, we are selecting and resequencing all the unique exons on the X chromosome in the same 10 HapMap samples. Initial results demonstrate a call rate of more than 80%; with an accuracy rate of greater than 99%; as compared to the HapMap. MGS offers an inexpensive (~ $300 per sample) and rapid (~ 3 days from sample to sequence ready DNA) protocol that can enable the comprehensive resequencing of genomic regions, chromosomes, linkage peaks or collections of genes identified in systems biology analyses from complex eukaryotic genomes with next-generation sequencing technologies.

46

THE PATTERN OF RET MUTATIONS AND VARIANTS IN HIRSCHSPRUNG DISEASE: A MEDICAL SEQUENCING CASE STUDY. *L. Hao[1], S. Arnold[1], J. Albertus[1], M. Dao[1], A. Rea[1], P. Cruz[2], J. Mullikin[2], A. Young[2], E.D. Green[2], A. Chakravarti[1].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Gene-based medical sequencing is critical to our understanding of complex diseases, following the discovery of new genes by association studies. One limitation for medical sequencing studies is the difficulty of interpreting base changes when functional annotation is incomplete. We present here a case study for a model complex genetic disorder, Hirschprung disease (HSCR), in which enteric ganglion cells are absent along variable lengths of the GI tract. RET, encoding a receptor tyrosine kinase, is functionally necessary, but not sufficient, for normal enteric development. Genetic data suggest that RET mutations must exist in each affected despite the involvement of other genes and, thus, many RET mutations and polymorphisms interact to produce disease. To identify both common and rare genetic variants, we are sequencing all 20 exons and 20 additional conserved non-coding regions at RET from 680 individuals including 237 probands and their families (~20Mb). Based on our analysis of 67% of the data, we have identified very high genetic variability with a total of 239 variants including 10 indels and 37 coding alterations, most of which are novel, rare and non-synonymous. The comparison of the frequency of sequence changes associated with transmitted and non-transmitted alleles in HSCR families validated the association of a previously identified RET enhancer variant. Interestingly, we identified a new premature stop mutation in the RET kinase domain that appears to interact with the non-coding enhancer mutation and contribute to the severest forms of HSCR. In addition, sequencing in families has allowed us to identify a few potentially large indels from Mendelian inconsistencies that would have been missed without family data. Our data answers questions such as the contribution of RET to HSCR, the parental origin of mutation and the role of rare and common mutations and, thus, their genetic mechanisms of action.

47

Clinical and Molecular Analysis of Arylsulfatase E in Patients with Brachytelephalangic Chondrodysplasia Punctata. *N. Braverman[1], C. Matos[1], M. Maeda[1], L. Chen[1], J. Allanson[2], C. Armour[2], C. Greene[3], M. Kamaluddeen[3], D. Rita[4], L. Medne[5], E. Zackai[5], S. Mansour[6], A. Superti-Furga[7], A. Lewanda[8], M. Bober[9], K. Rosenbaum[10], M. Nino[1].* 1) Inst. Genetic Medicine, Johns Hopkins Univ., Baltimore, MD; 2) Dept. of Genetics, CHEO, Ottawa, CA; 3) Dept. of Pediatrics, Univ. of Maryland, Baltimore, MD; 4) Dept. of Genetics, Lutheran General Hosp., Park Ridge, IL; 5) Division of Genetics, CHOP, PA; 6) Regional Genetics Dept., St Georges NHS Trust, London, UK; 7) Dept. of Pediatrics, Univ. of Freiburg, Germany; 8) Dept. of Pediatrics, Inova Fairfax Hosp., Falls Church, VA; 9) Dept. of Pediatrics, The Alfred I. duPont Hosp., Wilmington, DE; 10) Division of Genetics, CNMC, Washington DC.

X-linked Recessive Chondrodysplasia Punctata (CDPX1) is due to a defect in arylsulfatase E (*ARSE*), located on Xp22.3. Neither the substrate nor function of the encoded warfarin sensitive arylsulfatase has been identified and molecular analysis remains the only confirmatory diagnostic test. Nevertheless, the majority of patients evaluated have not had identifiable mutations in *ARSE*, and thus far 23 probands have been reported. The major clinical features in these patients are also present in a group now recognized as phenocopies, due to vitamin K deficiency in early gestation or maternal autoimmune disease. We evaluated the *ARSE* gene in 11 probands who met clinical criteria for CDPX1. We amplified all exons and intronic flanking sequence from each patient, and investigated suspected deletions or rearrangements by southern analysis. We identified mutations in 7 probands, including 3 novel mutations and two gene deletions. Of the remainder, 3 of 4 probands had maternal conditions that further expand the phenocopy group. Thus, this group might represent a proportion of the mutation negative patients in previous studies. We extracted clinical information from all prior reports over the past decade and show that there are few distinguishing features on examination between these two groups of patients. This study supports heterogeneity for CDPX1-like phenotypes and sorting these out will help to define the biological pathway and genetic contributors.

48

A novel form of cerebellar ataxia with increased free sialic acid in cerebrospinal fluid. *F. Mochel[1,6], F. Sedel[2], U.F.H. Engelke[3], J. Barritault[4], E. Morava[5], M. Timmons[6], F. Seguin[4], A. Brice[1,7], R. Schiffmann[6], A. Durr[1,7], R.A. Wevers[3].* 1) INSERM U679, Hôpital La Salpêtrière; 2) Fédération des Maladies du Système Nerveux, Hôpital La Salpêtrière; 3) Laboratory of Pediatrics and Neurology, Radboud University Nijmegen Medical Center; 4) INSERM E324, Hôpital La Milêtrie, Poitiers, France; 5) Department of Pediatrics, Radboud University Nijmegen MC, Nijmegen, The Netherlands; 6) NINDS, NIH, Bethesda, USA; 7) Département de Génétique et Cytogénétique, Hôpital La Salpêtrière, Paris, France.

In vitro Nuclear Magnetic Resonance (NMR) spectroscopy has contributed to the identification of new inborn errors of metabolism. We used 1H-NMR spectroscopy to identify new metabolic markers in cerebrospinal fluid (CSF) and urine of a large cohort of patients with complex neurodegenerative disorders for which extensive metabolic and genetic work-up was negative. In 5 adult patients, including 2 sisters, 1D and 2D 1H-NMR analyses revealed a significant elevation of free sialic acid in the CSF, ranging from 37.5 to 62.0 μmol/l. Normal free sialic acid in CSF, in 190 controls and patients with various neurodegenerative conditions, was 8.9± 4.0 μmol/l. Urine sialic acid excretion was normal and no mutation was found in the SLC17A5 and UDP-GlcNAc 2-epimerase genes, excluding the 2 known free sialic acid storage diseases. Other disorders associated with increased free sialic acid levels in CSF (pyogenic meningitis, brain tumors) were ruled out. The phenotype of all patients associated cerebellar ataxia, peripheral neuropathy and cognitive decline. Other features included myoclonic dystonia (1/5), deafness (2/5), pigmentary retinopathy (1/5), growth retardation (2/5) and glomerulosclerosis (1/5). On cerebral MRI, periventricular (3/5) and cerebellar (4/5) white matter was abnormal, with variable cerebellar atrophy (5/5). Bilateral hyperintensities of the basal ganglia (2/5) and the brainstem (3/5) were also observed. Despite clinical and radiological heterogeneity, even manifest in the 2 affected sisters, our NMR findings are consistent with a new sialic acid storage disease for which cerebellar ataxia is the leading symptom.

49

Saposin B deficiency in mice leads to multiple glycosphingolipids accumulation and slowly developing neurological deficit. *Y. Sun[1], H. Ran[1], M. Zamzow[1], B. Quinn[1], M.T. Williams[2], C.V. Vorhees[2], D.P. Witte[3], H. Cheng[4], X. Han[4], G.A. Grabowski[1].* 1) Div Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) Div Neurology, Cincinnati Children's Hosp, Cincinnati, OH; 3) Div Pediatric Pathology, Cincinnati Children's Hosp, Cincinnati, OH; 4) Dept Medicine, Washington University School of Medicine, St. Louis, MO.

Saposin B is one of four saposins derived from prosaposin. Saposin B functions as an activator for multiple lysosomal enzymes in the glycosphingolipids (GSL) degradation pathway. Patients with a saposin B mutation present a metachromatic leukodystrophy-like disease. To gain insight into the physiological function of saposin B, saposin B null mice (B-/-) were generated by knock in of a cysteine mutation in exon 7 of the prosaposin locus. No saposin B protein was detected in B-/- mice while saposin A, C and D were expressed at the normal levels. The saposin B-/- mice developed a neurological phenotype at approximately one year old as demonstrated by a significant increase in latency and foot slips on the narrow bridges test. Slight tremor of the head also was visible at 15 month. Sulfatide levels were increased in both brain and kidney, whereas ceramide levels were unchanged. The sulfatide was detected in urine of B-/- mice. Sulfatide storage cells stained by alcian blue were present in brain, spinal cord and kidney. Myelin basic protein levels were not altered in B-/- brain, which suggested that accumulation of sulfatide did not affect myelination. Lactosylceramide (LacCer), globotriaosylceramide (TriCer) and gangliosides were accumulated in B-/- mice at about a year of age, indicating saposin B participated in degradation of LacCer, TriCer and gangliosides in vivo. Activated microglial cells stained with CD68 and activated astrocytes labeled by GFAP demonstrated the proinflammatory response in B-/- mice. These findings indicate that saposin B plays an important role in vivo in degradation of multiple GSLs in lysosomes. Collectively, saposin B deficient mice are a useful model for understanding the contributions of saposins to the GSL metabolism and homeostasis.

50

Urinary globotriaosylceramide excretion correlates with the genotype in children and adults with Fabry disease. *C. Auray-Blais[1], D. Cyr[1], A. Ntwari[1], M.L. West[2], J. Cox-Brinkman[3], D.G. Bichet[4], D.P. Germain[5], R. Laframboise[6], S.B. Melançon[4], T. Stockley[7], J.T.R. Clarke[1], R. Drouin[1].* 1) Dept Pediatrics, Service of Genetics, Université de Sherbrooke,Sherbrooke, Qc, Canada; 2) Halifax, NS, Canada; 3) Amsterdam, The Netherlands; 4) Montreal, Qc, Canada; 5) Paris, France; 6) Québec, Qc, Canada; 7) Toronto, Ont, Canada.

Background: Fabry disease is a complex, multisystemic and clinically heterogeneous disease, with elevated urinary excretion of globotriaosylceramide (Gb3), the principal substrate of the deficient enzyme alpha-galactosidase A. Our first aim was to develop and validate a simple and rapid multiplex Gb3/creatinine methodology using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of urine samples collected on filter paper. The second aim was to evaluate the relationship between urinary Gb3/creatinine excretion and the genotype of children and adult patients with FD. **Methods**: The analysis of Gb3/creatinine was developed and validated for use with a short multiplex LC-MS/MS run of 2.6 minutes. We studied the relationship between the urinary levels of total Gb3/creatinine excretion and four types of mutations in the *GLA* gene (missense, nonsense, frameshift, and splice-site defects) in 32 children and 78 adult patients with FD. **Results**: The mean recoveries of Gb3 and creatinine from the urine filter paper standards were 91% and 97%, respectively with good precision, reproducibility, and linearity. The statistical analysis using the independent variables of sex, age, types of mutations and treatment showed that the mutation factor is statistically significant ($p = 0.0006$). This means that the levels of urinary excretion of Gb3/creatinine in children and adults with Fabry disease are directly related to the type of mutation. The same correlation has been found for the sex ($p < 0.0001$) and treatment ($p = 0.0005$). **Conclusions**: We found a highly significant correlation between the urinary excretion levels of Gb3 and the types of mutations in adults and children with Fabry disease. The results also indicate that the urinary excretion of this specific glycosphingolipid biomarker is directly related to sex and treatment, but not age.

51

In Chediak-Higashi syndrome melanocytes, giant melanosomes do not target to the dendritic tip's actin network. *W. Westbroek, A. Helip-Wooley, H. Dorward, W.A. Gahl.* Medical Genetics Branch, NHGRI/MGB/NIH, Bethesda, MD.

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder caused by mutations in the CHS1 gene. Clinical characteristics include partial oculocutaneous albinism, recurrent infections, a bleeding diathesis, enlarged lysosomes in every cell type, and late-onset progressive neurological impairment. We report a CHS patient with two truncating CHS1 mutations, i.e., a nonsense (p.R514X)) and a frameshift mutation (p.F3298fsX3304). This patient had significant hypomelanosis of the skin, hair and eye. In normal melanocytes, melanosomes undergo microtubule and actin-dependent transport toward the dendritic periphery. Actin-mediated transport is dependent on the Rab27a/Melanophilin/Myosin Va tripartite complex. Rab27a-GTP interacts through its geranylgeranyl lipid tail with the melanosomal membrane, where it acts as a receptor for its effector, Melanophilin, and the Myosin Va motor protein. We investigated whether the melanosomes in CHS were correctly tethered to the actin filaments in the dendritic tips. Bright field microscopy revealed that CHS melanocytes harbor enlarged melanosomes that localized to the cell body and dendrites, but not to the dendritic tips, as observed in normal melanocytes. Confocal microscopy showed that Rab27a did not associate with enlarged melanosomes in cultured CHS melanocytes. Furthermore, Melanophilin and Myosin Va did not co-localize with the enlarged melanosomes; in normal melanocytes, Melanophilin and Myosin Va nearly always co-localized with peripheral melanosomes. Next, we employed a melanosome-specific transcript of Myosin Va fused to GFP for additional studies. In normal melanocytes, Myosin Va-GFP co-localized with Rab27a, Melanophilin, and melanosomes, while in CHS co-localization occurred only with Melanophilin in the dendritic tips. This investigation showed that the Rab27a/Melanophlin/Myosin Va tripartite complex did not form on enlarged CHS melanosomes. Absence of melanosome tethering to the actin in dendritic tips of melanocytes could explain the skin hypomelanosis associated with CHS.

52

Up-regulation of ARH1 in Galactose-stressed, Isogenic Human Fibroblasts deficient in Galactose-1-phosphate Uridyltransferase. *K. Lai[1,2], M. Tang[2,3], X. Yin[2], H. Klapper[2], K. Wierenga[1,2], L.J. Elsas[1,2,3].* 1) Dept Pediatrics, U. Miami, Miami, FL; 2) The Dr. John T. Macdonald Foundation Center for Medical Genetics, U. Miami, Miami, FL; 3) Dept. Biochemistry & Mol. Biology, U. Miami, Miami, FL.

The cause and mechanisms for premature ovarian failure (POF) and cerebellar impairment commonly manifested among patients with inherited deficiency of galactose-1-phosphate uridyltransferase (GALT) remain unsolved. GALT-knockout mouse models do not manifest either ovarian failure or ataxia. Here we studied primary fibroblasts derived from patients homozygous for a 5kb deletion or the Q188R missense mutation in their GALT genes. Using gene expression microarrays, we found that the human tumor suppressor gene aplysia ras homolog I (ARHI) was up-regulated six-fold in cells treated with 0.5mM galactose for two hours, and the level of up-regulation rose to 11-fold at the end of a 24-hour incubation. Up-regulation of ARHI was not observed in normal cells similarly challenged. The microarray data were confirmed by quantitative real-time PCR. It is noteworthy that the murine ARHI gene was lost as a consequence of evolutionary chromosome rearrangement (Fitzgerald and Bateman, 2004). Over-expression of the human ARHI gene in transgenic mouse models produced failure of folliculogenesis and loss of neurons in the celebellar cortex (Xu et al., 2000). We conclude that increased expression of ARHI is a novel result of galactose toxicity in GALT-deficient cells, and may explain both the lack of a phenotype in the GALT-knockout mice, and the organ-specific effects of GALT-deficiency in humans.

53

A silent substitution in the MCAD gene causes exon 2 skipping by disruption of a crucial SRp40 binding exonic splicing enhancer which is fundamental for MCAD gene expression. *B.S. Andresen[1,2], A.V. Jensen[1,2], L.D. Schroeder[1,2], E. Naylor[3], L. Halaby[4], C.A. Stanley[4], N. Gregersen[2].* 1) Inst of Hum Genet, Aarhus University,Denmark; 2) Res Unit f. Molec Med, Aarhus University Hospital, Denmark; 3) Dept. Pediat, Med College of S. Carolina, USA; 4) Dept Pediat, The Children's Hosp of Philadelphia, USA.

Correct splicing of exons is determined by a fine balance between cis-acting regulatory sequences like exonic splicing enhancers (ESE) and exonic splicing silencers (ESS). Mutations that create or disrupt ESS/ESEs may disturb this balance and cause missplicing and disease. Two unrelated newborns who were identified by MS/MS screening were found to be compound heterozygous with the prevalent c.985A>G mutation and a synonymous c.87A>G substitution. Analysis of cells from one of the newborns and her father showed skipping of exon 2. To investigate if this is caused directly by c.87A>G or alternatively by an undetected intronic mutation we used a MCAD minigene. Transfection studies confirmed that c.87A>G causes exon 2 skipping. In silico analysis indicated that c.87A>G disrupts a binding motif for the splicing regulatory protein SRp40. We used a heterologous splicing reporter minigene to confirm that c.87A>G also causes missplicing in another genetic context and tested other substitutions to delineate the consensus sequence of this ESE. Using nuclear extracts and RNA affinity purification with wild type and c.87A>G RNA oligonucleotides we confirmed that c.87A>G disrupts binding of SRp40. This suggests that an ESE encompassing position c.87 harbors a SRp40 binding ESE, which is fundamental for splicing of MCAD exon 2. The present study provides an detailed example on how synonymous substitutions can be deleterious by disrupting the finely tuned balance between splicing regulatory elements in constitutive exons. Moreover, it could be speculated if this SRp40 regulated ESE may cause vulnerability to fasting stress, and thus contribute to the general pathology of MCAD deficiency, since SRp40 activity is known to be regulated by insulin levels, and MCAD exon 2 skipping in patient cells seems to be influenced by the metabolic status.

54

Deciphering Synergistic Heterozygosity in the Fatty Acid Oxidation Pathway. *K.M. Griffin[1], S. Ji[1], D. Matern[2], P. Rinaldo[2], J.D. Sharer[1], T.R. Schoeb[1], J. Vockley[3], P.A. Wood[1].* 1) Dept. of Genetics, University of Alabama at Birmingham; 2) Dept. of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine; 3) Dept. of Human Genetics, University of Pittsburgh.

Usually considered monogenic traits, mitochondrial β-oxidation (FAO) disorders may involve two or more mutant genes in a heterozygous state contributing to the disease phenotype; a concept called synergistic heterozygosity (SH). We hypothesize that SH is revealed when genetic combinations shift the control of pathway flux from key regulatory sites and alter metabolite pool sizes. Recent experiments introduced heterozygous deficiencies of a transcriptional regulator of FAO, peroxisomal proliferator activated receptor-α (PPARα), or the rate limiting enzyme of liver FAO, carnitine palmitoyltransferase-1a (CPT-1a, liver isoform) with long-chain acyl-CoA dehydrogenase (LCAD). Using mice 6-9 weeks of age and cold tolerance as a metabolic challenge, we observed ~33% cold intolerance in LCAD +/-//PPARα +/- (n=20, $p<0.01$), and also in PPARα +/- (n=6) mice. No detrimental interaction was detected in LCAD +/-//CPT-1a +/- mice (n=5). Acylcarnitine analysis revealed a significant increase of $C_{18:1}$ and C_{16} species only in the PPARα +/- group (n=5), and fatty liver scores were considerably decreased in LCAD +/-//PPARα +/- compared to wild-type, as if the double heterozygotes were "protected." Similar studies were also conducted with carnitine palmitoyltransferase-1b +/- (CPT-1b, muscle isoform)//CPT-1a +/- mice. Cold intolerance or fatty liver was not detected in CPT-1 single (n=20 for CPT-1a, n=14 for CPT-1b) or double heterozygotes (n=22). Since the CPT-1 isoforms are reciprocally expressed, they demonstrate some mechanistic specificity to the heterozygous combinations and development of metabolic intolerance. Overall, heterozygous enzyme deficiencies may synergize to increase susceptibility to environmental triggers of metabolic decompensation, whereas other combinations may have little effect or perhaps provide a novel increased resistance to metabolic disease phenotypes.

55

Apoptosis inducing effects of the signal sequence mutation in preproparathyroid hormone explains autosomal dominant familial isolated hypoparathyroidism and is corrected by a chemical chaperone. *R. Datta, A. Waheed, G.N. Shah, W.S. Sly.* Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, MO.

Autosomal dominant familial isolated hypoparathyroidism (AD-FIH) is caused by a Cys to Arg mutation (C18R) in the hydrophobic core of the signal peptide of human preproparathyroid hormone (PPTH). Although it has been shown that this mutation prevents secretion of the mutant hormone, the mechanism by which it produces an autosomal dominant disease is unexplained. The primary objective of this study was to clarify the pathogenic mechanism of AD-FIH. We hypothesized that impaired processing of the mutant hormone leads to endoplasmic reticulum (ER) stress and apoptosis of the hormone-producing cells, which would explain the autosomal dominant effects of the mutation. We also proposed that the chemical chaperone, PBA (4-phenylbutyric acid), would correct the adverse effects of the mutation.

Our data confirm that very little C18R PPTH is secreted into the media. The processing-incompetent mutant hormone is trapped intracellularly, predominantly in the ER. The ER retention of the mutant hormone was found to be toxic for the cells, which exhibited clear signs of apoptosis, as evident from the dramatic increase in cell staining positive for Annexin V binding and for the TUNEL reaction. Cells producing the mutant hormone also had marked upregulation of the ER stress markers, BiP and PERK, as well as the proapoptotic transcription factor, CHOP. Upregulation of these markers of the unfolded protein response suggested a causal link between the ER stress and the cell death cascade. When the C18R PPTH was expressed in the presence of 2 mM PBA, intracellular accumulation was reduced and normal secretion was restored. This treatment also produced remarkable reduction of ER stress signals and protection against cell death.

These data implicate ER stress induced cell death as the underlying mechanism for AD-FIH and suggest that pharmacological manipulation of this pathway using chemical chaperones offers a novel therapeutic option for treating this disease.

56

High frequency of uroporphyrinogen decarboxylase gene mutations in sporadic porphyria cutanea tarda patients. *K.H. Astrin[1], I. Nazarenko[1], E. Gehrie[1], K.E. Anderson[2], C. Lee[2], M. Yasuda[1], R.J. Desnick[1].* 1) Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Preventive Medicine & Community Health, University of Texas Medical Branch, Galveston, TX.

Porphyria cutanea tarda (PCT), the most common disorder of heme biosynthesis, presents with characteristic light-induced blistering skin lesions. Familial PCT (F-PCT) is an austosomal dominant disorder with low penetrance and all heterozygotes have uroporphyrinogen decarboxylase (URO-D) mutations and half-normal erythrocyte URO-D activities. In contrast, sporadic PCT (S-PCT) patients have normal URO-D genes and erythrocyte URO-D activities. Both have decreased hepatic URO-D activities when symptomatic presumably due to the specific inhibition of the hepatic enzyme. S- and F-PCT are precipitated by multiple factors including alcohol, iron overload, and viral infections. S- and F-PCT are diagnosed biochemically by markedly elevated urinary uroporphyrin and heptacarboxylate porphyrin levels. The erythrocyte URO-D activities are problematic for identifying F-PCT patients, due to the overlap of mutation-positive patient and normal activities. Also, urinary porphyrins are not diagnostically increased in asymptomatic F-PCT patients. To determine if PCT patients with no family history had URO-D mutations, the entire ~3.5 kb gene and 1000 bases upstream and downstream were sequenced in 27 biochemically documented patients and 16 patients referred with only a clinical diagnosis. URO-D mutations were identified in 6 of the 27 (22.2%) biochemically diagnosed patients (Q9H, P44L, A80S, G210D, H220P, and 648insT) and in 4 of the 16 (25%) clinically diagnosed patients (R142X, G281V, H331R, and g645del1053ins10). Overall 10/43 or 23.3% of the PCT patients without a family history had URO-D mutations. Five of the 10 URO-D mutations were novel (Q9H, P44L, G210D, H331R and 648insT). These findings indicate that 20-25% of PCT patients without a family history actually have F-PCT, Therefore, it is recommended that all PCT patients be screened by mutation analysis, that members of F-PCT families have diagnostic mutation testing, and that heterozygotes be counseled about their risk and PCT precipitating factors.

57

Maternal Cigarette Smoking, Metabolic Gene Polymorphisms, and Preterm Delivery: New Insights on GxE Interactions and Pathogenic Pathways. *H.-J. Tsai[1], X. Liu[1], K. Mestan[1], X. Yu[1], S. Zhang[1], C. Pearson[2], K. Ortiz[2], B. Zuckerman[2], H. Bauchner[2], S. Cerda[2], P. Stubblefield[2], X. Xu[3], X. Wang[1].* 1) Children's Memorial Hospital and Children's Memorial Research Center; Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL; 2) Boston University School of Medicine and Boston Medical Center, Boston, MA; 3) Center for Population Genetics, University of Illinois at Chicago School of Public Health, Chicago, IL.

Background: While we have investigated previously genetic susceptibility and gene-environment interaction in low birth weight and gestational age, this paper extends this work to preterm term delivery (PTD) which is largely unexplored. Such data may help elucidate pathogenic pathways for PTD. **Methods:** This report included 1,749 multi-ethnic mothers (571 with PTD and 1,178 controls) enrolled at Boston Medical Center. Regression analyses were performed to detect individual and joint associations of maternal smoking, two functional variants of CYP1A1 and GSTT1 with PTD and with preterm subgroups after adjusting for important covariates. False discovery rates were applied to correct for multiple testing. **Results:** We observed a moderate effect of maternal smoking on PTD (OR: 1.6; 95% CI: 1.1-2.2). Consistent with our earlier report, we found that compared to non-smoking mothers with low-risk genotypes, there was a significant joint association of maternal smoking, CYP1A1 (Aa/aa) and GSTT1 (absent) genotypes with gestational age (β: -3.37; SE: 0.86; $P = 9x10^{-5}$). With a larger sample size, we further demonstrated the joint association with PTD (OR: 5.8; 95%CI: 2.0-21.1). Such joint association was particularly strong in certain preterm subgroups, including spontaneous PTD (OR: 8.3; 95%CI: 2.7-30.6), PTD < 33 weeks (OR: 10.3; 95%CI: 2.9-42.4), and PTD accompanied by histologic chorioamnionitis (OR: 15.6; 95%CI: 4.1-76.7). We also showed similar patterns across ethnic groups. **Conclusions:** Maternal smoking significantly increased the risk of PTD among women with high-risk CYP1A1 and GSTT1 genotypes. Such joint associations were strongest among PTD accompanied by histologic chorioamnionitis.

58

A Large Scale High-throughput Candidate Gene Association Study of Preterm Birth. *D.R. Velez[1,2], R. Menon[3], P. Thorsen[3], S.M. Williams[1,2], S.J. Fortunato[3].* 1) Division of Cardiovascular Medicine, Vanderbilt University, Nashville, TN, USA; 2) Department of Medicine and Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA; 3) The Perinatal Research Center, Nashville, TN, USA.

Spontaneous preterm birth (<37 weeks gestationPTB) occurs in ~12% of pregnancies in the United States, and is the largest contributor to neonatal morbidity and mortality. PTB is a complex disease, potentially induced by several etiologic factors from multiple pathophysiologic pathways. To dissect the genetic risk factors of PTB a large-scale high-throughput candidate gene association study was performed examining 1442 SNP in 134 genes of PTB pathways. Maternal and fetal DNA from 370 Caucasian (C) birth-events (172 cases and 198 controls) was examined. Single locus association analyses were performed separately on maternal and fetal samples. For maternal samples the strongest associations were found in genes in the coagulation-complement pathway that are likely related to decidual hemorrhage in PTB. In this pathway 3/6 genes examined had SNPs significantly associated with PTB. These include Factor V (F5) that was previously associated with PTB (rs9332624, p = 3.0x10-3) and Plasminogen activator tissue (PLAT). The single strongest effect was observed in PLAT marker rs879293 (allelic association p = 2.00x10-3; genotypic association p=2.0x10-6). The odds ratio (OR) for this SNP was 2.80 (CI 1.77 - 4.44) for a recessive model. Given that 6/8 markers in PLAT were statistically significant, sliding window haplotype analyses were performed and 4 marker haplotype in PLAT (p = 0.006) was found. The single strongest effect in C babies was observed in the inflammatory pathway at rs17121510 in the interleukin-10 receptor antagonist (IL-10RA) gene for allele (p = 0.01) and genotype (p = 3.34x10-4). The OR for the IL-10RA genotypic additive model was 1.92 (CI 1.15-3.19). Sliding window haplotype analyses of IL-10RA revealed several haplotypes associated with PTB. These results support a role for genes in both the coagulation and inflammation pathways, and potentially different maternal and fetal genetic risks for PTB.

59

Racial differences in genetic association of cytokine concentrations in the presence and absence of bacterial vaginosis. *K.K. Ryckman[1,2], M.A. Krohn[3], H.N. Simhan[3], S.M. Williams[1,2].* 1) Center of Human Genetics , Vanderbilt Univ, Nashville, TN; 2) Department of Medicine, Vanderbilt Univ, Nashville, TN; 3) Department of OBGYN, Magee-Womens Research Institute, Pittsburgh, PA.

Bacterial vaginosis (BV) is one of the most prevalent vaginal disorders in adult women and is characterized by alterations in the normal vaginal flora. BV is associated with pelvic inflammatory disease (PID) and spontaneous preterm delivery (sPTD). BV-related changes in the vaginal flora are accompanied by changes in cervical cytokine levels. To assess the role that genetic variation plays in changes in cytokines that occurs in relationship to BV during the first trimester of pregnancy we examined 52 African-Americans (AA); 20 with normal flora and 32 with BV and 64 Caucasians (CA); 44 with normal flora and 20 with BV. Analyses were stratified by race. BV by genotype analysis of variance (ANOVA) was performed for 15 cytokines and 376 single nucleotide polymorphisms (SNPs) in 28 cytokine-related genes. The two-way ANOVA with BV and each SNP revealed many significant overall model associations and several of these showed dramatic differences between AA and CA. In particular, there were 102 out of 119 SNPs that had significant full model associations for IL-1α in AA, whereas only 16 SNPs had significant full model associations in CA. The opposite was true for IL-1β; there were 45 out of 123 SNPs that had significant full model associations in CA and only 13 full model associations in AA. The most significant BV by SNP interaction for IL-1α in African-Americans was rs1469007 (p-value=0.012) in the interleukin receptor 1 accessory protein. The recessive model for this SNP explained 24.7% of the variation in IL-1α concentration. In CA, the most significant BV by SNP interaction for IL-1β was rs7628250 (p-value=0.007). This dominant model included a significant BV by SNP interaction and explained 18.1% of the variation in IL-1β concentration. In conclusion, we found significant interactions between BV status and single SNPs for several cytokine concentrations. The patterns of associations between genotype and cytokine concentration differed by race.

60

WHOLE GENOME ANALYSIS IDENTIFIES A SUSCEPTIBILITY LOCUS TO HIV-1. *S. Deutsch[1], C. Loeuillet[2], A. Ciuffi[2], D. Robyr[1], M. Munoz[2], P. Taffé[3], M. Rotger[2], J.S. Beckmann[4], S.E. Antonarakis[1], A. Telenti[2].* 1) Genetic Medicine and Development, University of Geneva, Switzerland; 2) Institute of Microbiology, University of Lausanne, Switzerland; 3) Swiss HIV Cohort Study Data Center, Lausanne, Switzerland; 4) Medical Genetics, University of Lausanne, Switzerland.

Susceptibility to lentiviral infection (including HIV) is a quantitative trait that is influenced by genetic variability of the host. To identify loci underlying this trait we established an in vitro approach in lymphoblastoid cells (LCLs), based on a lentiviral GFP reporter system. We phenotyped 198 LCLs from 15 three-generation CEPH families to calculate heritability and perform quantitative linkage (QTL) analysis. Heritability calculations showed that the phenotype has a strong genetic component with a h2r of 0.53. QTL analysis using variance components, led to the identification of a significant locus on chromosome 8q (p=2E-04). The empirical significance of the locus was confirmed by performing simulation studies. To further dissect the locus, we phenotyped 57 LCLs from the CEU HapMap collection, and performed an association analysis using tag SNPs in a 3Mb region around the marker with the highest LOD-score. This resulted in the identification of a single intergenic SNP (p=7.7E-05) that remained significant after correction for multiple testing. To confirm the association, we infected CD4 cells from 128 healthy blood donors with replicating HIV. Individuals heterozygous for the SNP were on average 57% more susceptible to infection than non-carriers (p=0.02). In addition, we genotyped 496 HIV positive individuals that were followed up for 7 years without anti-retroviral treatment to determine whether the genotype had an effect on disease progression. Carriers of the SNP showed increased levels of viremia (p= 0.009) and a faster depletion of CD4 cells (p=0.046) compared to non-carriers. Since the SNP is located in an intergenic region, we performed a 3C experiment to detect potential interactions with genes within a 100 Kb region. Our data identify a novel locus on chromosome 8 that modulates HIV susceptibility in vitro and in vivo and provide candidates genes that might underlie this biological effect.

61

Insight on the role of maternal age and recombination in chromosome 21 nondisjunction. *T. Oliver[1], E. Feingold[2], K. Yu[3], S. Sherman[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Human chromosome nondisjunction (NDJ) occurs at a high frequency in humans and is known to be influenced by maternal age and recombination. In previous studies, we found that the presence of a single meiotic exchange within the most telomeric 5.2Mb of 21q and the most proximal 3.5Mb of 21q were associated with maternal MI and MII NDJ respectively. In addition to the altered placement of recombination, increased maternal age is another risk factor for NDJ. We examined the association of these two known risk factors among maternal chromosome 21 NDJ stratified by meiosis I (MI, n=400) and meiosis II (MII, n=278) errors. Specifically we looked at the number of recombinant events and the location of recombination in women belonging to one of three maternal age groups: women <29, women 29-34 and women >34. Results showed that among women who experienced MI NDJ of chromosome 21 there was no difference in the distribution of the number of recombinant events between women belonging to our youngest and eldest age groups. In addition among cases with a single recombinant event, we found that as maternal age increased, the location of recombination shifted from the telomere towards the middle of the chromosome. Among women who experienced MII NDJ of chromosome 21 the number of cases with greater than 1 recombinant event increased with increasing maternal age. In addition among cases with a single recombinant we found that as maternal age increased the location of recombination shifted towards the centromere. These results suggest that multiple mechanisms lead to NDJ. For example, single telomeric exchanges appear to predispose to NDJ irrespective of maternal age. However, for MII errors, recombination-related factors appear to interact with maternal age related factors. In order to better understand recombination-related NDJ we have initiated studies to characterize recombination breakpoints of the maternal MI and MII susceptible recombinant events.

62

Testing models of human aneuploidy: age-related variation in recombination in trisomic meioses. *H. Hall[1], U. Surti[2], T. Hassold[1].* 1) School of Molecular Biosciences and Center for Reproductive Biology, Washington State University, Pullman, WA; 2) Magee-Womens Research Institute, Pittsburgh, PA, 15213.

Trisomy affects about 4% of clinically recognized pregnancies. To date, only two factors have been linked to the origin of trisomy, recombination and maternal age. We have been interested in testing models that explain the relationship between these two predisposing factors. In the course of these studies, we have examined the parental/meiotic origin and recombination status of over 300 new cases of trisomies 13, 16, and 22. Combined with previous studies of trisomy, our results suggest three types of nondisjunctional mechanisms: those shared by all chromosomes, those by groups of chromosomes, and those specific to individual chromosomes. As a test of the relationship between recombination and maternal age, we evaluated recombination levels in maternal meiosis I derived trisomies for our new data set and for previously published data on trisomies 15, 18, 21, and sex chromosome trisomies. We tested these data against two popular models of human nondisjunction: the production line model and the two-hit hypothesis. The production line model predicts declining recombination levels with maternal age in both normal and trisomy-generating meioses. We found no evidence of this for any of the trisomies we examined; thus, this model is unlikely to explain the maternal age effect. The results for the two hit model were more complicated. This model proposes that nondisjunction-prone chiasmata configurations (the first hit) occur in a proportion of fetal oocytes and with age, these become more likely to nondisjoin due to degradation of meiotic processes (the second hit). Thus, in its simplest form, this model predicts similar levels of susceptible configurations in trisomies involving younger and older women. Our results indicate this is the case for some, but not all trisomies; for most trisomies, we observed a decrease in susceptible events with maternal age. Thus, it seems unlikely that either of the models satisfactorily explains the maternal age effect, suggesting a more complicated, and likely chromosome-specific, relationship between age, recombination, and nondisjunction.

63

Down Syndrome: Genomic analyses link genes for GI malformation and leukemia. *T. Tirosh-Wagner[1], J.O. Korbel[2], A.E. Urban[2], X-N. Chen[1], M. Snyder[2], J.R. Korenberg[1].* 1) Medical Genetics, Cedars-Sinai, Los Angeles, CA; 2) Yale University, New Haven, CT.

Down syndrome (DS) is a major cause of mental retardation (MR), gut disease and increased risk for leukemia. The question remains as to which of the 352 genes or clusters contribute most to cognitive or disease risks in persons with DS. Usually caused by trisomy 21, rare individuals with duplication of small regions provide opportunities to identify genes whose increased copy number are sufficient to cause DS features. We present multi-disciplinary data of 14 partial trisomy 21 people, combined with their molecular breakpoints and focus hypotheses linking genes to DS. Molecular analyses of following resolutions; 1) 100kb-3Mb by high resolution FISH with 350 BACs (13 subjects) and Southern blot dosage (13 sub) and 2) 50bp-300bp by high density isothermal oligomer microarrays employing 355,083 oligomers/chip, tiled at ~1/100bp unique genomic sequence, from 21p (10Mb) to 21qter (12 sub). Results: Cases: 1) MR, duplication (dup): pter-27.5Mb, 42.3-44.7Mb, 45.9Mb-qter; 2) MR, dup: pter-30.2Mb; 3) Low Normal function, dup: pter-33.0Mb & 46.7Mb-qter; 4) MR, 4 copies: pter-30.6Mb; 5) MR, Duodenal stenosis, dup: 24.8-41.5Mb; 6) MR, dup: 28.9-41.4Mb; 7) MR, dup: 34.1-46.6Mb; 8) MR, AMKL, dup: 17.9-31.4Mb, 36.1-42.9Mb; 1 copy: 42.9Mb-qter; 9) Transient leukemoid reaction (TLR), dup: 16.5-41.3Mb; 4 copies: 41.3Mb-qter; 10) MR, dup: 19.2Mb-qter; 11) MR, duodenal stenosis, dup:19.5Mb-qter; 12) MR, Hirschsprung's Disease, dup: 33.5-46.2Mb; 13) MR, Hirschsprung's Disease, dup: 28.8-46.9Mb; 14): imperforate anus,TRL. dup:30.4Mb-qter; Conclusions: The candidate region (CR) for leukemia and TLR (3/14 cases) includes maximum limits 30.4-42.9 Mb, minimum 36.1-42.9. Genes include CLDN17 - TSGA2(max); exclude TPTE-GRIK (max) and TIAM (min). The CR for duodenal stenosis and imperforate anus (3/14) includes the region from 30.4-41.5Mb, genes SOD1 to BACE2; for Hirschsprung's Disease (2/14) includes the region from 33.5-46.2Mb, genes IFNAR1 to COL18A. These results focus animal and cellular models targeting treatment of DS and identify dosage sensitive steps for disease in the normal population.

64

An Intragenic Genomic Duplication Resulting in Loss of Function and other Novel Mutations in *NLRP7* in Women with Recurrent Biparental Hydatidiform Moles. *Y. Kou[1], L. Shao[1], R. Rosetta[1], D. Del Gaudio[2], H. Peng[1], T. AL-Hussaini[3], I. Van den Veyver[1, 2].* 1) Depts of Ob-Gyn, Baylor College of Medicine, Houston, TX; 2) Depts of Molecular Human Genet, Baylor College of Medicine, Houston, TX; 3) Dept of Ob-Gyn, Assiut University, Assiut, Egypt.

Hydatidiform mole (HM) is an abnormal development of the placenta with hyperproliferative trophoblast. Biparentally inherited HM (BiHM) have normal diploid biparental inheritance and are not androgenetic. Linkage using consanguineous pedigrees of women with BiHM refined a major locus to chromosome 19q13.42. Recently, mutations in the NACHT, leucine rich repeat (LRR) and PYD containing 7 (*NLRP7*) gene were identified in DNA of women with recurrent BiHM whose mutation maps to this region. We studied kindreds with several affected women and isolated cases of recurrent BiHM of confirmed biparental inheritance and first performed bisulfite genome sequencing of regulatory DMRs at several imprinted loci (*NESP55*, *KCNQ1OT1*, *PEG3*, *H19*, *SNRPN*) on DNA from BiHM tissue. We found failure to acquire or maintain DNA methylation that is established at imprinted DMRs during oogenesis. We sequenced coding exons of *NLRP7* using DNA of women with recurrent BiHM and found new missense and splice-site mutations in isolated cases. We identified a homozygous missense mutation c.2234C>G (p.L745V) affecting a conserved leucine in the 2nd LRR of *NLRP7*, a compound heterozygous c.2234C>G, an exon 9 splice donor mutation (c.2796+2T>G) and a previously described c.2457+1G>A mutation. Southern analysis and quantitative RT-PCR (qPCR) revealed a 4Kb tandem intragenic duplication spanning exons 2-5 of *NLRP7* in 5 patients from 3 unrelated Egyptian families but not in unaffected controls, suggesting the presence a founder effect in this population. The resulting mutant mRNA is predicted to translate into a truncated protein containing a frameshift of six amino acids and a stop codon after Thr710 and lacking all LRRs. This is second report confirms that *NLRP7* deficiency is a major cause of BiHM.

65

Gastroschisis and Genitourinary Infections. *M.L. Feldkamp[1], L.D. Botto[1], J. Reefhuis[2], J. Kucik[2], S. Krikov[1], A. Wilson[1], C. Moore[3], J.C. Carey[1].* 1) Pediatrics, University of Utah, Salt Lake City, Ut; 2) Center on Birth Defects and Developmental Disabilities; 3) Office of Genomics and Disease Prevention, Centers for Disease Control and Prevention, Atlanta, Ga.

Gastroschisis is a pathogenetic and epidemiologic dilemma. Competing pathogenetic views consider gastroschisis as a late fetal disruption (after normal morphogenesis), or, alternatively, a primary malformation of ventral wall closure. Epidemiologically, gastroschisis is unique for its strong association with very young maternal age and its recent increase in occurrence. We focused on genitourinary (GU) infections as a possible risk factor for gastroschisis, because such infections, which include urinary tract infections (UTI) and sexually transmitted infections (STI), are common in sexually active young women and their frequency may be increasing. We analyzed data from the National Birth Defects Prevention Study, an ongoing multi-center, population-based case-control study of risk factors for major birth defects. The study included 515 case-infants with gastroschisis and 5008 unaffected controls. Women were considered exposed if they reported a GU infection at any time from one month prior through the third month after conception. An STI was reported by 21 cases (4.1%) and 99 controls (2.0%), and a UTI was reported by 68 cases (13.2%) and 341 controls (6.8%). Odds ratios for gastroschisis, adjusted for maternal age, were 1.2 (95% confidence interval: 0.7, 2.2) for STI alone, 1.4 (1.1, 1.9) for UTI alone, and 3.8 (1.3, 10.5) for STI with UTI. Within maternal age strata, the risk for STI with UTI was highest for women <25 years: 2.9 (0.6, 14.4) for women <20 years and 5.7 (1.4, 22.9) for women 20-24 years. The risk for gastroschisis remained elevated for STI with UTI at 4.6 (1.4, 15.4) after controlling for many covariates. These findings suggest that GU infections, particularly as a combination of an STI and a UTI, are risk factors for gastroschisis. If the association is causal, and the rate of infections is increasing, GU infections may account for some of the increase in reported rates of gastroschisis. The role of a specific susceptibility in young women, fever, or medications, remains unclear.

66

Genetic Screening of Usher Syndrome in Children. *W.J. Kimberling[1], R.J.H. Smith[2], E.M. Stone[2], R.G. Weleber[3], C. Moller[4], C. Carney[1], M. Jensen[1], K. Trzupek[3].* 1) Boys Town Hospital, Omaha, NE; 2) Univ. Iowa, Iowa City, IA; 3) Oregon Health Sciences Center, Portland, OR; 4) Swedish Institute for Disability Research, Orebro, Sweden.

Past estimates of the frequency of Usher syndrome (US) in children relied on clinical diagnosis of the associated retinitis pigmentosa (RP) and generally reported that 5% of deaf children manifested Usher syndrome. Population studies yielded frequencies in the range of 1 in 25,000. New molecular testing offers a more accurate and less expensive alternative. We conducted two pilot studies designed to determine the frequency of US. The first involved high school children from the state of Oregon who are in the special education program for the deaf and hard of hearing (D/HOH). DNA samples were collected by mail using a Genotek saliva collection kit (dnagenotek.com) and genotyped using the an US chip (asperophthalmics.com). Out of 78 children who were genotyped, eight (10.5%) had at least one pathologic mutation. Mutations were observed in CDH23(3), MYO7A(2), or USH2A(3). A second study was carried out on children who had received cochlear implants at the University of Iowa. Fifty-five children were genotyped using the US chip. GJB2 positive children were eliminated from the Usher genotyping. Of these, 7 (12.7%) were observed to have mutations in MYO7A, CDH23, USH1C, or USH2A. Adjusting for the elimination of GJB2 positive children yielded an estimate of the frequency of US at 8.2%. Both pediatric populations gave similar estimates of US frequency and a similar distribution of the genetic subtypes. Assuming that the US chip has a 50% sensitivity, between 15 and 21% of D/HOH children have or will develop RP. Assuming also that the frequency of childhood Deafness is 1/1000, the population frequency of US is between 1/5000 and 1/10000 births, a value much greater than previously believed. Further, this study shows that molecular screening for US is a cost effective and accurate means for early diagnosis. Early diagnosis is particularly relevant since recent research suggests several possibilities of intervention, all of which would be expected to more effective if instituted early.

67

The human Y-encoded testis-specific protein (TSPY) interacts functionally with the eukaryotic translation elongation factor 1A (eEF1A), a putative oncoprotein. *Y. Lau, T. Kido.* Dept Medicine/ VAMC-111C5, Univ California, San Francisco, San Francisco, CA.

Testis specific protein Y-encoded (TSPY) gene is a candidate for the gonadoblastoma locus on the Y-chromosome (GBY). It is expressed in normal testicular germ cells and tumor germ cells in gonadoblastoma cells of XY sex-reversed females and testicular germ cell tumors (TGCTs). It is hypothesized to serve a normal function(s) in male germ cell proliferation and early meiotic division, dysregulation of which could contribute to tumorigenesis. TSPY belongs to the TSPY/SET/NAP1 protein family and harbors a highly conserved SET/NAP-domain. SET/TAF-Iβ, the best characterized TSPY/SET/NAP1 family member, is located in the nucleus and is demonstrated to regulate transcription by interacting with histones and transcription factors, such as COUP-TF, CREB-binding protein and ER1α. TSPY is located on both cytoplasm and nucleus. To explore the possible function(s) of TSPY in tumorigenesis, we performed a yeast two-hybrid screen of a fetal gonadal cDNA library using the TSPY SET/NAP domain as bait. The translation elongation factor, eEF1A, was consistently identified as a binding partner for TSPY. eEF1A is essential for protein elongation of the protein synthesis machinery. It is also a putative oncoprotein involved in the development of ovarian and breast cancers. TSPY and eEF1A were colocalized in the cytoplasm and were coimmunoprecipitated from transfected COS7 cells. Immunostaining of human TGCTs demonstrated that TSPY and eEF1A were highly expressed and colocalzied in both the premalignant precursor, carcinoma in situ (CIS), of both seminomas and nonseminomas and tumor germ cells of seminomas. Significantly, over-expression of eEF1A increased the expression of a reporter gene in cultured cells. Such enhancement could be further amplified in the presence of TSPY. Since cell proliferation requires significant metabolism and growth nutrients, the interaction between TSPY and eEF1A accelerates the protein synthesis machinery, thereby exacerbating the respective tumor-promoting functions in TGCTs.

68

MicroRNA profiling in hypoxia identifies HSA-MIR-210 as an independent prognostic predictor in breast cancer. *C. Camps[1], F. Buffa[2], S. Colella[1], J. Moore[2], H. Sheldon[2], A.L. Harris[2], J. Gleadle[3], J. Ragoussis[1].* 1) Genomics, Wellcome Trust Centre for Human Genetics, Oxford , OXXON, United Kingdom; 2) Cancer Research UK Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, United Kingdom; 3) Oxygen Sensing Group, The Henry Wellcome Building for Molecular Physiology, University of Oxford, Oxford OX3 7BN, United Kingdom.

Many cancers are characterised by areas of hypoxia, enhanced HIF levels and increased expression of hypoxically regulated genes, all of which correlate both with tumour aggression and patient outcome. Recently MicroRNA expression alterations have also been associated with carcinogenesis.We used microarrays to determine changes in microRNA expression under hypoxia and validations were performed by quantitative-PCR. hsa-miR-210 was identified as a hypoxically, early induced microRNA in MCF7 cells. This induction (4-fold, $p<0.001$) was also confirmed in a range of other cancer cells. Using siRNA against HIF1α and HIF2α as well as RCC4 cells transfected with VHL we demonstrated that the regulation by hypoxia was mediated by the HIF1α -VHL transcriptional system but not HIF2α. We analysed the expression of hsa-miR-210 and hsa-miR-21, as a control, in 219 early breast cancers with long term clinical follow up. Correlation with clinical parameters was performed using Pearson and Spearman's rank tests, univariate and Cox multivariate analysis. We determined that the hsa-miR-210 expression correlated with a previously identified hypoxia signature based on the expression of 96 genes in 73 samples (Spearmanρ=0.54, p2-tailed<0.001). The expression of has-miR-210 correlates highly significantly with recurrence free and overall survival, both in univariate and multivariate analysis (Log Rank (Mantel-Cox) $\chi 2$=16.6, df=1, $p<0.001$) in contrast to has-miR-21. In conclusion hsa-miR-210 expression is induced by hypoxia in a HIF-1α and VHL dependent fashion and is an independent prognostic predictor in breast cancer.

69

Identification of a clinical and biological role for TGIF in leukemogenesis and hematopoiesis. *R. Hamid[1], J. Patterson[1], S. Brandt[2].* 1) Dept Pediatrics, Div Med Gen, Vanderbilt Univ Sch Medicine, Nashville, TN; 2) Department of Medicine, Vanderbilt University, Nashville, TN. 37232.

To identify genes with prognostic potential in acute myeloid leukemia (AML), we applied microarray analysis and real-time PCR to RNA from cryopreserved bone marrow or venous blood samples from 61 patients with newly diagnosed or relapsed AML, and the expression of one gene, TG-interacting factor (TGIF) was found to be highly predictive of relapse and survival (p=0.00001). TGIF is a transcriptional repressor belonging to the TALE (three amino acid loop extension) class of homeobox proteins, and deletion or mutation of a single allele of TGIF is associated with the craniofacial genetic disorder holoprosencephaly (HPE). In order to better understand its role in hematopoiesis, we characterized an in vitro model system to study the effects of its knockdown and enforced expression. We show that TGIF increases during granulocytic differentiation of both leukemic and normal myeloid precursors, likely indicating that adequate expression of TGIF has to be achieved along with monocyte/neutrophil/ maturation of myeloid progenitor cell lines as well as normal hematopoietic stem cells (HSC). shRNA mediated TGIF knockdown results in significant growth inhibition of myeloid progenitor cell lines while enforced expression results in accelerated growth. Cell cycle analysis indicates that this growth inhibition is due to a block at G2M stage of cell cycle. Our results suggest a new role for TGIF: as a prognostic indicator in AML. TGIF is a modifier (inhibitor) of TGFβ pathway, which plays a significant role in both normal and leukemic hematopoiesis by its action of HSC quiescence and renewal. Decreased TGIF may then be expected to lead to increased TGF-β pathway activity resulting in HSC quiescence and thus escape from the toxic effects of chemotherapy. Our data raises the intriguing possibility that baseline levels of TGIF (a genetic trait) in individuals could predict a priori their prognosis if they develop leukemia in future. This and further definition of biological and genetic role of TGIF in leukemogenesis continues to be an active area of research in our laboratory.

70

Heritability of susceptibility to ionizing radiation induced apoptosis of human lymphocyte subpopulations. *A. Schmitz[1], J. Bayer[1,3], N. Dechamps[1,3], L. Goldin[2], G. Thomas[2,3].* 1) Institut de Radiobiologie Cellulaire et Moléculaire, DSV/CEA , Fontenay aux roses, France; 2) Division of Cancer Epidemiology and Genetics, NCI/NIH Bethesda MD; 3) Fondation Jean Dausset-CEPH, Paris, France.

Association between major changes in chromosomal radiosensitivity and increased susceptibility to cancer has been demonstrated for several rare cancer prone monogenic conditions, It has also been suggested that chromosomal radiosensitivity may be an inherited trait in families of breast cancer patients. In mice, the Prkdc-gene was identified as a candidate for a gene controlling in vivo thymocyte sensitivity to radiation induced apoptosis, as well as for a gene controlling radiation lymphomagenesis. These observations support the possibility that subtle inter-individual variation in radiosensitivity in humans may contribute to cancer susceptibility in the general population and that this trait may in part be genetically determined. To evaluate heritability of intrinsic radiosensitivity, induction of apoptosis in lymphocyte subpopulations was determined by flow cytometry immunophenotyping on samples from 334 related individuals belonging to 38 large kindred-families. Intra-familial correlations and heritability were computed on 199 father-mother-offspring trios. Marked differential susceptibility to ionizing radiation induced apoptosis of naïve and memory T lymphocytes was demonstrated, and although age and sex were significant covariates, their effects only accounted for a minor part of the inter-individual variation. Parent-offspring and sib-sib correlations were significant for radiosensitivity of B cells, T4, T8, and of effector memory (EM) T4- and T8 subpopulations. In the T4-EM subpopulation, the phenotype showed correlations most consistent with dominant or additive genetic effects and segregation analysis in the pedigrees was consistent with the contribution of a bi-allelic dominant locus. Thus, heritability was demonstrated for the susceptibility to ionizing radiation induced apoptosis of lymphocyte populations and segregation of the T4-EM radiosensitivity phenotype was consistent with a Mendelian transmission model involving one major gene.

71

Molecular mapping of the 12q13-15 amplicon and identification of new target oncogenes in well-differentiated liposarcomas. *L. Bianchini, A. Italiano, F. Keslair, F. Pedeutour.* Lab. of Tumor Genetics, Univ. Hospital Nice and CNRS UMR 6543, Nice, France.

The characteristic supernumerary rings and giant chromosomes of well-differentiated and dedifferentiated liposarcomas (WDLPS/DDLPS) are composed of amplified material from chromosome 12q13-15. The MDM2 and CDK4 genes are usually considered as the targets of the 12q amplicons. However, most data were obtained before the availability of precise and complete maps and were based on small series of WDLPS/DDLPS. Our goal was to precisely define the structure of the 12q13-15 amplicon and to identify new potential target genes of amplification. We investigated a series of 38 WDLPS/DDLPS using fluorescence in situ hybridization (FISH) analysis with a panel of BAC probes encompassing the CDK4-MDM2 region. We studied MDM2, CDK4, CHOP/DDIT3, HMGA2 and GAS41 expression in 11 of 38 cases, using real time quantitative RT-PCR (Q-RT-PCR). We showed the presence of two discrete amplicons centred around MDM2 and CDK4, respectively. In all cases, the centromeric border of the CDK4 amplicon was located precisely downstream to the 5' end of CHOP at 12q13 suggesting that CHOP might be deregulated by the close proximity of the amplicon. Moreover, CHOP is already known for being the seat of the translocation breakpoint in myxoid/round cell liposarcoma. We found that CHOP was overexpressed in 9 of 11 cases demonstrating that CHOP is involved in the development of WDLPS/DDLPS. We found that HMGA2, located between CDK4 and MDM2, was amplified and structurally rearranged in all cases. HMGA2 is known to be the seat of structural rearrangements in lipoma. Our results suggest that the HMGA2 region might be a "fragile zone"; its disruption would be a major event in the pathogenesis of both benign and malignant adipose tissue tumors. We finally found that GAS41, originally described to be frequently amplified in gliomas, was co-amplified with MDM2 in 83% of cases. Overexpression of GAS41 was detected in 91% of cases. Our results suggest that GAS41 may play an important role in the tumorigenesis of WDLPS/DDLPS and provide evidence for several oncogenes besides MDM2 and CDK4 in the pathogenesis of WDLPS/DDLPS.

72

Defective chromosome segregation and telomere dysfunction in aggressive Wilms tumours. *Y. Stewénius[1], Y. Jin[1], I. Øra[2,3], A. Frigyesi[4], J. Alumets[5], B. Sandstedt[6], A.K. Meeker[7], D. Gisselsson[1].* 1) Department of Clinical Genetics, Lund University Hospital, Sweden; 2) Department of Pediatric Oncology, Lund University Hospital, Sweden; 3) Department of Human Genetics, AMC, the Netherlands; 4) Department of Cardiology, Lund University Hospital, Sweden; 5) Department of Pathology, Lund University Hospital, Sweden; 6) Department of Pathology, Karolinska Hospital, Stockholm, Sweden; 7) Department of Pathology, Division of Genitourinary Pathology, The Johns Hopkins Institutions, Baltimore, MD, USA.

Wilms tumour (WT) is a paediatric renal tumour with a 5-year survival rate at approximately 90 %. One of the challenges in WT management is to refine the risk assessment on which treatment is based. In other childhood neoplasms it has been possible to define prognostic subgroups based on the pattern of chromosome changes in the tumour cells. In WT, such sub-classification has been hampered by the fact that WT exhibits a diverse and relatively unspecific pattern of chromosomal imbalances. In adult tumours, complex genomic changes are often generated by abnormal mitotic segregation of chromosomes, caused by disrupted telomeric protection. We here show that similar mechanisms of mitotic instability are present in a sub-group of WT. Molecular cytogenetic analysis of 12 WT showed a strong association between abnormal telomere shortening, karyotypic complexity, and specific cell division abnormalities, including anaphase bridges and multipolar mitoses. Anaphase bridges led to structural rearrangements and single chromosome losses, whereas multipolar mitoses led to more extensive variation in chromosome number. Assessment of mitotic figures in tissue sections from 41 WT revealed that anaphase bridges and multipolar mitoses were predominantly, but not exclusively, present in blastemal predominant and diffuse anaplastic tumours. The presence of anaphase bridges and multipolar mitoses in the primary tumour was a significant predictor of poor event-free and overall survival, independent of stage. Thus, chromosomal instability is rare in WT but may nevertheless have an important pathogenetic role by accelerating clonal evolution in cases that respond poorly to therapy.

73

The leukemogenic CALM/AF10 fusion protein alters the subcellular localization of the lymphoid regulator Ikaros. *P.A. Greif[1], B. Tizazu[1], A. Krause[1], E. Kremmer[2], S.K. Bohlander[1].* 1) Medicine III , Universität München / GSF, München, Germany; 2) Molecular Immunology , GSF , München , Germany.

The t(10;11)(p13;q14) translocation leads to the fusion of the CALM and AF10 genes. This translocation can be found as the sole cytogenetic abnormality in acute lymphoblastic leukemia, acute myeloid leukemia and also in malignant lymphomas. The expression of CALM/AF10 in primary murine bone marrow cells triggers the development of an aggressive myeloid leukemia in a murine bone marrow transplantation model. Interestingly, the leukemia propagating cell shows lymphoid characteristics including immunoglobulin rearrangements and B220 surface markers. Here we show that AF10 interacts with the lymphoid regulator Ikaros in yeast-two-hybrid assays. Ikaros is required for normal development of lymphocytes and aberrant expression of Ikaros has been found in leukemia. In a murine model, the expression of a dominant negative isoform of Ikaros causes leukemias and lymphomas. The Ikaros interaction domain of AF10 was mapped to the leucine zipper domain of AF10, which is required for malignant transformation by both the CALM/AF10 and the MLL/AF10 fusion protein. The interaction between AF10 and Ikaros was confirmed by GST-pulldown and co-immunoprecipitation. In contrast to AF10, CALM/AF10 alters the nuclear localization of Ikaros. The transcriptional repressor activity of Ikaros is reduced by CALM/AF10 but not by AF10. These results suggest that CALM/AF10 might have a dominant negative effect on Ikaros, and thereby block differentiation of the leukaemia propagating cell in CALM/AF10 positive leukemias.

74

Cryptic Xq duplications in *ETV6/RUNX1*-positive acute lymphoblastic leukemia. *H. Lilljebjörn[1], M. Heidenblad[1], B. Nilsson[1], C. Lassen[1], A. Horvat[1], J. Heldrup[2], M. Behrendtz[3], B. Johansson[1], A. Andersson[1], T. Fioretos[1].* 1) Department of Clinical Genetics, Lund University Hospital, Lund, Sweden; 2) Department of Pediatrics, Lund University Hospital, Lund, Sweden; 3) Department of Pediatrics, Linköping University Hospital, Linköping, Sweden.

Seventeen *ETV6/RUNX1*-positive pediatric acute lymphoblastic leukemias (ALLs) were investigated by high resolution array-based comparative genomic hybridization (array CGH), gene expression profiling, and fluorescence in situ hybridization (FISH). Comparing the array CGH and gene expression patterns revealed that genomic imbalances conferred a great impact on the expression of genes in the affected regions. The array CGH analyses identified a high frequency of cytogenetically cryptic genetic changes, e.g., del(9p) and del(12p). Interestingly, a duplication of Xq material, varying between 30 and 60 Mb in size, was found in 6 of 11 males (55%). Genes on Xq were found to have a high expression level in cases with dup(Xq); a similar overexpression was confirmed in t(12;21)-positive cases in an external gene expression data set. By studying the expression profile and proposed function of genes in the minimally gained region, several candidate target genes (*SPANXB, HMGB3, FAM50A, HTATSF1, RAP2C*) were identified. Among them, the testis-specific *SPANXB* gene was the only one showing a high and uniform overexpression, irrespective of gender and presence of Xq duplication, suggesting that this gene plays an important pathogenetic role in t(12;21)-positive leukemia.

75

Detection of balanced translocations by DNA microarrays. *C.C. Lau[1], C. Davis[1], P. Rao[1], R. Selzer[2], P. Eis[2].* 1) Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX,; 2) NimbleGen Systems, Inc. Madison, WI.

Balanced translocations are hallmarks of many human cancers and some of them are also used as prognostic markers. With pediatric acute lymphoblastic leukemia (ALL), several translocations including t(12;21), t(1;19), t(4:11) and t(9;22) are used as part of a prognostication algorithm to stratify patients to risk-based therapy. These translocations are detected clinically by a combination of G-banding and FISH but the precise location of the breakpoints, which might have further prognostic significance, is not identified by either one of these techniques. We report here preliminary results using long oligonucleotide tiling-path microarrays to simultaneously detect the presence of these translocations and precisely map the breakpoints. Using a 390K feature microarray designed and manufactured by NimbleGen to interrogate specific translocation breakpoints with a median probe spacing of 5 bp, we analyzed 8 bone marrow samples from pediatric ALL patients, including 7 from fresh frozen specimens and 1 matched sample from cell pellet previously fixed in methanol-acetic acid. The 3 samples with t(12;21) all showed microdeletions ranging from 200 bp mapped within intron 5 of the TEL/ETV-6 gene on chr 12p13 to 60 kb of the 3'-end of TEL/ETV6 starting from intron 5. Only 1 out of 3 t(12;21) samples showed a 600 bp microdeletion approximately 36 kb 5' of exon 1 of the AML-1 gene on chr 21q22. The four cases of t(1;19) we examined included a matched pair of fresh frozen and fixed samples from the same patient. The matched samples showed identical results with duplication of sequences at both breakpoints of the PBX1 gene on chr 1q23 and the E2A/TCF gene on chr 19p13.3. One sample showed loss of intron 16 in the E2A/TCF3 gene but no change in the PBX1 gene breakpoint. One sample with t(4;11) was also analyzed which showed a microdeletion of 900 bp within intron 3 of the AF-4 gene on chr 4q21 and 200 bp within intron 14 of the MLL gene on chr 11q23. Overall, we identified the correct translocations in 7 out of 8 samples analyzed so far. Finally, we also detected additional changes involving other breakpoints that were missed by G-banding and FISH.

76

Accelerating genome and tumor research with Single Cell Arrays. *H. Weier[1], J.F. Weier[1,2], S. Baehring[3], J. Laubenthal[1].* 1) Life Sci Div, UC-Lawrence Berkeley Natl. Lab, Berkeley, CA 94720; 2) UCSF, San Francisco, CA 94550; 3) Medical Faculty of the Charite, Franz Volhard Clinic, Wiltberg Strasse 50, 13125 Berlin, FRG.

Present technology for genome-wide screening and analysis fails to detect subtle genetic changes such as small balanced translocations or accurately characterize gene amplifications in tumors. We propose the development of a fluorescence in situ hybridization (FISH)-based technology platform capable of analyzing very small amounts of tissue with unprecedented sensitivity, accuracy and resolution. In a typical FISH procedure, the efficiency of FISH and thus the ability to detect a specific target inside a cell nucleus, depends on the penetration of probes and detection reagents as well as the accessibility of the hybridization target. In clinical samples, hybridization efficiencies are typically low and, in combination with photobleaching of detection reagents, limit the in situ detection of genes of interest to about 100kb or larger. Our laboratory investigates the application of a technology termed 'single cell arrays (SCAs)' to study a spectrum of human genetic conditions including the characterization of genetic changes in breast cancer specimens, the delineation of rearrangements in familial autosomal-dominant hypertension and quantitative analysis of gene amplification in thyroid tumors. We completed proof-of-concept experiments showing that a) individual cells can be arrayed on glass slides inside specially designed micro-channels (individually or in pools using a micromanipulator or flow cytometry sorting), b) cells can be treated physico-chemically to release chromatin, c) the entire chromatin can be stretched in a linear fashion, d) the extent of stretching (ranging from a few microns to 10-12 mm) can be adjusted by controlling the stretching force and environmental parameters, e) stretched chromatin can be analyzed by FISH providing a resolution of up to 5-15 kb, f) the method is suitable to address tumor heterogeneity by preparing chromatin arrays of at least 32 single cell spreads per slide, and g) the method works equally well with fresh, frozen or fixed cells as starting material.

77

A novel high resolution genome-wide method identifies over 1,500 very small deletion CNVs and triallelic SNPs. *L. Franke[1], C.G.F. de Kovel[1], Y.S. Aulchenko[2], D.A. van Heel[3], L.R. Cardon[4], P. Deloukas[5], R.A. Ophoff[1], L.H. van den Berg[6], C. Wijmenga[7].* 1) DBG-Dept Medical Genetics, UMC Utrecht, Netherlands; 2) Department of Epidemiology & Biostatistics, Erasmus MC Rotterdam, Netherlands; 3) Centre for Gastroenterology, Institute of Cell and Molecular Science, Queen Mary University of London, UK; 4) Wellcome Trust Centre for Human Genetics, Oxford, UK; 5) Wellcome Trust Sanger Institute, Hinxton, UK; 6) Department Neurology, UMC Utrecht, Netherlands; 7) Genetics Department, UMC Groningen, Netherlands.

Various disorders are associated with chromosomal aberrations. Recently, the availability of genome wide DNA chips have allowed for the identification of considerable amounts of common copy number variation (CNV) throughout the genome. However, the methods employed for detecting these have a limited resolution as they identify only regions of multiple consecutive SNPs. We have developed a novel method that can identify deletion CNVs and triallelic SNPs within genome wide SNP chips in a SNP-by-SNP way. Our method is based on a maximum likelihood estimation procedure, assuming Hardy-Weinberg equilibrium under a triallelic model. It is straightforward and can be applied to single SNPs. Additionally it provides functionality for performing association tests for these SNPs as it can estimate allele frequencies. Analyses in two samples (> 2,700 individuals) with the Illumina HumanHap300 and HumanHap550 platforms identified ~1,500 common triallelic SNPs. Only 20% of these triallelic SNPs map within known deletion CNV regions of the Database of Genomic Variants, whereas only 10% of the 1,500 SNPs span multiple consecutive SNPs included on the platorms, suggesting the presence of many very small deletion CNVs that have not yet been identified before. Interestingly, many of the identified triallelic SNPs have major consequences for haplotype based analyses, as many loci turn out to have incorrect haplotype structures when not assuming these SNPs are triallelic. We will present our method and will show the results of this CNV association analysis in genome-wide data for celiac disease (778 cases, 1422 controls).

78

***ATM* gene deletion in children with *TEL-AML1*-positive acute lymphoblastic leukemia.** *M. Shago[1,2], S.H. Hong[1,2], G. Nie[1], M. Abdelhaleem[1,2], I. Teshima[1,2], O. Abla[1,2].* 1) Departments of Paediatric Laboratory Medicine and Paediatrics, The Hospital for Sick Children, Toronto, ON, Canada; 2) Departments of Laboratory Medicine and Pathobiology and Pediatrics, University of Toronto.

The t(12;21) translocation, which results in the fusion of the *TEL* and *AML1* genes, is present in ~25% of pediatric B-precusor acute lymphoblastic leukemia (ALL) patients. Although initially thought to be a favorable prognostic indicator, the t(12;21) was associated with a similar rate of relapse as t(12;21)-negative pre-B ALL in subsequent studies. While secondary cytogenetic abnormalities are often present, their prognostic significance is unknown. We characterized cytogenetic changes in 57 patients diagnosed with t(12;21)-positive ALL between 2000-2005. One of the recurrent changes was a deletion of 11q, detected in 3/57 (5%) of patients.

Using FISH analysis, we determined that the 11q deletions in these 3 patients included the *ATM* gene. *ATM* (11q22.3) is one of the master genes controlling signaling pathways for DNA repair. *ATM* mutations, deletions, or loss of heterozygosity have been reported in a number of sporadic cancers. In CLL, ATM protein deficiency is associated with aggressive disease, while in adult ALL, loss of heterozygosity of the *ATM* gene is associated with a favourable prognosis. Our aim was to further characterize the incidence and significance of the *ATM* deletions. In total, *ATM* deletions were detected in 16 of 56 patients. In 10 of the 16 *ATM*-deleted patients, the deletion extended distally to the *MLL* gene region (11q23). Five of the 57 children (9%) in this cohort have developed a relapse of ALL. None of the patients with *ATM* deletions have relapsed.

Our data support the speculation that *ATM* gene deletion may have a positive impact on patient survival. In the majority of *ATM*-deleted cases in our cohort, the deletion in 11q was not detected by conventional G-band analysis. This emphasizes the significance of procedures such as FISH and microarray analyses in more accurate definition of genomic alterations in childhood ALL.

79

Dissecting clinical heterogeneity relevant to early development in autism by copy number variations. *P.I. Lin[1,2,3], S. Yoon[1], K. Ye[4], M. Wigler[1], J. Sebat[1].* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Department of Medicine, University of Maryland, Baltimore, MD; 3) Department of Psychiatry, University of Maryland, Baltimore, MD; 4) Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY.

Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder. Despite the high estimated heritability of ASD, no risk gene has been conclusively identified yet, which may be partly due to heterogeneous phenotype-genotype relationships. Previous evidence indicates that developmental milestones may be modulated by genetic components. Therefore, we proposed to study the association between gene copy number variations (CNVs) and quantitative traits relevant to early development in ASD. We analyzed DNAs collected from 1,485 subjects by using representational oligonucleotide microarray analysis (ROMA) that consisted of 85K probes. The probe intensity ratio of tested DNA to reference DNA corresponds to gain or loss of the DNA. Genotypes were inferred from intensity ratio distributions by clustering analysis (i.e., partition around medoids algorithm). We performed factor analysis to identify four subdomains of clinical correlates related to early development such as age-at-onset (AAO), head circumference (HC), age-at-first-sitting (AFS), age-at-first-word (AFW), which represented four distinct sub-domains, respectively. We then performed ANOVA to test whether copy number variations were associated with these traits. We found that the duplication within the COL5A1 gene on 9q34 was significantly associated with AAO ($p = 0.00002$), the deletion within the MGAM gene on 7q34 was significantly associated with HC ($p = 0.0001$), the deletion within the NTRK3 gene on 15q25.3 was significantly associated with AFS ($p = 0.0002$), and the duplication within the STARD13 gene on 13q13 ($p = 0.0003$) was significantly associated with AFW. These results suggest that CNVs may play a role in clinical heterogeneity relevant to early development in ASD.

80

Novel deletion in chromosome 22 in schizophrenia patients from an internal isolate of Finland. *O.P.H. Pietilainen[1,2], T. Paunio[1,3], A. Loukola[1], A. Tuulio-Henriksson[4,5], J. Suvisaari[4,5], T. Varilo[1,2], J. Lönnqvist[3,4], H. Stefansson[6], L. Peltonen[1,2,7].* 1) National Public Health Institute, Dept of Molecular Medicine, Helsinki, Finland; 2) University of Helsinki, Dept of Medical Genetics, Helsinki, Finland; 3) University of Helsinki and Helsinki University Central Hospital, Dept of Psychiatry, Helsinki, Finland; 4) National Public Health Institute, Dept of Mental Health and Alcohol Research, Helsinki, Finland; 5) University of Helsinki, Dept of psychology, Helsinki, Finland; 6) deCODE genetics, Reykjavik, Iceland; 7) The Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Schizophrenia (SZ) is thought to result from an interaction between numerous genes and environment. Copy Number Variations (CNVs) may confer risk to complex diseases by disrupting gene functions either qualitatively or quantitatively. The new era of high-throughput genotyping platforms provides the first generation of tools to collect information on CNVs in a genome-wide manner. We utilized genome-wide SNP-array data from Illumina 317K genotyping platform on a Finnish sample of 200 cases and 200 controls with well established genealogy to search for CNVs potentially predisposing to SZ. Half (47%) of the sample originated from an internal isolate of Finland with an exceptionally high prevalence of SZ (3% versus 1.2% for the general population) representing an outcome of multiple population bottle necks. The sample was a part of a large international EU funded consortium, SGENE, a collaboration of total of 1500 SZ patients and 1600 controls of European origin. In the Finnish sample we identified a novel 238 kb heterozygous deletion in chromosome 22 in 13 patients and three related controls ($P=.025$) all originating from the isolate. Among the patients with deletion, 11 had generalized cognitive impairment. We assessed the prevalence of the deletion in samples of non-Finnish origin by extending the investigations to the rest of SGENE sample and 14,000 additional Icelandic individuals. From the total of 15,600 individuals the deletion was found in four individuals implying that this deletion is enriched especially in SZ patients in this population isolate. Detailed analyses of the regional gene are ongoing.

81

Searching for genes contributing to autism in WAGR syndrome by oligo array CGH. *S. Xu[1], J. Han[2], A. Morales[1], C. Menzie[2], K. Williams[3], Y. Fan[1].* 1) Cytogen R&D Lab, MC Child Dev, Univ Miami, Miami, FL; 2) National Institute of Child Health Human Development, NIH, Bethesda, MD; 3) International WAGR Syndrome Association, Manassas, VA.

WAGR syndrome (Wilms tumor, Aniridia, Genitourinary anomalies and mental Retardation) is a genomic disorder caused by a deletion in 11p12-14 region. Autistic features are seen in about 25% of patients. While deletion of PAX6 and WT1 results in aniridia and an increased risk of Wilms tumor, the genes contributing to mental retardation and autism remain undetermined. We have characterized the 11p12-14 region in 25 WAGR patients with CGH using an array containing >44,000 oligo probes. 11 of the patients had autism spectrum disorder (ASD). Our study revealed a deletion in 11p12-14 in all 25 patients with a size of 2.65-19.13 Mb involving 30-70 mapped genes. In addition to PAX6 and WT1, the deletions involved several genes known to be related to neuron development and brain function, including PRRG4, BDNF and SLC1A2 (in 25 cases, 100%; 14 cases, 56%; and 16 cases, 64% respectively). Of the 11 patients with ASD, 7 had deletion of BDNF (64%), 7 had deletion of SLC1A2 (64%), and 5 had deletion of both BDNF and SLC1A2 (45%). Genome-wide linkage analyses have suggested linkage of 11p11.2-13 region with autism. There is evidence that BDNF is a crucial signaling molecule between microglia and neurons. The recent results of linkage and CNV analysis have implicated 11p12-13 as the candidate locus that includes PRRG4 and SLC1A2. Both PRRG4 and SLC1A2 are related to glutamate synaptic function and brain development. The mapping positions of the autism candidate genes relating to WAGR can be described as tel-BDNF-PAX6-WT1-PRRG4-SLC1A2-cen. Our results suggest that haploinsufficiency of PRRG4, BDNF and SLC1A2 may contribute to mental retardation and autism in WAGR patients. Further expansion and statistical analysis of our data in correlating patient's sex, age of onset and inheritance of the deletion may provide additional insights about the possible mechanisms involved in development of autism in WAGR patients.

82

Inferring carrier of copy number variation in Bipolar linkage region with novel Expectation-Maximization algorithm. *S. Zollner[1,2], Y. Chen[1], G. Su[3], M.G. McInnis[2], M. Burmeister[2,3].* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Psychiatry, Univ Michigan, Ann Arbor, MI; 3) Program of Bioinformatics,Univ Michigan, Ann Arbor, MI.

Copy Number variations (CNVs) are polymorphic features of the human genome that provide exciting candidates for risk variants of complex trait phenotypes. Efforts are underway to generate a CNV-map by collecting all common CNVs in the human genome. To assess the phenotypic impact of these polymorphisms, case-control designs will be a useful aproach. Testing for association of CNV carrier status and diseases status in such studies will require scoring such common CNVs in all individuals in the sample by evaluating the signal intensity of SNP genotyping assays. However, such analyses are hampered by a lack of appropriate statistical tools. Here we present a novel statistical method that is designed to perform such inference and apply this method to a known CNV in a Bipolar linkage region. Using an Expectation-Maximization algorithm we infer the carrier status of a CNV in each individual of a sample by modeling the signal intensity as a mixture of multiple normal distributions allowing for locus-specific and allele-specific distributions. Thus we generate a maximum-likelihood estimate for the carrier status of each individual in the sample. We applied the method in a sample of 3512 individuals to a known deletion on 8q24, a region implicated in the etiology of Bipolar Disorder. We unambiguously inferred 172 heterozygous and 1 homozygous deletion carrier among 3512 individuals from 737 families. We confirmed several inferred carriers by PCR amplification and detected no inconsistencies in Mendelian transmission of the deletion. However, we observed no significant association between bipolar disorder and carrier status. Finally, we assessed to power of this EM-algorithm to detect CNVs by sub-sampling from the SNPs covered by this deletion. We demonstrated that our EM algorithm produces precise estimates for CNVs covering 6 or more SNPs.

83

Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication. *B. Ballif[1], J. Coppinger[1], G. Gowans[2], J. Hersh[3], S. Madan-Khetarpal[4], K. Schmidt[4], R. Tervo[5], L. Escobar[6], C. Friedrich[7], M. McDonald[8], J. Ming[9], E. Zackai[9], B.A. Bejjani[1], L.G. Shaffer[1].* 1) Signature Genomic Laboratories, Spokane, WA; 2) Weisskopf Child Evaluation Center, Louisville, KY; 3) University of Louisville, KY; 4) Children's Hospital of Pittsburgh, PA; 5) Gillette Children's Specialty Healthcare, St. Paul, MN; 6) St. Vincent Children's Hospital, Indianapolis IN; 7) University of Mississippi Medical Center, Jackson, MS; 8) Duke University Medical Center, Durham, NC; 9) Children's Hospital of Philadelphia, PA.

Interstitial deletions of 3q29 have been recently described as a novel microdeletion syndrome mediated by nonallelic homologous recombination between low-copy repeats resulting in an ≈1.5 Mb common-sized deletion. Given the molecular mechanism causing the deletion, the reciprocal duplication is anticipated to occur with equal frequency. To our knowledge, the duplication has not been reported in the literature. We have analyzed >13,000 cases by array CGH using a targeted BAC microarray which includes a high-resolution, near-tiling-path coverage of the most distal 5 Mb of 3q29. Among these cases, we have identified 12 patients with microdeletions of 3q29 including one family with a mildly affected mother and two affected children. We have also identified 14 patients with duplications of 3q29, five of which appear to be the reciprocal duplication product of the 3q29 microdeletion and nine with duplications that flank, span, or partially overlap the common deletion region. Examination of eight 3q29 microdeletion patients revealed variable clinical presentations but identified some common features not previously appreciated. The clinical features of seven 3q29 duplication cases were also examined and, like 3q29 microdeletions, were found to be variable with few common features. Furthermore, de novo and inherited abnormalities were found in both the microdeletion and microduplication cohorts illustrating the need for parental samples to fully characterize these abnormalities. Our report demonstrates that array CGH is especially suited to identify chromosome abnormalities with unclear or variable presentations.

84

Molecular delineation of the 9p deletion syndrome: Phenotypic diversity of a common syndrome and the search for genes. *S. Schwartz[1], R. Anderson[1], S. Biton[2], M. Graf[3], H.K. Vance[4], D.J. Waggoner[1], C.A. Crowe[5].* 1) Univ of Chicago, Chicago, IL; 2) Univ of Toronto, Toronto, Canada; 3) TGEN, Phoenix, AZ; 4) Roswell Park Cancer Institute, Buffalo, NY; 5) MetroHealth Hospital, Cleveland, OH.

The 9p deletion was first described by Alfi in 1976. However, while there have been many reports there has been little phenotype correlation with molecular analysis. We have ascertained 135 patients with chromosome 9 abnormalities and have both detailed phenotypic and breakpoint information (using both BAC and array delineation of breakpoints). Phenotype/breakpoint analysis of 64 cases, where only a pure deletion is present, revealed four general groups of patients: (I) - Patients with general 9p deletion features and trigonencephaly; (II) - Patients with general 9p deletion features and mild face/cranium changes, but not trigonencephaly; (III) - Patients with minor phenotypic abnormalities; (IV) - Patient's phenotype not related to the 9p phenotype. All of the patients (100%) in the Groups I, II and III have hypotonia, mental retardation and specific behavior problems. Results from these studies are interesting and reveal important information including: (1) This is the largest study of 9p deletions to date and reinforces the importance of both precise clinical information and breakpoint analysis and the importance of including only individuals with pure deletions; (2) A putative candidate gene has been identified for trigonencephaly along with suggested putative genes for the other facial features seen in the 9p deletion syndrome as well as the neurological manifestations; (3) 9p subtelomeric deletions are not pathogenic as they have been identified in normal individuals and in individuals whose phenotype is not consistent with the 9p deletion syndrome; (4) In understanding the relationship of the loss of genes to the phenotype, it is extremely important to determine which genes have been shown, in studies of copy number variation, to be deleted in normal individuals; (5) The proximal region of 9p is relatively gene poor and several genes have been shown to be deleted in the general population thus limiting the number of genes that may potentially be important in the etiology of this syndrome.

85

Recurrent genomic rearrangements of 17q12 are involved in a wide range of phenotypes: renal disease, diabetes and epilepsy. *H. Mefford[1,2], S. Clauin[3], A. Sharp[1], R. Moller[4,5], R. Ullmann[6], R. Kapur[7], D. Pinkel[8], G. Cooper[1], M. Ventura[1,9], H. Ropers[10], N. Tommerup[5], E. Eichler[1,10], C. Bellanne-Chantelot[3,11].* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Division of Medical Genetics, University of Washington, Seattle, WA; 3) Center of Molecular Genetics and Cytogenetics, AP-HP Pitié-Salpetrière, Paris, France; 4) Danish Epilepsy Centre, Dianalund, Denmark; 5) Wilhelm Johannsen Centre for Functional Genome Research; 6) Max-Planck Institute of Molecular Genetics, Berlin, Germany; 7) Department of Laboratories, Children's Hospital and Regional Medical Center, Seattle, WA; 8) Comprehensive Cancer Center, University of California San Francisco; 9) Department of Genetics and Microbiology, Universitá di Bari, Italy; 10) Howard Hughes Medical Institute, Seattle, WA; 11) University Pierre et Marie Curie, Paris, France.

Most studies of genomic disorders have focused on patients with cognitive disability and/or peripheral nervous system defects. In an effort to broaden the phenotypic spectrum of this disease model, we assessed 155 autopsy samples from fetuses with well-defined developmental pathologies in regions predisposed to recurrent rearrangement by array CGH. We found that 6% of fetal material showed evidence of microdeletion or microduplication, including 3 independent events that likely resulted from unequal crossing-over between segmental duplications. One of the microdeletions, in a fetus with multicystic dysplastic kidneys, encompasses the *TCF2* gene on 17q12, previously shown to be mutated in maturity-onset diabetes as well as a subset of pediatric renal abnormalities. Fine-scale mapping of the breakpoints in different patient cohorts reveals a recurrent 1.5 Mb *de novo* deletion in individuals with phenotypes ranging from congenital renal abnormalities to maturity-onset diabetes of the young type 5. Breakpoints lie in flanking polymorphic segmental duplications. The reciprocal duplication was also identified and is enriched in samples from patients with epilepsy and mild mental retardation. We describe the first example of a recurrent genomic disorder associated with diabetes.

86

Cryptic deletions are a common finding in "balanced" reciprocal and complex chromosome rearrangements: a study of 43 cases. *M. De Gregori[1], R. Ciccone[1], F. Cifuentes[2], P. Magini[1], S. Gimelli[1], J.R. Vermeesch[3], J. Messa[1], O. Zuffardi[1,4].* 1) Patologia Umana ed Ereditaria , Università di Pavia, Pavia, PV, Italy; 2) Agilent technologies Santa Clara, California 95051, USA; 3) Center for Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium; 4) IRCSS Policlinico San Matteo, Pavia, Italy.

We report array-CGH findings (44B Agilent kit, resolution of 100 kb) in 26 cases of de-novo reciprocal translocations and 17 complex chromosome rearrangements (CCRs), all but one interpreted as balanced through conventional cytogenetic examinations. Thirteen CCRs were detected in individuals with abnormal phenotypes, two in females with repeated abortions and the remaining two in fetuses investigated for advanced maternal age. Fifteen (twelve patients with "chromosomal phenotype", one of the women with abortions and the two fetuses) resulted unbalanced with up to four deletions present either at the breakpoints or elsewhere. Thus, genome-wide array is recommendable in patients with a phenotype and a "balanced" CCR. Regarding the reciprocal translocations, seventeen were detected in patients with abnormal phenotype and six resulted unbalanced after array-CGH screening with three having a deletion at one derivative and three having a deletion elsewhere with one case having three deletions. All twenty-one imbalances originated at the paternal meiosis. Thus, among patients with a "chromosomal phenotype" and an apparently balanced translocation 35% is unbalanced. We analyzed nine fetuses with an apparently balanced translocations that resulted normal at the array-CGH screening. The size of all imbalances ranged from 0.37 Mb to 35 Mb. Using a customized array for seven CCRs we narrowed the deletion breakpoints to few hundreds of bp and no peculiar motif of DNA sequences associated to the imbalance was detected. Our findings demonstrate that phenotypic abnormalities, reported in half of the cases of apparently balanced de novo rearrangements, are mainly due to cryptic deletions not exclusively at the breakpoints and that male gametogenesis is more prone to create chaotic multiple chromosome imbalances and reciprocal translocations than female one.

87

Disruption of an AP-2 binding site upstream of *IRF6* is commonly associated with nonsyndromic cleft lip and palate. *F. Rahimov[1], M.J. Hitchler[2], F.E. Domann[2], A. Jugessur[3], R.T. Lie[3], A.J. Wilcox[4], K. Christensen[5], E.D. Green[6], M.L Marazita[7], B.C. Schutte[1], J.C. Murray[1].* 1) Dept Pediatrics, Univ Iowa; 2) Dept Rad Onc, Univ Iowa; 3) Univ Bergen, Norway; 4) NIEHS, Durham, NC; 5) Univ Southern Denmark; 6) NHGRI, NIH; 7) Center Craniof Dent Genet, Univ Pittsburgh.

Nonsyndromic cleft lip and palate (NSCLP) is a common craniofacial birth defect. We discovered that mutations in *IRF6* underlie Van der Woude syndrome (VWS), an orofacial clefting disorder where lower lip pits are the only features distinguishing VWS from NSCLP. Subsequently, we reported a strong association between SNPs in the *IRF6* locus and NSCLP. We observed a particularly strong overtransmission of the ancestral allele V of the rs2235371 (V274I) SNP in individuals of Asian and South American ancestry. However, the frequency of the risk allele is over 97% in European and African populations making it an unlikely candidate for the etiological mutation. Direct sequencing of the coding regions of *IRF6* did not detect potential causative mutations. We postulated that the causative variant(s) are in linkage disequilibrium with V274I and could reside in the regulatory element(s) of *IRF6*. Using comparative genomic sequence analysis from 14 vertebrate species, we detected a highly conserved region 9.7kb upstream of *IRF6*. Family-based association analysis in Norwegian, Danish and Filipino populations showed strong overtransmission of a conserved SNP (rs642961) in this region ($p<2x10^{-8}$). The ancestral allele G and the derived allele A of rs642961 split the V allele of V274I into two haplotypes. The V-A haplotype is significantly overtransmitted ($p<3x10^{-8}$), whereas transmission of the V-G haplotype is not distorted (p<0.7). Gel shift assays showed that the A allele of rs642961 disrupts binding activity of the transcription factor AP-2 alpha. *TFAP2A* is highly expressed in craniofacial structures and knockout mice have multiple facial anomalies. A ChIP assay showed that AP-2 binds to its consensus binding sites *in vivo* suggesting that it could function upstream of *IRF6*. In total, our data suggests that a common functional variant upstream of *IRF6* contributes to NSCLP and implicates AP-2 in the IRF6 developmental pathway.

88

FAF1 a new gene for Cleft Palate and Pierre Robin Sequence. *M. Ghassibe[1], L. Desmyter[1], O. Boute[2], B. Bayet[3], Ph. Pellerin[4], N. Revencu[1], H. Poirel[5], J. Vermeesch[6], L. Backx[6], R. Vanwijck[3], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute and Université catholique de Louvain, Brussels, Belgium; 2) Centre de Génétique, CHU de Lille, Lille, France; 3) Centre Labiopalatin, Division of Plastic Surgery, Cliniques universitaires St Luc, Brussels, Belgium; 4) Service de chirurgie plastique et reconstructives, CHU de Lille, Lille, France; 5) Center for Human Genetics, Cliniques universitaires St Luc and Université catholique de Louvain, Brussels, Belgium; 6) Center for Human Genetics, Leuven University Hospital, Leuven, Belgium.

Cleft lip and/or cleft palate is the most frequent craniofacial malformation in humans (~ 1/700). Genetic factors involved in cleft lip with or without the palate (CL/P) are thought to be different from those having a role in cleft palate only (CPO). There is a significant challenge in identifying genetic and environmental components of isolated clefts since it is a multifactorial disease with complex etiology. We show that the FAF1 gene (Fas-Associated Factor 1) is disrupted, by a reciprocal translocation, in a patient with Pierre Robin sequence (PRS), characterized by a cleft of the palate and a micrognathia resulting in glossoptosis. Moreover, association study showed that FAF1 predisposes to cleft palate and Pierre Robin sequence. Screening of the gene revealed several substitutions occurring in highly conserved domains which might thus be responsible of the cleft condition in five separate families. Finally, by in-situ hybridization we show high levels of Faf1 mRNA along the medial edge epithelium of the fusing palate, at the fusing superior lips and the tongue. This expression declines after fusion. Human FAF1 is a Fas-associating molecule with the ability to initiate apoptosis. It is a member of the Fas death-inducing signaling complex and a suppressor of NF-kB activity. Taken together, our observations demonstrate that FAF1 is a novel gene playing an important role in the ethiopathogenesis of syndromic and non-syndromic cleft palate by preventing the medial edge epithelial cells (MEE) from undergoing apoptosis. (vikkula@bchm.ucl.ac.be).

89

A genome-wide association study of Kawasaki disease identifies multiple new loci validated in a family based follow-up study. *D. Burgner[1], S. Davila[2], T.W. Kuijpers[3], S.B. Ng[2], W.B. Breunis[3], M. Levin[4], J.C. Burns[5], V.J. Wright[4], M.L. Hibberd[2], US KD Genetics Consortium.* 1) Sch Pediatrics, Univ Western Australia, Perth, Australia; 2) Genome Institute of Singapore; 3) Emma Children's Hospital,Netherlands; 4) Paediatrics,Imperial College London; 5) Pediatrics,UCSD,La Jolla,CA.

Background: Kawasaki disease (KD) is a common pediatric vasculitis that damages the coronary arteries in 25% of untreated and 5% of treated children. Epidemiologic data suggest that KD is probably caused by unidentified infection(s) in genetically susceptible children. We undertook a genome-wide association (GWA) study to identify novel genetic determinants. **Methods:** In a staged study design, 119 Dutch Caucasian KD cases and 136 matched controls where genotyped using the Affymetrix 250K NSP chip. SNPs that deviated significantly from HWE, had significant Mendelian errors or failed genotyping QC were excluded. Nominally associated SNPs were ranked by significance and 1,176 top variants were genotyped in 1,903 members of 583 KD families from Australia, UK and US, including 498 trios, by a custom Illumina Oligo Pool Assay. Analysis of the 1,087 SNPs successfully genotyped was performed with Illumina BeadStudio software. Associated genes were investigated for putative biological relationships by gene ontology using PANTHER. **Results:** At replication, 61 SNPs remained significant, of which 31 lie in or within 50 kb of 29 known genes. These include an intronic SNP in an expressed gene (P= 2x10-4) and 2 SNPs within introns of a known transcription factor (P= 1.6 and 2.3x10-3). Systems analysis identified function for 26 of 29 genes and clustered 4 of them to calcium-mediated signaling (Pc = 0.005). **Discussion** This is largest GWA study of any paediatric inflammatory disease. Our initial power and genome coverage were modest, but we used a large replication sample to minimize type I errors. We describe several novel biologically plausible variants in KD in gene regions that are currently being fine-mapped. Functional KD-associated variants may lead to novel interventions and may highlight common pathways in adult cardiovascular disease.

90

A Whole Genome Scan For Polymorphisms Influencing Warfarin Dosing. *M.J. Rieder[1], G.M. Cooper[1], J.D. Smith[1], M.H. Wong[1], E.A. Johanson[1], D.L. Veenstra[2], A.E. Rettie[3].* 1) Genome Sciences; 2) Pharmacy; 3) and Medicinal Chemistry, University of Washington, Seattle, WA.

Warfarin is the most commonly prescribed oral anticoagulant and has a narrow therapeutic range which determines the stabilized warfarin dose for individual patients. Warfarin dosing is affected by both clinical and genetic factors, with the vitamin K epoxide reductase complex 1 (*VKORC1*) and cytochrome P450 2C9 (*CYP2C9*) genes having the largest known overall effect. To identify potentially novel associations with moderate to large genetic effect sizes (> 5% of stabilized dosage variance), we genotyped 190 warfarin stabilized patients of European-descent using the Illumina 550K BeadChip. Genotyping call rates for 561,466 SNPs averaged >98% across all samples. Regression analyses were used to identify novel, significant SNP/dose associations under three different base models: 1) independent SNP effects 2) adjusted for clinical covariates (i.e. age, sex, and medication use) 3) combined clinical and genetic (i.e. *VKORC1* and *CYP2C9*) factors. While the overall distribution of correlation measures revealed no systematic biases in our analysis, we find evidence for associations at a small minority of loci with uncorrected p-values below 10^{-4}. Under the clinical covariate model, polymorphisms within or in linkage disequilibrium with *VKORC1* and *CYP2C9* SNPs had p-values of $6x10^{-15}$ and 10^{-5}, explaining 25-30% and 10% of the variance in stabilized dose, respectively. *VKORC1* SNPs showed the strongest signal of association, suggesting that no other candidate gene has a greater contribution to stabilized warfarin dose in this dataset. SNPs below a p-value threshold of 10^{-4} were further explored for associations with warfarin dose, and may explain an additional 10-20% of variance in stabilized dose. Lastly, we tested all genotyped SNPs for significant statistical interactions with a highly predictive *VKORC1* polymorphism(rs10871454). In this analysis one polymorphism showed a significant interaction term (p-value < $8.0x10^{-7}$) despite having no independent effect on dose. Replication studies to confirm these findings have been initiated.

91

Biological and genetic interplay between the asthma susceptibility genes Neuropeptide S receptor 1 and Tenascin C. *C. Orsmark-Pietras[1], E. Melén[2], J. Vendelin[3], M. van Hage[4], F. Nyberg[2], G. Pershagen[2], A. Scheynius[4], M. Wickman[2], J. Kere[1,4], the PARSIFAL Genetics Study Group.* 1) Dept. of Biosciences and Nutrition, Karolinska Institutet, Sweden; 2) Institute of Environmental Medicine, Karolinska Institutet, Sweden; 3) Dept. of Medical Genetics, University of Helsinki, Finland; 4) Dept. of Medicine, Karolinska Institutet, Sweden.

Neuropeptide S receptor 1 (NPSR1, GPRA) was recently identified as a susceptibility gene for asthma and high total serum IgE levels. The ligand for NPSR1, Neuropeptide S (NPS), activates signalling through NPSR1 and microarray analysis has identified Tenascin C (TNC) as a target gene of NPS-NPSR1 signalling. Several studies have linked TNC to asthma through its altered expression in asthmatic tissue but few genetic analyses have been performed. As both NPSR1 and TNC have been implicated as susceptibility genes for asthma, and since TNC is upregulated by NPSR1 activation, our objective was to study the joint risk modifying effect of different TNC and NPSR1 allele combinations. Regulation of TNC was investigated using NPS stimulated NPSR1 transfected cells. Using the cross-sectional PARSIFAL study (n=3,113) we genotyped 12 TNC SNPs and performed single SNP association, haplotype association and TNC and NPSR1 gene-gene interaction analysis. Our results confirm and show a NPS dose-dependent expression of TNC. The genotyping results indicate single SNP and haplotype associations to several SNPs in TNC for asthmatic phenotypes with the most significant association for haplotype TGGT (p=0.0005) in rhinoconjunctivitis. Evidence of significant gene-gene interaction was found between several of the TNC and NPSR1 SNPs. We here show that the asthma susceptibility gene TNC, previously thought of as a phenotypic marker for inflammation also contributes to the asthmatic phenotype on a genetic level. We show an example of gene-gene interaction, based on both a regulatory relationship between NPSR1 and TNC and genetic interaction between NPSR1 and TNC, modifying the phenotype in asthma-related traits. These results join previously independent pathways of importance in the development of asthma and allergic diseases.

92

Genomic and genetic approaches identify IREB2 as a novel susceptibility gene for chronic obstructive pulmonary disease. *D.L. DeMeo[1,2], T. Mariani[2], C. Lange[1], S. Bhattacharya[2], S. Srisuma[2], S. Shapiro[3], R. Bueno[2], E. Silverman[1,2], J. Reilly[1].* 1) Channing Laboratory; 2) Pulmonary/Critical Care Division, Brigham and Women's Hospital,Boston,MA; 3) University of Pittsburgh School of Medicine, Pittsburgh, PA.

As a complex human lung disease, chronic obstructive pulmonary disease (COPD) is influenced by genetic and environmental factors. To date, the only proven genetic cause is a severe deficiency of alpha 1-antitrypsin. We integrated results from gene expression microarrays of lung tissue samples from 56 individuals with a broad range of pulmonary function values to select 69 genes for association studies. LD-tagging SNP panels were genotyped in 389 severe COPD cases from the National Emphysema Treatment Trial (NETT) and 424 smoking controls with normal spirometry from the Normative Aging Study (NAS). After a case/control analysis of 1052 SNPs, 88 SNPs in 16 genes demonstrated nominal significance (p=1x10-5 to 0.05). These 88 SNPs were evaluated for associations with spirometric phenotypes in a family-based study of 127 probands with severe, early-onset COPD and 822 of their family members in the Boston Early-Onset COPD Study. Forced expiratory volume in the first second (FEV1), an important quantitative spirometric phenotype for COPD severity, was the primary phenotype in the family-based association analysis. We used Fisher's exact method for combining p values from the case/control analysis of COPD susceptibility and the family-based analysis of FEV1, setting p=5 x 10-5 as the threshold for significance for the combined p value after Bonferroni correction. Three SNPs in iron regulatory protein 2 (IREB2) met this stringent threshold for significance, with 4 other SNPs in IREB2 demonstrating combined p values < 0.01. IREB2 controls iron metabolism in vivo, with murine data suggesting that iron regulatory protein family genes are modulated by tissue oxygen tension. Hypoxemia is a common feature in individuals with COPD, suggesting that polymorphic variation in a key iron metabolism gene may contribute to phenotypic features of COPD. Support: NIH K08 HL072918, HL71885, HL72303, P01 HL083069.

93

Genetic associations with ancestral differences in gene expression in the small airway epithelium in response to cigarette smoking. *T.P. O'Connor[1], B-G. Harvey[1], W. Wang[2], A. Clark[2], J. Mezey[2], P. Schweitzer[2], J. Salit[1], I. Dolgalev[1], T. Raman[1], N.R. Hackett[1], R.G. Crystal[1]*. 1) Weill Cornell Medical College, New York, NY; 2) Cornell University, Ithaca, NY.

Epidemiologic data suggest that Americans of African ancestry are more susceptible to cigarette smoking than those of European ancestry, with faster rates of lung function decline and increased mortality. In the context that the small airway epithelium (SAE) is the initial site of smoking-associated disease, we hypothesize that: (1) the SAE gene expression profile of individuals of African ancestry responds differently to cigarette smoke compared to individuals of European ancestry; and (2) genome-wide SNP genotyping will reveal *cis*-acting single nucleotide polymorphisms (SNPs) correlated with expression of differentially responsive genes. Gene expression levels in SAE were assessed with Affymetrix HG-U133A Plus 2.0 arrays in 24 healthy smokers (14 of African and 10 of European ancestry) and 18 healthy non-smokers (10 of African and 8 of European ancestry). Smoking-responsive genes in each ancestral group were independently identified as significant with a fold-change >2 and a p value <0.01 in smokers compared to non-smokers. Smokers of European ancestry showed a greater number of smoking-responsive genes (n=356 genes) than smokers of African ancestry (n=188 genes) and, in general, a greater magnitude of differential expression between smokers and non-smokers. For example, xenobiotic metabolism genes were up-regulated in smokers of both groups, but cytochrome P450 1A1 and 1B1 were upregulated 16 and 20-fold, respectively in smokers of African ancestry, while the same genes were upregulated 40 and 150-fold, respectively, in smokers of European ancestry. Genome-wide SNP profiles were obtained on genomic DNA from blood samples from a large cohort of individuals using Affymetrix 5.0 SNP arrays. Significant associations of SNPs within 25,000 base pairs of many of the genes that were differentially responsive to smoking in the two ancestral groups were identified using a likelihood ratio test.

94

Assessing biological pathways using genome-wide association data reveals evidence for excess association of variants in the cell cycle, Wnt signaling and Adherens Junction pathways with type 2 diabetes. *J.R.B. Perry[1], H. Lango[1], N.J. Timpson[2], E. Zeggini[2], R.M. Freathy[1], C.M. Lindgren[2], K.S. Elliott[2], N.W. Rayner[2], B. Shields[1], C.J. Groves[2], A.T. Hattersley[1], M.I. McCarthy[2], T.M. Frayling[1], M.N. Weedon[1]*. 1) Peninsula Med School, Exeter, UK; 2) OCDEM, Oxford, UK.

Initial results from genome wide association studies indicate that many genuine risk alleles may not reach genome wide significance. This means replication is important and methods are needed to decide how best to prioritise SNPs for follow up. After selecting the most significant SNPs, one approach is to identify biological pathways where there was a significant excess of associations compared to that expected under the null distribution. The Wellcome Trust Case Control Consortium (WTCCC) recently completed a GWAS comparing 1924 UK type 2 diabetes patients and 2938 UK population controls using the Affymetrix GeneChip Human Mapping 500k Array Set. We used data from the WTCCC, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) to test 198 human pathways. We totaled the trend test chi-sq for all SNPs in all genes (as defined by the NCBI and 25kb flanking sequence) in a KEGG defined pathway. This observed chi-sq total was compared to that of a distribution of 100,000 permuted chi-sq totals from an equivalent-sized random selection of genic SNPs from across the genome. The pathways showing the strongest association were the WNT signaling ($P<0.00001$), Adherens Junction ($P<0.00001$), and cell cycle ($P < 0.00001$) pathways. The WNT signaling and Adherens Junction pathway associations remained even when TCF7L2 variants were removed from the analyses ($P = 0.03$). The cell cycle pathway association was not explained by the recently identified CDKN2A/B or CDKAL1 signals ($P=0.00002$). There was no evidence for other candidate pathways, for example oxidative phosphorylation $P = 0.99$. In conclusion, identifying pathways with an excess of association signals may be an effective way of prioritizing SNPs for follow up of genome wide studies.

95

GENETIC AND NON-GENETIC SOURCES OF PHENOTYPIC VARIATION IN HUMAN LYMPHOBLASTOID CELL-LINES. *R. Yelensky[1,5,7], E. Choy[1,2,7], S. Bonakdar[1], R.M. Plenge[1], P.L. de Jager[1,3], R. Saxena[1,2], E. McFarland[6], C. Wolfish[1,3], E. Kieff[6], D.A. Hafler[1,3], M. Daly[1,4], D. Altshuler[1,2,4]*. 1) Broad Institute, Cambridge, MA; 2) Molecular Biology, MGH, Boston, MA; 3) Neurology, B&W Hospital, Boston, MA; 4) CHGR, MGH, Boston, MA; 5) HS&T, MIT, Cambridge, MA; 6) Channing Lab, B&W Hospital, Boston, MA; 7) equal contributors.

Lymphoblastoid cell lines (EBV-transformed human B-cells) are an exciting new ex-vivo model for population genetics combining advantages of model organisms with benefits of working directly on human biology. Genotyped LCLs have already been successfully used to study gene expression and attempts have been made to identify DNA variants that influence response to radiation and chemotherapy. However, for LCLs to be broadly useful as a generic ex-vivo human model, it is critical to understand the relative contributions of genetic and non-genetic influences to cellular phenotypes. The field has focused on genes with cis-eQTLs, but in fact these make up only a small fraction of expressed RNAs. Pharmacogenetic studies in LCLs, while promising, have thus yielded few replicated results.

We sought to advance our knowledge of this important new model system by elucidating both genetic and, importantly, non-genetic factors influencing LCL phenotypes. We profiled essential cell-line properties - EBV genome, cell surface receptor and cytokine make-up (i.e. the B-cell "immuno-phenotype"), in vitro growth rates and metabolic state - and assessed their influence on RNA expression and drug response. We find that while a small fraction of phenotypic variation can be consistently related to DNA (~5% of RNAs in current studies), a much greater proportion is explained by these cell-line dependent factors. For instance, 24% of all well-measured, varying genes in LCLs appear to be determined by EBV and the secreted cytokine milieu, while chemotherapeutic response is markedly influenced by baseline growth rate and metabolic state. These findings reveal important, previously uncharacterized, modifiers/confounders of genetic effects in LCLs and will guide both interpretation of prior studies and design of future work.

96

Cis regulation of gene expression is an important target for selection in the human genome. *S. Kudaravalli[1], B.E. Stranger[2], E.T. Dermitzakis*[2], J.K. Pritchard*[1]*. 1) Dept Human Genetics, University of Chicago, Chicago, IL; 2) Population and Comparative Genomics, Sanger Institute, Hinxton, Cambridge, UK.

*Joint supervision.

Previous studies have used genome-wide genotype data from the International HapMap project with genome-wide expression data to identify SNPs that are associated with gene expression differences in lymphoblastoid cell lines (e.g. Stranger et al, 2007). In this study we find SNPs showing haplotype-based signals for selection (Voight et al, 2006) are significantly more likely to be associated with cis gene expression differences than are matched control SNPs. This effect remains highly significant even after controlling for various confounding factors and is observed in all three HapMap population groups. Our results argue that selection on gene expression is an important and widespread mode of human adaptation.

97

The mutational spectrum of the novel HSP gene REEP1 suggests haploinsufficiency and microRNA target site involvement. *S. Zuchner[1], C. Beetz[2], R. Schüle[3], T. Deconinck[4], J. Beats[4], KN. Trans Viet[5], H. Zhu[6], N. Nagan[6], T. Deufel[2], C. Braastad[6], L. Schöls[3], P. de Jonghe[4], M. Pericak-Vance[1].* 1) Univ. of Miami, MIHG, Miami,FL; 2) Univ. Jena,Germany; 3) Univ. of Tübingen,Germany; 4) Univ. of Antwerp,Belgium; 5) Duke Univ.,NC; 6) Athena Diagnostics,MA.

Hereditary spastic paraplegia (HSP) is a genetically and clinically heterogeneous disease affecting the upper motor neuron. HSP shows clinical and genetic overlap with other diseases of the motor neuron system, such as ALS, dHMN, and axonal neuropathies. The most common forms of HSP are SPG4 and SPG3A that account for about ~40% and ~10%, respectively. Other HSP genes appear to be rarely affected (<1%).Although more than 30 chromosomal loci have been mapped only 15 HSP genes have been identified thus far. We studied two families that showed significant linkage (TP-LOD 4.7) to chromosome 2p12 thereby establishing a new HSP designation, SPG31. Candidate gene sequencing revealed the novel gene REEP1 as the underlying cause for SPG31. Screening of 627 HSP samples from multiple academic centers and a commercial testing laboratory revealed 17 REEP1 mutations in 20 cases/families (3.2%). The majority of mutations (71%) caused frame shifts or splice-site mutations and was predicted to lead to haploinsufficiency. Another 20% of mutations occurred in conserved microRNA target sites suggesting a novel mechanism for HSP and potentially for a wider range of genes involved in neurodegeneration. Detailed genotype/phenotype correlations revealed a pure clinical phenotype and suggested a bimodal distribution of the age of onset. We further designed specific REEP1 antibodies and showed in cell culture and on Western blots that REEP1 localizes to mitochondria and likely resides in the mitochondrial membrane. This fits well into the proposed pathways for HSP and other neurodegenerative disease, where mitochondrial transport, fusion, and oxidative phosphorylation appear to be central molecular themes. Taken together, REEP1 is an important new HSP gene with a considerable frequency that justifies regular testing. The further characterization of its molecular properties, especially the haploinsufficiency and microRNA mechanism, is under way.

98

Genome-wide association study of sporadic amyotrophic lateral sclerosis identifies ITPR2 as a susceptibility gene. *M.A. van Es[1], P.W. van Vught[1], H. Blauw[1], L. Franke[2], C.G.J. Saris[1], P.M. Anderson[3], L. Vandenbosch[4], A. Birve[3], V. de Jong[5], F. Baas[5], H.J. Schelhaas[6], K. Sleegers[7], C. van Broeckhoven[7], J.H.J. Wokke[1], C. Wijmenga[2], W. Robberecht[4], J.H. Veldink[1], R.A. Ophoff[2,8], L.H. van den Berg[1].* 1) Neurology, University Medical Centre Utrecht, Utrecht, Utrecht, Netherlands; 2) Complex Genetics Section, Department of Biomedical Genetics, University Medical Center Utrecht, The Netherlands; 3) Institute of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden; 4) Department of Neurology, University Hospital Gasthuisberg, Leuven, Belgium; 5) Department of Neurology and Neurogenetics, Academic Medical Center, Amsterdam, The Netherlands; 6) Department of Neurology, Radboud University Medical Center, Nijmegen, The Netherlands; 7) Department of Molecular Genetics, University of Antwerp, Antwerpen, Belgium; 8) Department of Human Genetics and Neuropsychiatric Institute, University of California, Los Angeles, USA.

Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by progressive degeneration of motor neurons in the brain and spinal cord. We performed a genome-wide association study in 461 patients with sporadic ALS and 450 matched controls. After replication in two independent sample series we identified rs2306677 located in the Inositol 1,4,5-triphosphate receptor 2 (ITPR2) gene to be significantly associated with ALS. Combined analysis of all samples (total: 1,337 cases and 1,356 controls) gave an overall odds ratio (OR) of 1.58, with 95% confidence interval (CI) of 1.30-1.91. ITPR2 is an important regulator of intracellular Ca2+-levels and is involved in glutamate-mediated neurotransmission. We further observed significantly elevated gene expression levels of ITPR2 in peripheral blood of 126 ALS cases compared to 126 healthy controls (P = 0.00016). Elevated ITPR2 levels have been shown to play a major role in apoptosis. Since ITPR2 is involved with glutamate, Ca2+ and apoptosis, it is a strong biological candidate for a susceptibility gene in ALS.

99

Interactions involving nitric oxide synthase genes and environmental risk factors in Parkinson's disease. *D.B. Hancock[1], E.R. Martin[2], J.M. Vance[2], W.K. Scott[2].* 1) Duke University, Durham, NC; 2) University of Miami, Miami, FL.

Nitric oxide synthase (NOS) genes (*NOS1, NOS2A,* and *NOS3*) are biological candidate genes for Parkinson's disease (PD), as excess nitric oxide (NO) levels are associated with dopaminergic neuronal depletion in the substantia nigra. NO levels may also be influenced by the putative PD risk factors cigarette smoking, caffeine, nonsteroidal anti-inflammatory drugs, and pesticides. Thus, combinations of NOS-related genetic and environmental factors might be important in the etiology of PD. We genotyped 27 *NOS1* coding and tagging SNPs, 18 *NOS2A* SNPs, and 5 *NOS3* SNPs in 337 families with no history of PD (337 cases, 389 relative and other unrelated controls) and examined allelic associations with PD using the Association in the Presence of Linkage (APL) test and the Pedigree Disequilibrium Test (PDT). In those with environmental risk factor data (163 cases and 178 controls), interactions between the risk or minor allele of each SNP and exposure history of each risk factor (ever vs. never) were assessed with generalized estimating equations. Significant associations were found for the *NOS1* SNPs rs3782218, rs11068447, rs7295972, rs2293052, rs12829185, rs3741475, and rs2682826 (p=0.00083-0.046) and the *NOS2A* SNPs rs2072324, rs944725, rs12944039, rs2248814, rs2297516, rs1060826, and rs2255929 (p= 0.0000040-0.047) using APL and/or PDT in at least one of three family subsets, in which the case reported an age-at-onset less than 40, 45, or 50 years old. There were no significant associations of *NOS3* SNPs with PD. Significant interactions (p<0.05) between pesticides and two of the *NOS1* SNPs (rs12829185 and rs2682826) and between smoking and two of the *NOS2A* SNPs (rs2248814 and rs1060826) were detected. These data support *NOS1* and *NOS2A* as genetic risk factors for PD and demonstrate that their interactions with established environmental PD risk factors may influence susceptibility to PD development.

100

Characterization and replication of a novel locus for late-onset Parkinson's disease detected in a genome-wide association study in an isolated population. *Z. Bochdanovits[1,2], P. Rizzu[1,2], K. Rak[1,2], L. Pardo-Cortes[1,2], P. Heutink[1,2].* 1) Section Medical Genomics, Department of Clinical Genetics, VUMC, Amsterdam, the NetherlandsClinical Genetics, VU Medical Center, Amsterdam, Noord Holland, Netherlands; 2) Center for Neurogenomics and Cognitive Research, VU/VUMC, Amsterdam, the Netherlands.

Genetically isolated populations have two major advantages above general populations when conducting genome-wide association studies: 1- more extended LD allows for using less markers and 2- less genetic heterogeneity, hence a higher relative risk of individual susceptibility alleles. We have performed a genome-wide association study in a young genetic isolate in Turkey for late onset Parkinson's disease. This isolate exhibits increased prevalence of the disease relative to the general population suggesting that susceptibility alleles of relatively high risk are segregating in this homogeneous population. We used the Affymetrix 10K SNPChip to genotype 31 late-onset Parkinson's disease patients and 27 unrelated controls. Strong LD (r2>0.8) was commonly found up to approximately 150kb, hence the 10K SNPChip is sufficient to cover the entire genome in this isolate. Single SNP associations were carried out followed by a permutation test to determine the genome wide significance threshold in this dataset. One SNP, rs1492592, was found to be significantly associated with PD, with an empirical p-value of 4x10-6. Subsequently, 30 additional SNPs covering a 1.1 Mb region surrounding the initial SNP have been tested. Multiple SNPs from this panel confirmed the association. Within 1.5 Mb of the locus, several interesting candidate genes are located, most notably GRIN3A and PPP3R2. A known limitation of using genetic isolates is that the loci detected in one population might not be of relevance in other isolates or the general population. Therefore we screened two additional genetic isolates and the general Dutch population for association with tagging SNPs covering six candidate genes. We confirm our locus by showing that multiple tagging SNPs are significantly associated with late-onset Parkinson's disease in the additional isolates.

101

High-density linkage screen identifies potential dementia loci in the Amish. *L. Jiang[1], J.L. McCauley[1], P.J. Gallins[2], N. Schnetz-Boutaud[1], A.E. Crunk[1], L.L. McFarland[1], D. Fuzzell[1], C. Knebusch[1], M. Creason[2], L. Caywood[2], C.E. Jackson[3], W.K. Scott[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) University of Miami School of Medicine, Miami, FL; 3) Scott & White, Temple, TX.

Although a role for the *APOE* gene in late-onset Alzheimer's disease (AD) is apparent, it accounts for less than half of the susceptibility and thus other genetic variations are likely to be involved. Genetic heterogeneity is a major complicating factor hindering further gene identification in AD, as is evident with the recent findings implicating a role for the *SORL1* gene in AD risk. To minimize heterogeneity, we have been collecting individuals with dementia from the genetically isolated Amish populations in Ohio and Indiana. We have assessed over 1550 individuals who have consented to participate. Through use of the Anabaptist Genealogy Database (AGDB) and its query software PedHunter, we interrogated the family structure of our sample using kinship coefficients. Subsequently, we used the GREFFA program to construct sub-pedigrees, clustering individuals based on kinship scores ≥ 0.0156 (second-cousins), from our complex multi-generational extended Amish pedigree (n=4,220 over 11 generations). We have undertaken a whole-genome SNP linkage screen (Illumina Linkage Panel IVb) using 672 Amish individuals (103 with AD) within 21 sub-pedigrees ranging in total size from 18 to 157 individuals. We performed 2-pt linkage analysis using both dominant and recessive models, on 5,645 SNPs using the Superlink program. Preliminary analysis found 150 SNPs with lod scores ≥1.0. Suggestive linkage to AD was found for 13 SNPs across 12 independent loci (2-pt lod scores ≥2.0: 1q, 2p, 2q, 3q, 4q, 5q, 6q, 7q, 14q, 18q, 20p, and 21q). Two SNPs in strong linkage disequilibrium, on 5q give our highest lod scores (3.88 and 3.72 recessive; 2.41 and 2.65 dominant), in a region independently suggested to be linked to AD. These results provide evidence for multiple AD risk genes with our strongest result providing additional evidence for a novel gene at chromosome 5q.

102

Genome-Wide Association for Late-Onset Alzheimer Disease (LOAD) Confirms Risk locus on Chromosome 12. *G. Beecham[1], E. Martin[1], Y.-J. Li[2], R. Carney[1], M. Slifer[1], J. Gilbert[1], J. Haines[3], M. Pericak-Vance[1].* 1) MIHG, University of Miami, Miller School of Medicine, Miami FL; 2) CHG, Duke University, Durham NC; 3) CHGR, Vanderbilt University, Nashville NC.

The heritability of late-onset Alzheimer disease (LOAD) is ~80%. Despite its strong genetic component, only apolipoprotein E (APOE) has been consistently associated with LOAD. While APOE has a strong effect on LOAD, the risk allele is neither necessary nor sufficient for LOAD. At most, APOE accounts for half of the genetic component of LOAD and the risk allele is not even present in a third of LOAD cases. To identify the remaining risk loci, we performed a genome-wide association (GWA) study for LOAD. Data was generated using the Illumina Infinium platform on 518 cases and 531 controls for 550,000 SNPs. All cases met NINDS-ADRDA criteria for probable or possible AD and all controls were cognitively normal on MMSE exams. The data were analyzed for population substructure using STRUCTURE and Eigenstrat, and numerous quality control tests were used to ensure the integrity of the data. We tested for association using Armitage's Trend test. SNPs on chromosomes 1, 2, 12, 13, 14, and 19 have P-values <0.00001, uncorrected, suggesting multiple regions that need to be investigated in more detail. Not surprisingly, three SNPs in APOE exceeded a genome-wide association FDR of 0.20, and served as positive controls. Most importantly, one additional SNP on chromosome 12 also exceeded this threshold (FDR = 0.172). The Chromosome 12 SNP, RS11610206 (~46Mb), lies within a very narrow linkage peak (LOD score 4.2) that we recently reported using a completely independent dataset. Two potential candidates (45-47Mb) are AMIGO2 involved in neuronal survival and SENP1, involved in the processing of SUMO family genes. The convergence of the previous linkage and current association data provide overwhelming evidence for a risk allele near this SNP.

103

Nonallelic variants of neuronal sorlitin-related receptor (SORL1) associated with distinct Alzheimer disease (AD) processes observed by magnetic resonance imaging (MRI). *K.T. Cuenco[1], K.L. Lunetta[1], L.A. Cupples[1], A. McKee[1], H. Chui[2], C. DeCarli[3], P. St.George-Hyslop[4], R.C. Green[1], C. Baldwin[1], L.A. Farrer[1], MIRAGE Study Group.* 1) Boston Univ.,MA; 2) USC,Los Angeles,CA; 3) UC-Davis,CA; 4) U-Toronto,Canada.

AD is hypothesized to involve neurodegenerative and cerebrovascular disease mechanisms.Recently, associations between AD and SORL1 gene variants in two distinct regions were reported in independent samples from diverse ethnic backgrounds.

We evaluated association of 30 SORL1 SNPs with 4 MRI traits in 55 African American (AA) and 266 Caucasian (CA) sibships from the MIRAGE Study.Measures of general cerebral (GA) and hippocampal (HA) atrophy, white matter hyperintensities (WMH) and overall cerebrovascular disease (CVD) were derived from MRI.Family-based association tests were used to perform single- and 3-SNP sliding window haplotype analyses, adjusting for age at MRI and AD status.In CA, SNPs 8, 9, and 10 were associated with WMH and CVD ($0.0006 \le p \le 0.02$);SNP 20 with GA ($p=0.004$);and SNP 15 with HA ($p=0.003$).WMH, CVD, and GA were associated with haplotypes in the SNP 6-10 region ($0.0002 \le$ global $p \le 0.05$);GA with SNP 3-5 haplotypes ($p=0.00006$);and HA with the SNP 4-6 haplotypes ($p=0.026$).In AA, associations were observed for WMH and CVD with haplotypes spanning SNPs 20-24 and for CVD with haplotypes spanning SNPs 1-4, but results were based on few informative families.Of note, AD risk was previously associated with haplotypes of SNPs 8-10 in groups of CA, Hispanics (HS) and Israeli-Arabs, and with haplotypes of SNPs 23-25 in CA and AA.A SNP 4-6 haplotypes were associated with AD risk in a study of HS.

Results suggest that multiple nonallelic functional SORL1 variants influence AD risk through neurodegenerative and cerebrovascular pathways.We are currently evaluating whether these haplotypes are differentially associated with accumulation of amyloid β in cerebrovascular and cortical regions in extensively characterized brains of ~200 unrelated AD cases and controls.

104

Investigation of genetic susceptibility to Late-onset Alzheimer disease through genomic convergence. *X. Liang[1], M. Slifer[2], E.R. Martin[2], N. Schnetz-Boutaud[1], J. Bartlett[1], B.M. Anderson[1], S. Zuchner[2], J. Gilbert[2], M.A. Pericak-Vance[2], J.H. Haines[1].* 1) Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Miami Institute for Human Genomics, Miami, FL.

With the exception of APOE gene, no universally accepted genetic association has been identified for Late-onset Alzheimer Disease (LOAD). A broad region of chromosome 10 (chr10) has engendered continued interest from both preliminary genetic linkage and candidate gene studies, including VR22/LRRTM3, PLAU and IDE. However, there is a very extensive heterogeneity on chr10. Therefore, we converged linkage analysis and gene expression data using the concept of genomic convergence that suggests that genes showing positive results across multiple different data types are more likely to be involved in AD. We identified and examined 28 genes on chr10 for association with AD in a case-control dataset of 1064 individual Caucasians (506 cases and 558 controls) with substantial clinical information. The cases were all Late-onset Alzheimer disease (minimum age at onset (AAO)≥60 years). Both single marker and haplotypic associations were tested in the overall dataset and 8 subsets defined by age, gender, ApoE status and clinical status. PTPLA showed allelic, genotypic and haplotypic association in the overall dataset. SORCS1 was significant in the overall data sets ($p=0.0025$) and most significant in the female subset ($p=0.00002$). Odds Ratio of SORCS1 in the female subset was 1.7 ($p<0.0001$). SORCS1 encodes sortilin-related VPS10p domain containing type 1 receptor. It is a homologue of SORLA that has been associated with AD by inhibiting the generation of amyloid β peptide (Aβ),one of the hallmarks of Alzheimer disease. SORCS1 is also a substrate of γ-secretase that cuts amyloid precursor protein (APP) and generates Aβ. Genetic variations in PTPLA and SORCS1 may be associated and have modest effect to the risk of AD by affecting Aβ pathway. The replication of the effect of these genes in different study populations and search for susceptible variants and functional studies of these genes are necessary to get a better understanding of the roles of the genes in Alzheimer disease.

105

A major genetic risk factor of periodic limb movements and restless legs syndrome. *H. Stefansson[1], D. Rye[2], A. Hicks[1], H. Petursson[1], A. Ingason[1], T.E. Thorgeirsson[1], S. Palsson[1], T. Sigmundsson[3], A.P. Sigurdsson[3], I. Eiriksdottir[4], L.M. Trotti[2], D. Bliwise[2], J.M. Beck[2], A. Rosen[2], S. Waddy[2], U. Thorsteinsdottir[1], A. Kong[1], J. Gulcher[1], D. Gudbjartsson[1], K. Stefansson[1].* 1) Dept Population Genomics, Decode Genetics, Reykjavik, Iceland; 2) Department of Neurology and Program in Sleep, Emory University; 3) Landspítalinn University Hospital, 101 Reykjavik, Iceland; 4) Clinical Research Center, Nóatún 17, 105 Reykjavik, Iceland.

We have discovered a variant associated to periodic limb movements in sleep (PLMs) and Restless Legs Syndrome (RLS). RLS, a major cause of sleep disruption, is a common neurologic disorder characterized by an irresistible urge to move the legs. PLMs are detectable in most RLS subjects and represent an objective physiologic metric. In an Icelandic RLS discovery sample of subjects with RLS and PLMs, we observed a genome-wide significant association to SNP rs3923809 ($P = 2x10\text{-}9$, OR = 1.8) in an intron of the BTBD9 gene on 6p21.2. This association was replicated in a second Icelandic sample ($P = 4x10\text{-}4$, OR = 1.8) and a U.S. sample ($P = 4x10\text{-}3$, OR = 1.5). For RLS with PLMs, the population attributable risk of this variant is approximately 50%. Association of the variant to PLMs without RLS, and lack of its association to RLS without PLMs suggests that we have identified a genetic determinant of PLMs ($P = 1x10\text{-}17$, OR = 1.9). Ferritin index, a measure inversely related to body iron stores, was increased by 5.5% per A allele of marker rs3923809 (95% CI 1%-10%, P = 0.02). In line with this observation, serum ferritin was decreased by 13% per A allele (95% CI 5%-20%, P = 0.002). The inverse correlation with iron stores is consistent with the suspected involvement of iron depletion in the pathogenesis of the disease. This is the first susceptibility variant discovered for PLMs and RLS, supporting that RLS with PLMs is a genuine syndrome with an ascertainable phenotype and biologic basis.

106

An enhancer in the intron 2 deletion regulates DCDC2 gene expression, and is associated with dyslexia. *H. Meng[1], J.R. Gruen[1], N.A. Cope[1], A. Citterio[2], G. Menozzi[3], M.L. Lorusso[2], M. Molteni[1], Y. Wang[4], J.J. LoTurco[4], C. Marino[2,3].* 1) Dept Pediatrics, Yale Univ, New Haven, CT; 2) Scientific Institute "Eugenio Medea", Dept Child Psychiatry, Bosisio Parini (LC), Italy; 3) CRULRG, Dept Psychiatry, Laval Univ, Québec, Canada; 4) Dept Physiology and Neurobiology, Univ of Connecticut, Storrs, CT.

Dyslexia is the most common neurobehavioral disorder of children. The prevalence is 5-20%, and heritability is 44-71%. We reported a deletion and highly polymorphic purine-rich compound STR (BV677278) in intron 2 of DCDC2 (DYX2) that showed strong TDT-association ($p=0.00002$) with dyslexia phenotypes in 153 families from the US (Meng et al, PNAS, 102: 17053). To test the hypothesis that BV677278 alleles modify DCDC2 expression, we first showed that competitively binding human brain nuclear extract to oligos in BV677278 shifted their electrophoretic mobility in-vitro. Next we showed a 3-fold range of in-vivo enhancer activity among the common BV677278 alleles in luciferase constructs paired with a DCDC2-specific promoter. Finally, to show that the enhancer assays had a significant clinical correlation, we tested an independent cohort of 218 dyslexic families from Italy (151 offspring with reading-related phenotypes). In this cohort, BV677278 alleles without DCDC2-specifc enhancer activity (null and allele 1) in the luciferase constructs, had the strongest association with a dyslexia phenotype, Single Non-word Spelling (QTDT analyses $p=0.004$, estimated additive effect= -0.659). These data confirm the DCDC2 association and dyslexia in a second non-English speaking sample, and show an inverse relationship between BV677278 enhancer activity and dyslexia association.

107

Population structure in European American populations - Impact on the design and analysis of Genome-Wide Association Studies (GWAS). *K. Yu[1], Z.M. Wang[2], Q.Z. Li[1], S. Wacholder[1], R. Hoover[1], D. Hunter[3], S. Chanock[1,4], G. Thomas[1], CGEMS project team.* 1) DCEG, NCI, Rockville, MD; 2) SAIC-Frederick, Frederick, MD; 3) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 4) Pediatric Oncology BRanch, NCI, Bethesda, MD.

Population stratification can lead to bias in estimates of disease association in GWAS due to the existence of population sub-structure differences in cases and controls. The several study designs and correction methods proposed to overcome these difficulties have seldom been evaluated on large datasets to date. The genome-wide scans performed as part of the Cancer Genetic Markers of Susceptibility (CGEMS) has identified the genotypes of over 500,000 SNPs for approximately 4,400 individuals participating in two prospective studies. All are self-described as of European origin. The two studies were conducted independently with different centers of recruitment. The participants from NHS in the study of breast cancer are females working in the medical area; the PLCO participants in the study of prostate cancer are males who volunteered for a trial of cancer screening. Despite these differences, the structure of the populations recruited in PLCO and NHS appears similar, demonstrating two major and one minor significant axis of genomic variation when a set of 6,000 uncorrelated SNPs is used in a principal component analysis. The application of the STRUCTURE program on the combined genotypes of CGEMS and HapMap reveals a small number of individuals whose ancestral origin is shared between Europe Africa or Asia. However, such intercontinental admixture may not account for all the population structure observed in the CGEMS data. By combining cases and controls within and among studies we are exploring potential inflation in false positive rate and loss in statistical power that will result from the use of groups of cases and controls that are recruited independently. Population stratification may cause an inflated false positive rate. There is at least a theoretical possibility that markers appear to be associated with disease only because they are associated with the phenotype of being a nurse or being a trial volunteer.

108

A permutation test for estimating the number of subpopulations using whole-genome SNP data. *K. Bryc, H. Gao, C.D. Bustamante.* Dept Biol Stats and Comp Biol, Cornell Univ, Ithaca, NY.

Population substructure is a potentially confounding factor in whole-genome association mapping. Many current approaches for correcting population stratification require knowledge as to the number of subpopulations, K, in the data (e.g., Yu 2006, Montana and Pritchard 2004). As the availability of large genome-wide marker data sets increases, the need for a computationally efficient method for estimating K grows. Recently, a principal component method, Eigenstrat, generated much interest due to its speed as well as accuracy (Patterson 2006). This algorithm approximates the p-value distribution used to identify the number of significant clusters in the data via the Tracy-Widom distribution. Using coalescent simulations under a litany of demographic scenarios (Hudson 2002), we find that the Tracy-Widom distribution may be a poor fit and result in spurious detection of substructure for a given nominal p-value. The problems potentially stem from two sources: (1) applying PCA analysis to correlated, non-symmetrical, and non-Gaussian data, and (2) general poor-performance of the sample variance-covariance matrices in finite samples. We show that the Eigenstrat algorithm performs as expected on clearly defined subpopulations (no admixture) only when extremely aggressive p-values are chosen. However, in certain population settings, choosing extremely small p-values will result in poor power to detect substructure. In short, our simulations indicate that the choice of a significance level alters Eigenstrat's performance, which affects real world utility. We propose an empirical method for estimating K that does not make any assumptions about the distribution of the data. We replace the Tracy-Widom approximation with permutations of the original data, and find that the bias introduced by resampling is within an acceptable tolerance range. In our comparison of our method to Eigenstrat, we provide general insights into the advantages and limitations of principal component analysis for detecting population structure, as well as implications for association mapping.

109

Informative heterogeneity and failed replication: lessons from genome-wide association data for type 2 diabetes. *M.I. McCarthy[1], T.M. Frayling[2], N.J. Timpson[1,3], M.N. Weedon[2], C.M. Lindgren[1], H. Lango[2], K.S. Elliott[1], J.R.B Perry[2], N.W. Rayner[1], R.M. Freathy[2], A.T. Hattersley[2], E. Zeggini[1], The Wellcome Trust Case Control and UK Type 2 Diabetes Genetics Consortia.* 1) University of Oxford, UK; 2) Peninsula Medical School, Exeter, UK; 3) University of Bristol, UK.

Replication is central to effective follow-up of putative associations emerging from genomewide association (GWA) analyses: signals which fail to replicate are typically dismissed from further evaluation. Recently, we showed that variants within the *FTO* gene were strongly associated with type 2 diabetes (T2D) in both the Wellcome Trust Case Control Consortium GWA scan (n=4862: OR=1.27 [1.16,1.37], $p=2x10^{-8}$) and replication samples (n=9103: OR=1.22 [1.12,1.32], $p=5x10^{-7}$). This T2D-susceptibility effect was mediated exclusively through an impact on adiposity and was not detected in other well-powered GWA scans for T2D which had explicitly (or implicitly) matched cases and controls for BMI.

To explore the impact of ascertainment scheme on the profile of highly-significant findings, we re-analysed the WTCCC scan comparing the same 2938 common controls separately with lean and obese T2D subgroups (each n~968) stratified by median case BMI ($30.3kgm^{-2}$). In the "obese T2D" GWA, *FTO* was clearly the strongest T2D-effect (OR=1.48, $p=1.4x10^{-13}$) with only weak evidence for *TCF7L2* (OR=1.21, p=0.001), even though this was the strongest signal in the combined scan. In the "lean-T2D" GWA scan, the contributions were reversed with the *FTO* association undetectable (OR=1.07, p=0.2) and *TCF7L2* predominant (OR=1.52, $p=1.3x10^{-14}$).

These data clearly demonstrate that: (a) the profile of extreme signals emerging from GWAs can be profoundly affected by the ascertainment scheme; (b) failure to detect replication in other well-powered studies does not always indicate a spurious association; (c) "informative" heterogeneity can deliver valuable mechanistic insights (in this example, identification of adiposity as the factor mediating the *FTO* effect on T2D-susceptibility revealed the functional mechanism); (d) from the point of view of major genetic determinants, lean and obese T2D represent quite distinct phenotypes.

110

Combining SNP genotype data and hybridization intensity to simultaneously detect and test deletions for disease association. *J.R. Kohler, D.J. Cutler.* Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Elucidating the role of copy number variation in complex disease has proven difficult. Showing association between any variant and complex disease requires large study designs and accurate estimates of frequency for the variant. Our program, *microdel*, is the first tool capable of using trio-based SNP genotype data to both detect deletions at high power and assign accurate estimates of the deletion frequency, thereby facilitating association testing. In realistic simulations using 100 trios with 1 SNP per 6 kb, *microdel* can detect deletions as small as 20 kb or as rare as 5% frequency with greater than 80% power. Using this approach on the HapMap 16c data, we report 693 deletions with 253 validated by previous studies.

Our improved version, *microdel v2*, is the first tool capable of combining both genotype data and hybridization intensity to detect deletions in SNP genotyping studies. *Microdel v2* handles inherent error in the methods used to call copy number from hybridization intensity, effectively increasing power to detect real deletions while eliminating false positives as our false positive deletion detection rate is $\sim10^{-6}$ per SNP. Using 1000 trios, *microdel v2* is limited only by SNP density and deletion frequency and in realistic simulations has ~100% power if a SNP falls within the deletion and the deletion has frequency > 0.5%.

Genotyping studies may or may not have family data. Thus, it is important to develop tools capable of analyzing both family-based and unrelated sample data. *Microdel v2* facilitates analysis of unrelated individuals, alone or in combination with family data. To accomplish the former, prior information concerning error rates and missing data rates at each SNP must be available, easily derived from previous applications of *microdel v2* to familial samples or perhaps from clustering characteristics of hybridization intensities. Thus, these tools incorporate all information from SNP arrays into a single framework and enable simultaneous discovery and testing of deletions for disease association using both family-based and unrelated sample designs.

111

Using LD to predict CNVs and test for disease associations. *N. Cardin[1], C. Barnes[2], V. Plagnol[3], D. Clayton[3], M. Hurles[2], P. Donnelly[1], J. Marchini[1] on behalf of the WTCCC CNV Analysis Group.* 1) Dept Statistics, Univ Oxford, Oxford, United Kingdom; 2) Genome Dynamics and Evolution, The Wellcome Trust Sanger Institute, United Kingdom; 3) Diabetes and Inflammation laboratory, Cambridge Institute for Medical Research, United Kingdom.

There has been recent and growing evidence that copy number variants (CNVs) within human populations are a major source of genetic variation. An extremely important aspect of CNVs is that such variation in the genome is a strong candidate for increased risk of disease and reports of CNV associations are starting to emerge in the literature. Detection and typing of copy number variants in humans remains a difficult task and there is currently no consensus on the best analysis methods. This work is focussed on an approach which uses linkage disequilibrium to allow imputation of copy number polymorphisms by drawing on previously called variation in the International Hapmap data. We have shown that this method is highly effective at inferring bi-allelic CNVs using cross-validation to assess performance on a set of 65 bi-allelic CNVs within the Hapmap data. This is a substantial improvement in prediction over simpler approaches: the median r2 using our method was 0.81, while the median for a single-marker approach was only 0.64. The algorithm is extremely fast and this allows imputation to be performed in very large samples. We illustrate the method by applying it to predict copy number and test for CNV associations in all 7 genome-wide studies carried out as part of the Wellcome Trust Case-Control Consortium.

112

A robust statistical method for genome-wide Copy Number Variation association studies. *C. Barnes[1], V. Plagnol[2], N. Cardin[3], J. Marchini[3], D. Clayton[2], M. Hurles[1] on behalf of the WTCCC CNV Analysis Group.* 1) Human Genetics, Wellcome Trust Sanger Institute, Cambridge, UK; 2) Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK; 3) Department of Statistics, University of Oxford, Oxford, UK.

Copy Number Variation (CNV) is pervasive in the human genome, and has previously been demonstrated to be involved in the aetiology of all classes of genetic disease. The functional impact of CNV cannot be captured in its entirety through linkage disequilibrium with SNPs. These two observations motivate the development of efficient statistical methods for performing direct CNV association studies. CNV can be mined from the quantitative allele intensity data underlying the current generation of genome-wide SNP genotyping platforms. We show through simulation that current chi-square testing of CNV association are prone to substantial inflation if underlying quantitative data are noisy, as is generally the case with current technologies. These simulations motivate the development of a more robust methodology for performing CNV association from quantitative data. We present a general statistical framework for performing case-control CNV association studies. This framework entails the fitting of mixture models separately to quantitative data from cases and controls and performing significance testing of the proportions of each population with different copy numbers at a given locus. We show that our method does not produce inflated p values. Moreover, we have integrated genetic models within this association testing framework, thus improving statistical power. The power of this approach is exemplified through the analysis of the Affymetrix 500k data on the ~17,000 samples of the Wellcome Trust Case Control Consortium, which represent two control and seven case populations.

113

Ordered Subset Analysis for Association Mapping. *R.H. Chung[1], S. Schmidt[1], X. Qin[1], X. Lou[1], E.R. Martin[2], E.R. Hauser[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Institute for Human Genomics, Univ of Miami, FL.

Complex diseases are caused by multiple factors such as sequence variants in multiple genes, environmental effects, gene-gene and gene-environment interactions. Genetic heterogeneity refers to different variants resulting in the same disease phenotype. Genetic heterogeneity can reduce the power for complex disease gene mapping since there may be only a portion of families carrying a specific disease susceptibility allele in collected samples. Ordered subset analysis (OSA) is a linkage test that identifies a subset of families that has the strongest linkage signal based on the ranking of covariates. The same strategy can be applied to association analysis to find a subset that has the most informative families. APL is a family-based association test that uses nuclear families with multiple affected sibs and can infer missing parental genotypes properly by accounting for linkage. We developed APL-OSA, which applies the OSA algorithm to the APL statistic. Each family is assigned a covariate based on the covariates for affected sibs in the family and then families are ranked according to their covariates. Each family is added one by one into a set S based on the ranking. Since the APL test statistic is not additive over families, unlike a LOD score for linkage, the APL test statistic is re-calculated each time a family is added into S. The most significant APL statistic, which is noted as the APL-OSA statistic, is chosen after all families have been added into S. The null hypothesis for APL-OSA is that there is no relationship between the family covariate and the APL-OSA statistic. A permutation procedure is used to approximate the distribution for the APL-OSA statistic under the null hypothesis. The permutation procedure randomly orders the families and then performs the APL-OSA analysis as before. We performed a comprehensive simulation study to verify that APL-OSA has the correct type I error rate under the null hypothesis. This simulation study also showed that APL-OSA has greater power than other association tests (APL, FBAT and FBAT with covariate adjustment) in the presence of genetic heterogeneity.

114

Population genomics of human gene expression. *E.T. Dermitzakis[1], B.E. Stranger[1], A. Nica[1], M.S. Forrest[1], A. Dimas[1], C.P. Bird[1], C. Beazley[1], C. Ingle[1], M. Dunning[2], P. Flicek[3], D. Koller[4], S. Montogomery[1], S. Tavare[2], M.E. Hurles[1], P. Deloukas[1].* 1) Department of Informatics, Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Department of Oncology, University of Cambridge, Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK; 3) European Bioinformatics Institute, Hinxton UK; 4) Computer Science Department, Stanford University, Stanford, CA 94305-9010, USA.

Genetic variation influences gene expression, and this can be efficiently mapped to specific genomic regions and variants (e.g. Stranger et al. Science 2007, 315: 848-853). We used gene expression profiling of lymphoblastoid cell lines of all 270 individuals of the HapMap to elucidate the features of genetic variation underlying gene expression variation. A detailed association analysis of over 2.2 million common SNPs per population (5% frequency HapMap) with gene expression identified at least 1348 genes with association signals in cis (FDR=5%)and at least 180 in trans (FDR= 30%). Replication in at least one independent population was achieved for 37% of cis- signals and 15% of trans- signals, respectively. Our results strongly support an abundance of cis-regulatory variation in the human genome. Detection of trans- effects is limited but suggests that cis- regulatory variation may be the key primary effect contributing to phenotypic variation in humans. We have expanded our previous analysis of effects of copy number variation (CNV) on gene expression looking for longer distance cis and trans effects. Trans effects of CNVs on gene expression come in two flavours: i) those where the CNV DNA is trans to the insertion point (e.g. duplicative transposition) but the effect on gene expression occurs in cis; ii) those where the insertion point is local to the CNV DNA (tandem duplication) but there is a trans effect on expression through a biological pathway. We will present new results on cis and trans CNV associations and data to distinguish the two trans scenarios. Finally, we explore a variety of statistical methodologies that provide new insights into gene expression genetics.

115

Searching for Master Regulatory Variants of Gene Expression. *J. Ding, G.R. Abecasis.* Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

Gene transcript levels can serve as an intermediate phenotype which bridges genotypes and more complex organismal phenotypes. Although many examples of cis-regulators of expression have now been mapped, identifying trans-regulators of expression has proved more challenging. Within the framework of genome-wide association studies of gene expression, we develop a method to search for single nucleotide polymorphisms (SNPs) that are associated with mRNA expression levels for multiple genes (master regulatory SNPs). Our approach should increase power to identify regulatory variants that influence gene expression for multiple genes.

While conventional methods assess significance of association for individual SNP-gene pairs by p-values and then highlight SNPs that are significantly associated with large numbers of gene transcript levels, our method proposes a new statistic to summarize all p-values for each SNP. It results in a summary statistic that takes into account both significance levels and the number of association signals simultaneously. In a genome-wide scan, we rank SNPs based on this summary statistic and determine significance by a permutation test. As an example, we apply our method to the gene expression and genotypic data of HapMap subjects (Stranger *et al., Science* **315**, 848 (2007).). We show that our method has advantages over conventional methods. In addition, we find common master regulatory SNPs of gene expression among four study populations. Our study can potentially shed light on the global regulation of gene expression by genetic variants.

116

Common Mitochondrial Haplogroups Do Not Predict Risk of Morbidity, Mortality, or Longevity in the General Population. *M. Benn[1], M. Schwartz[2], B.G. Nordestgaard[3, 4], A. Tybjærg-Hansen[1, 4].* 1) Dept. Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital; 2) Dept. Clinical Genetics, Rigshospitalet, Copenhagen. University Hospital; 3) Dept. Clinical Biochemistry, Herlev University Hospital; 4) The Copenhagen City Heart Study, Bispebjerg University Hospital.

Recently there have been numerous reports suggesting a role for mitochondrial haplogroups in the pathogenesis of common multifactorial disease and in longevity. These studies have in common that they are all case-control studies with a limited number of participants, and therefore are prone to selection bias and have limited power. In the present study, we tested the hypothesis that mitochondrial haplogroups predict risk of morbidity, mortality, and longevity in a large prospective study of a general population of European descent. We genotyped 9254 individuals from the Danish general population, The Copenhagen City Heart Study, for six polymorphisms (mt7028, mt10398, mt11719, mt12308, mt12612, mt15607) defining eight mitochondrial haplogroups, and determined the ability of these haplogroups to predict risk of morbidity, mortality, and longevity with, respectively, 25 and 11 years follow-up. Haplogroup frequencies were: H(45.9%), U(15.9%), T(9.9%), J(9.1%), K(6.2%), V(4.5%), W/I(3.8%), and Z(3.5%). Hazard ratios for hospitalization due to infectious diseases, respiratory disorders, cardiovascular disorders, malignant neoplasms, digestive disorders, musculoskeletal disorders, neuropsychiatric disorders, and miscarriages as a function of haplogroups were not significantly different from the most common haplogroup H. Multifactorially adjusted hazard ratios for death of all causes as well as for major causes of death as a function of haplogroups were also not significantly different from haplogroup H. Finally, longevity defined as percent surviving as a function of age by haplogroups did not differ when comparing each haplogroup with haplogroup H. Our results suggest that mitochondrial haplogroups are not major predictors of morbidity, mortality, or longevity in a general population of Northern European Descent.

117

Syne1 mutations cause a novel form of autosomal recessive pure cerebellar ataxia. *F. Gros-Louis[1], N. Dupré[3], P. Dion[2], M. Fox[4], S. Laurent[2], J.R. Sanes[4], J.P. Bouchard[3], G.A. Rouleau[2].* 1) CHUL Research Centre, Universite Laval, Quebec, PQ, Canada; 2) Centre for the Study of Brain Diseases, CHUM and Ste-Justine Hospital Research Centre, Université de Montréal, Montréal, QC, Canada; 3) Department of Neurological Sciences, CHAUQ - Enfant-Jesus Hospital, Quebec City, QC, Canada; 4) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, 02138, USA.

BACKGROUND: The recessive ataxias are a heterogeneous group of disorders comprised mainly of Friedreich ataxia, ataxia telangiectasia, ataxia with vitamin E deficiency, ARSACS, abetalipoproteinemia, AOA1, and AOA2. We have recently identified a cluster of families with a new recessive pure cerebellar ataxia phenotype that we named ARCA1. METHODS: (1) Clinical history and neurological examination was performed on each affected members of 27 families who originate from Quebec. 2) We conducted a genome-wide scan with 5 families of which 20 individuals were affected. (3) We screened candidate genes within the mapped area. RESULTS: (1) Based on the cases examined, ARCA1 is characterized mainly by: middle-age onset and slow progression; a cerebellar syndrome with dysmetria and wide-based gait; normal nerve conduction studies and severe cerebellar atrophy. (2) Genome-wide scan revealed one marker (D6S476) with a LOD score higher than 3. This linkage was followed up with additional markers and the maximum two-point LOD score was 6.84. (3) Sequencing analysis allowed us to uncover 5 different mutations within SYNE1, one of the biggest genes in the human genome. 4) We have identified 2 additionnal mutations detected amongs a cohort of unlinked recessively inherited ataxias. CONCLUSION: We report a novel form of recessive ataxia in a French-Canadian cohort and show that SYNE1 mutations are causative in all of our kindreds, making SYNE1 the first gene responsible for a recessively inherited pure cerebellar ataxia. Since 7 different mutations have been identified in a relatively homogenous population, we predict that mutations in this gene may be responsible for a significant fraction of all adult-onset autosomal recessive ataxia syndromes with cerebellar atrophy.

118

Oligosaccharyltransferase subunits mutations in non-syndromic mental retardation. *F. Molinari[1], S. Romano[1], F. Foulquier[2], W. Morelle[3], P. de Lonlay[1], P.S. Tarpey[4], J. Teague[4], S. Edkins[4], P.A. Futreal[4], M.R. Stratton[4], M. Partington[5], G. Turner[5], G. Matthijs[2], J. Gecz[6], A. Munnich[1], L. Colleaux[1].* 1) INSERM U781, Hopital Necker, Paris, France; 2) Laboratory for Molecular Diagnostics, University of Leuven, Belgium; 3) UMR CNRS/USTL 8576, Université des Sciences et Technologies, Lille, France; 4) Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton UK; 5) The Gold Service, Hunter Genetics, University of Newcastle, Australia; 6) Department of Genetic Medicine, Women's and Children's Hospital, Adelaide, Australia.

Mental Retardation (MR), defined as an intelligence quotient below 70, is the most frequent handicap among children and young adults. While a large proportion of X-linked MR genes have been identified, only three genes of autosomal recessive non-syndromic MR (AR-NSMR) have been described so far. Here, we report on a new gene involved in an AR-NSMR in two sibs born to first cousin French family. Autozygosity mapping led to the identification of a unique candidate region of 8 Mb on 8p23.1-p22. This interval encompasses the gene TUSC3/OST3 encoding one subunit of the oligosaccharyltransferase (OST) complex which catalyses the transfer of an oligosaccharide chain on nascent proteins, the key step of N-Glycosylation. Sequencing the OST3 gene identified one base-pair insertion in exon 6, c.787_788insC resulting in a premature stop codon, p.N263fsX300, and lead to mRNA decay. Remarkably, screening of an X-linked homologue gene, the OST6 gene, in patients with X-Linked NSMR also identified a missense mutation (c.932T>G, p.V311G) in two boys born from an Australian family. Recent studies of fucosylation and polysialic acid modification of neuronal cell adhesion glycoproteins have shown the critical role of glycosylation in synaptic plasticity (in particular their glycan structures). However, our data provide the first demonstration that a defect in N-Glycosylation can result in NSMR. Altogether, our results demonstrate that fine regulation of OST activity is essential for normal cognitive function development, providing therefore new insights into the understanding of the pathophysiological bases of MR.

119

Identification of mutations causing severe motoneuron disease. *H.O. Nousiainen[1], M. Kestilä[1], N. Pakkasjärvi[1], H. Honkala[1], S. Kuure[2], J. Tallila[1], K. Vuopala[3], J. Ignatius[4], R. Herva[5], L. Peltonen[1,6,7].* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Biochemistry and Developmental Biology, Institute of Biomedicine, University of Helsinki, Helsinki, Finland; 3) Department of Pathology, Lapland Central Hospital, Rovaniemi, Finland; 4) Department of Clinical Genetics, University of Oulu, Oulu, Finland; 5) Department of Pathology, Oulu University Hospital, Oulu, Finland; 6) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 7) The Broad Institute, MIT, Boston, MA, USA.

Motoneurons are affected in several neurological disorders with variable severity and age of onset. The most severe forms of motoneuron disease manifest already in utero and are characterized by arthrogryposis and fetal immobility. Among the lethal arthrogryposes are two entities called LCCS (MIM 253310) and LAAHD, which are caused by the lack of anterior horn motoneurons. Fetuses affected with LCCS present with joint contractures and pulmonary hypoplasia. Histopathological analysis reveals severe atrophy of the anterior horn motoneurons of the spinal cord and muscle atrophy. In LAAHD the neuropathological findings are similar to LCCS, but the degeneration of muscles and the spinal cord is less severe. Both syndromes are inherited autosomally recessively. We previously mapped the LCCS locus to 9q34 in Finnish LCCS families. By monitoring for shared haplotypes of affected individuals, we restricted the critical DNA region to 1Mb between markers D9S1827 and D9S752. We sequenced all 30 genes on this critical DNA region and identified mutations causative of both LCCS and LAAHD in one of them. The mutation-carrying gene is of known biological function and reveals a distinct pathway defective in motoneuron disease. In situ hybridization showed low ubiquitous expression of this gene in mouse embryonic tissues and marked expression in the ventral cell population of the neural tube, from which motoneurons differentiate. In vitro expression studies and analysis of expression array data are ongoing to collect functional evidence of the critical importance of this gene/pathway to normal motoneuron function.

120

Genotype-phenotype analysis in Retinal Vasculopathy with Cerebral Leukodystrophy with 3'-truncating mutations in human 3'-5' DNA Exonuclease TREX1. *A.M.J.M. van den Maagdenberg[1,2], A. Richards[3], J.C. Jen[4], D. Kavanagh[3], D. Spitzer[3], M.K. Liszewski[3], M.L. Barilla-LaBarca[3], G.M. Terwindt[2], Y. Kasai[5], M.G. Grand[6], K.R.J. Vanmolkot[1], P.T.V.M. de Jong[7], M. Dichgans[8], K.E. Kotschet[9], T. Hardy[10], S.F. Nelson[11], R.R. Frants[1], R.W. Baloh[4], M.D. Ferrari[2], J.P. Atkinson[3].* 1) Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Dept Neurology, Leiden Univ Medical Ctr, Leiden, Netherlands; 3) Dept Medicine Rheumatology, Washington Univ, St. Louis, MO; 4) Dept Neurology, UCLA, Los Angeles, CA; 5) Genome Sequencing Ctr, Washington Univ, St. Louis, MO; 6) Dept Opthalmology, Washington Univ, St. Louis, MO; 7) Dept Opthalmology, Academic Medical Ctr, Amsterdam, The Netherlands; 8) Dept Neurology, Klinikum Gross Hadern, Munich, Germany; 9) Dept Neruology, Monasch Medical Ctr, Victoria, Australia; 10) Dept Neurology, Concord Repatriation General Hospital, New South Wales, Australia; 11) Dept Human Genetics, UCLA, Los Angeles, CA.

Our International Consortium set out to identify the molecular defects in patients with Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL,MIM192315), an autosomal dominant microvascular endotheliopathy with middle age onset. The retinal vasculopathy resembles diabetic retinal vasculopathy and delayed post-radiation brain vasculopathy. A large Dutch family and two US families were linked to 3p21. Heterozygous carboxyl-terminal frameshift mutations were identified in TREX1 a ubiquitously expressed 3'-5' repair exonuclease. Frameshift mutations were identified in TREX1 in six additional RVCL families from different parts of the world: in total, five different truncating mutations, including one recurrent mutation present in five unrelated RVCL families. Despite the stereotypic 3'-truncating TREX1 mutations, RVCL families show inter- and intra-familial variation with respect to severity and clinical features, including progressive visual loss, migraine, Raynaud's phenomenon, liver and kidney dysfunction, stroke, and dementia-like features. TREX1 seems involved in maintenance of vascular integrity.

121

Comprehensive analysis of aberrantly spliced exons in myotonic dystrophy type 1 using Affymetrix Exon Array. *Y. Yamashita[1], T. Matsuura[1], J. Shinmi[1], T. Ibi[2], M. Kinoshita[3], T. Kimura[4], O. Yahara[4], K. Sahashi[5], K. Ohno[1].* 1) Div Neurogen & Bioinfo, Nagoya Univ Grad Sch Med, Nagoya, Japan; 2) Aichi Med Univ College of Nursing, Aichi, Japan; 3) Faculty of Health Sciences, Tokyo Metropolitan Univ, Tokyo, Japan; 4) Dept Neurol, National Dohoku Hosp, Asahikawa, Japan; 5) Dept Neurol, Aichi Med Univ, Aichi, Japan.

Myotonic dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophies and affects multiple organs. DM1 is caused by expanded CTG repeats in the 3' untranslated region of the *DMPK* gene located in 19q13.3. In DM1, the expanded CUG repeats form a stem and loop structure and leads to misregulation of trans-acting splicing regulators, such as MBNL1 and CUG-BP1, which then causes aberrant splicing of target exons of these regulators. Although DM1 exhibits diverse symptoms, no more than twenty aberrantly spliced exons have been reported to date. We here attempted to extensively identify aberrantly spliced exons using the Human Exon 1.0 ST array (Affymetrix) by comparing three DM1 and three normal skeletal muscles. We initially used two kinds of commercially available analysis software, but noticed that these pick up a lot of false positives. We then developed our own algorithm, in which exonic probesets only on the NCBI or Ensembl database are considered, and the variability of splice indices in a given gene was taken into account. The splice index is the signal intensity of a given probeset normalized for the expression level of the gene carrying the probeset. We additionally narrowed down candidate exons by the fold-change values and by the t-test scores as in other algorithms. We picked up 13 candidate exons with a stringent condition, and found by RT-PCR that 12 were indeed aberrantly spliced. Using less stringent conditions, we identified a total of 21 aberrantly spliced exons. Eleven of these were found even in other myopathies, whereas ten were unique to DM1. Identification of the entire catalog of aberrantly spliced exons will elucidate molecular mechanisms of diverse symptoms in DM1, and will lead to development of rational therapies to normalize the aberrantly spliced genes.

122

Mutations in the Na^+/H^+ exchanger gene *SLC9A6* cause an X-linked variant of Angelman Syndrome. *K.K. Selmer[1,2], G.D. Gilfillan[1], C.E. Schwartz[3], R.E. Stevenson[3], A.L. Christianson[4], M. Kyllerman[5], T. Egeland[2], M. Kroken[2], M. Mattingsdal[6], K. Eiklid[2], D.E. Undlien[1,2], P. Strømme[7].* 1) Inst. of Medical Genetics, University of Oslo, Oslo, Norway; 2) Dept. of Medical Genetics, Ullevaal University Hospital, Oslo, Norway; 3) JC Self Research Institute, Center for Molecular Studies, Greenwood Center, Greenwood, South Carolina, USA; 4) Division of Human Genetics, University of the Witwatersrand, Johannesburg, South Africa; 5) Dept. of Neuropediatrics, Queen Silvia Children's Hospital, Göteborg, Sweden; 6) Dept. of Medical Informatics, Rikshospitalet-Radiumhospitalet, Oslo, Norway; 7) Dept. of Pediatrics, Ullevaal University Hospital, Oslo, Norway.

Angelman syndrome (AS) is a severe neurological disorder characterized by developmental delay, ataxia, happy demeanor, speech impairment, microcephaly and seizures. The genetic cause of 85-90% of the patients clinically diagnosed with AS is a loss of function of the maternally imprinted gene *UBE3A* on 15q11-13. Linkage analysis on chromosome X identified a locus Xq24-Xq27.3 in a Norwegian family with an X-linked phenotype resembling AS. This region was reported to be linked to a similar phenotype in a large South African family in 1999. Sequencing of candidate genes led to the identification of deletions in the *SLC9A6* gene in both the Norwegian and the South African family. A further 67 males with genetically unexplained clinical AS were sequenced and a nonsense mutation was found in a Swedish patient. The *SLC9A6* gene encodes the organellar Na^+/H^+ exchanger NHE6, which is ubiquitously expressed and is suggested to take part in regulation of endosomal pH and Na^+ concentration. The complete function of this cation exchanger and how it can cause a AS like phenotype when function is impaired, remains to be explored. Both *UBE3A* and *SLC9A6* are involved in the intracellular protein processing pathway, but if they interact or affect a common pathway is currently unclear. In conclusion: Mutations in the *SLC9A6* gene cause an X-linked mental retardation syndrome similar to AS in two families and one sporadic patient of different geographical origin. Functional studies to address the potential consequences of NHE6 deficiency are ongoing.

123

Mutations in UPF3B, a member of the nonsense mediated mRNA decay surveillance complex, cause Lujan-Fryns and FG phenotypes and non-syndromic X-linked mental retardation. *J. Rodriguez[1*], P.S. Tarpey[2*], L.S. Nguyen[3*], F.L. Raymond[4*], A. Hackett[5], L. Vandeleur[3], R. Smith[4], C. Shoubridge[3], S.S. Bhat[1,9], M. Corbett[3], M.E. Porteous[6], G. Hoganson[7], D. Superneau[8], G. Turner[5], R.E. Stevenson[1], C.E. Schwartz[1], P.A. Futreal[2], M.R. Stratton[2], J. Gécz[3], A.K. Srivastava[1], *Contributed equally.* 1) Greenwood Genet Ctr, Greenwood, SC, USA; 2) Cancer Genome Project, Wellcome Trust Sanger Inst, Hinxton UK; 3) Dept of Genet Med, Women's and Children's Hosp., N. Adelaide, Australia; 4) Cambridge Inst of Med Res, Cambridge UK; 5) GOLD Service, Hunter Genet,Waratah NSW, Australia; 6) SE Scotland Genet Service, Edinburgh, Scotland; 7) Med Genet, Rockford Mem. Hosp., Rockford, IL, USA; 8) Genet Services of LA, LLC, Baton Rouge, LA 70884 4260, USA; 9) Pres add: Inst of Genet Med, Johns Hopkins Med Inst, Baltimore MD, USA.

We have recently identified two frameshift mutations, one nonsense mutation, and one missense mutation at a conserved amino acid in UPF3B in 4 of 368 families with putative X-linked mental retardation (PS Tarpey et al. submitted). Two families were diagnosed with Lujan-Fryns syndrome, one family had a FG phenotype and one family had non-syndromic X-linked mental retardation. Three of the mutations lead to the introduction of a premature termination codon and subsequent low levels of mutant UPF3B mRNA. Western blot analysis, using patient lymphoblastoid cell line protein lysates, revealed an absence of the wild-type or predicted truncated protein in two families with the frameshift mutations. A low level of the predicted truncated protein was detected in the family with the nonsense mutation. The family with the missense mutation showed apparently normal to mild overexpression of both UPF3B transcript and protein. UPF3B is an important component of the nonsense mediated mRNA decay (NMD) surveillance machinery and is expressed in a variety of tissues including brain and at different developmental stages. Our results show that NMD is only partially compromised in absence of UPF3B protein function and thus point to at least partial redundancy of NMD pathway. More importantly, the data therefore directly implicate mutations in a component of the NMD complex in human disease.

124

Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with peripheral RPE atrophy. *C. Chakarova[1], M. Papaioannou[1], A. Shah[1], N. Waseem[1], I. Lopez[2], B. Wissinger[3], E. Zrenner[3], C. Ponting[4], R. Koenekoop[2], S.S. Bhattacharya[1].* 1) Molecular Genetics, Institute of Ophthalmology, London, UK; 2) The McGill Ocular Genetics Laboratory, McGill University Health Centre, Montreal, Canada; 3) Department of Pathophysiology of Vision and Neuro-Ophthalmology, University Eye Hospital, Tubingen, Germany; 4) The MRC Functional Genetics Unit, University of Oxford, Department of Human Anatomy and Genetics, Oxford OX1 3QX, UK.

Purpose: To identify the disease-causing mutation in a large French-Canadian family with an autosomal dominant retinal degeneration and perivascular RPE atrophy which maps to 9p (RP31). To explain the molecular basis for photoreceptor cell death due to mutations in the RP31 gene. Methods: Linkage analysis was used on twenty-six individuals from the French-Canadian family after standard ophthalmological evaluations. To identify the disease causing mutation, 53 genes within the critical interval of 14 Mb underwent direct genomic sequencing. A variety of molecular and cell biological techniques were used (bioinformatics, Western blot, immunohistochemistry) in order to explore the disease mechanism involved in this particular retinal degeneration. Results: Here we report the identification of the gene for autosomal dominant retinitis pigmentosa (RP31). TOPOisomerase I - binding - RS protein (TOPORS) is mutated in two families of different origins (French and German). Both mutations cause frameshift leading to premature stop codon and are thus predicted to result in truncated proteins. RNA expression studies indicate the gene to be ubiquitously expressed. Functional studies show a specific cellular localization of TOPORS in the area of the connecting cilium of photoreceptor cells in human and mouse retina. Conclusions: This is the first report of a ubiquitous and multifunctional gene causing only retinitis pigmentosa. Due to the nature of the mutations (leading to truncated protein) we suggest haploinsufficiency as the disease mechanism.

125

Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency amongst the Amish. *H. Cross[1], K. Kalidas[2], K.G. Zahka[3], J. Tumbush[4], B.B. Keller[5], C. Galambos[6], K. Gurtz[7], M.A. Patton[2], A.H. Crosby[2].* 1) Department of Ophthalmology, University of Arizona School of Medicine, Tucson, AZ; 2) Clinical Developmental Sciences, St George's University of London, London, United Kingdom; 3) Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH; 4) Das Deutsch Center (DDC) Clinic for Special Needs Children, Middlefield, Ohio; 5) Department of Pediatrics, Hospital of Pittsburgh of UPMC, Pittsburgh, PA; 6) Department of Pathology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA; 7) Windows of Hope Genetic Studies, Kimmeridge Trail, OH.

Familial hypertrophic cardiomyopathy (HCM) is a leading cause of sudden cardiac death amongst young and apparently healthy individuals. Mutations within nine genes encoding sarcomeric proteins have so far been identified to act in an autosomal dominant fashion. We have identified an autosomal recessive form of HCM within a group of Amish children that is associated with very poor prognosis and death within the 1st year of life. Affected patients experienced progressive cardiac failure despite maximal medical therapy. Post-mortem histology revealed myofiber disarray and myocyte loss consistent with refractory clinical deterioration in affected infants. Assuming that a founder mutation was responsible we conducted a genome-wide screen for linkage and identified an autozygous region of chromosome 11 which cosegregates with the infant cardiac phenotype. This region contained the MYBPC3 gene, which has previously been associated with autosomal dominant adult onset HCM. Sequence analysis of the MYBPC3 gene identified a novel splice site mutation in intron 30 which was homozygous in all affected infants. All surviving patients with the homozygous MYBPC3 gene mutations (3330+2T>G) have been treated by orthotopic heart transplantation.

126

A novel gene is disrupted in a patient with balanced translocation t(3;X)(q12.3-q22.3) associated with Cerebral Cavernous Malformations. *F. Gianfrancesco[1], T. Esposito[1], S. Penco[2], V. Maglione[3], F. Letizia[1], C.L. Liquori[4], M.C. Patrosso[2], O. Zuffardi[5], A. Ciccodicola[1], D.A. Marchuk[4], F. Squitieri[3].* 1) Institute of Genetics and Biophysics, Italian National Research Council,Naples, Italy; 2) Medical Genetics Laboratory, Niguarda Ca' Granda Hospital, Milan, Italy; 3) Neurogenetics Unit, IRCCS Neuromed, Pozzilli (IS), Italy; 4) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA; 5) Department of Pathology and Medical Genetics, University of Pavia, Pavia, Italy.

Cerebral Cavernous Malformations (CCM) exhibit autosomal dominant inheritance, and accounts for 10-20% of all cerebrovascular abnormalities with prevalence in the general population between 0.1% and 0.5%. The past few years have seen rapid advances in our understanding of the genetics and molecular biology of CCM with the identification of the CCM1, CCM2, and CCM3 genes. A discrepancy in the frequencies of mutations in the three CCM genes between the values originally predicted by linkage in families and the values obtained by DNA mutation-analysis screens of probands suggest that another CCM gene exists on the chromosome 3. Recently, we have recruited a patient with a X/3 balanced translocation that exhibits CCM. We refined the critical region to an interval of 200-kb and identified the interrupted gene. Quantitative real-time PCR was used to quantify the amounts of this transcript in the lymphoblastoid cell line of our patient. We detected that the mRNA expression level of this gene is consistently decreased 2.5 fold versus control (P= 0.0006) with allelic loss of gene expression. Because CCM2 is required as a scaffold for MEKK3-mediated p38 MAPK phosphorylation during osmotic shock by sorbitol, we also investigated a possible role for our protein in the p38 MAPK pathway. We observed that the phosphorylation status of activated p38 MAPK was altered in response to mechanical stress associated with medium change. These data indicate that this protein may be part of the complex signaling pathway that, when perturbed, causes abnormal vascular morphogenesis in the brain, leading to CCM.

127

Moral distress and burnout among clinical genetics service providers (GSPs). *B.A. Bernhardt[1], K. Kolodner[2], G. Geller[2].* 1) Medicine, University of Pennsylvania, Philadelphia, PA; 2) Johns Hopkins School of Medicine, Baltimore, MD.

In providing patient care, GSPs may experience moral distress through threats to integrity and personal identity. To investigate the nature and consequences of moral distress, we surveyed 386 GSPs. The survey included the Maslach Burnout Inventory (MBI) and 38 items associated with moral distress identified through focus groups of genetic counselors (GCs), MD clinical geneticists (MD) and nurses in genetics. Of the 173 responses to date, the majority of GSPs reported experiencing a moderate or high degree of distress associated with 3 of the moral distress items: experiencing sadness or grief, and feeling inadequate about how to help a patient. Over one-third experienced similar distress by feeling frustrated by unreasonable patient expectations, worrying that a patient's decision will come back to haunt them, feeling unsupported by colleagues, and disrespecting a colleague's approach to patient care. Other common sources of distress differed by provider type. MDs were more likely to report distress from feeling angry at (41%) or disliking a patient (26%). GCs reported more distress from feeling disrespected by MDs (39%), feeling that they were overly optimistic (42%) or pessimistic (37%) in the information given patients, and difficulty reconciling their faith with being a GSP (24%). Nurses reported more distress from feeling disrespected by GCs (33%), and from not being able to make a recommendation because of emphasis on patient autonomy (28%). GCs had the highest mean MBI scores, followed by MDs and then nurses. The majority of GCs and MDs scored high on the depersonalization subscale. Many items on the moral distress inventory were correlated with burnout. 21% of MDs, 22% of GCs and 6% of nurses reported thinking about leaving patient care. Some sources of distress experienced by GSPs, especially those related to disrespect, reconciling faith, and inadequacy with regard to assisting patients with decision-making may be unique to genetics. Interventions are needed to assist GSP to minimize the consequences of moral distress so as to decrease burnout and increase the satisfaction derived from providing patient care.

128

Transitioning to self-management (TSM): Experience with Marfan syndrome (MFS). *R.E. Pyeritz[1], B.A. Bernhardt[1], E. Giarelli[2].* 1) Medicine, Univ PA, Philadelphia, PA; 2) Sch of Nursing, Univ PA, Philadelphia, PA.

Self-management requires disease knowledge, adherence to recommendations, and health-promoting behaviors. TSM occurs as parents transfer to the child management responsibility in partnership with providers. This study describes the process of TSM in people with MFS, and offers provider recommendations. A sample of 107 (15 providers, 39 parents and 53 MFS patients, 14-35yrs) were recruited through a genetics clinic and the National Marfan Foundation, and interviewed by phone. MFS patients described TSM as: becoming knowledgeable about MFS, their health history, and the health care delivery system; the gradual acceptance of MFS and adoption of realistic expectations and behaviors to monitor and promote health, and follow provider recommendations. Patients engaged in frequent self-surveillance including awareness of heart beat, attention to body pain and side effects of medications. Five concurrent shifts lead to successful TSM: perception (invisible malady to one perceived), orientation (present to future), ownership (parent to child), reasoning (competitive to cooperative), and sphere (private to public). Providers can facilitate a shift in perception by teaching patients how to "listen to their bodies", understand the relevance of symptoms, and when medical attention is required. A shift in orientation is facilitated by discussing future medical needs, reproductive plans, career, and impact of behaviors on future health. A shift in ownership is facilitated by encouraging parents to allow the child greater role in their health care, and the provider modeling respect for the child's opinions and concerns. A shift in reasoning is encouraged when a provider attends to the patient's perspective and seeks compromise, thereby transferring control. A shift in sphere is facilitated by encouraging the child to interact with others with MFS, and to discuss MFS with others. Transitioning is more than the transfer to adult care, and involves gradual changes in knowledge, attitudes and behavior that are influenced by parents and providers. TSM is an incremental and life-long series of adaptations to maintain control and hope in the context chronic illness.

129

Social support, communal coping and psychological status in sisters in Hereditary Breast and Ovarian Cancer (HBOC) families. *J.A. Peters[1], L. Koehly[2], L. Hoskins[1], N. Kuhn[2], A. Letocha[2], R. Kenen[3], J. Loud[1], M.H. Greene[1].* 1) Clinical Genetics Branch, DCEG, NCI/NIH/DHHS, Rockville, MD; 2) SBRB/NHGRI/NIH/DHHS, Bethesda, MD; 3) The College of New Jersey, Ewing, NJ.

Adult sisters in HBOC families often undergo genetic counseling and testing together but the social context of their long-term adjustment to genetic information is rarely a focus of research. We conducted a quantitative, descriptive, cross-sectional study of 65 sisters from 31 HBOC families within a larger Breast Imaging Study (NCI-01-C-009) for high risk women. The aims were to consider how the size of the sisters' social networks and which communal coping measures related to psychological distress. We performed social network analyses using data from the Brief Symptom Inventory-18 to determine anxiety, somatization and depression and the Colored Eco Genetic Relationship Map (CEGRM) to identify family and non-family members of participants' social support networks. Intra-family correlation coefficients suggest that these sisters share perceptions of breast cancer risk and worry, but not ovarian cancer risk and worry. Additionally, sisters indicated shared levels of anxiety and somatization, but not depressive symptoms. Communal coping indices of shared support resources were related to anxiety and somatization, with larger numbers of shared emotional supports associated with lower levels of anxiety and lower levels of somatization. Having more shared informants regarding cancer risk was positively associated with somatization. Having a large emotional support network was negatively associated with anxiety. Participants with lower depression scores had more persons playing multiple support roles and fewer individuals providing tangible assistance. In summary, we found that quantity, function, and communal aspects of social exchanges are differentially correlated with self-reported anxiety, somatization and depression. Understanding the specific ways in which quality, quantity and types of supportive relationships impact sisters' well-being will allow us to develop appropriate management strategies to help cancer-prone families better adjust to their cancer risk.

130

Reimbursement for genetic counseling and related services. *J. Dungan, C. Yates, A. Trivedi, T. Bamlett Sherman, L. Shulman.* Dept OB/GYN, Northwestern Univ Sch of Med, Chicago, IL.

Introduction: The AMA CPT Editorial Board and CMS recently introduced a new CPT code (96040) to cover genetic counseling (GC) visits provided by counselors only. Previously, GC-related consultations were billed as Evaluation & Management (E&M) visits that necessitated presence of a physician to qualify for payment from most third-party payors. We sought to determine to what extent counselor-only visits at our center billed with this new CPT code have been reimbursed.

Methods: We reviewed the billing statements and account information from patients who presented to our center seeking GC or related consultations during the months of January-March 2007. Services were provided by counselors and/or physicians in the Division of Reproductive Genetics, and include prenatal diagnosis and screening, as well as consultations for hereditary gynecologic cancer families. We categorized visits by CPT code and calculated the mean reimbursement for each code. Only CPT codes used on more than 5 occasions were evaluated.

Results: During the interval reviewed, we billed for 372 visits using CPT code 96040. From this group, excluding those visits still awaiting payment, third-party insurers did not cover any portion of the charges for 3.2% (12/372). Average reimbursement for 96040 was $53.87. In the small number of instances where multiple submissions of 96040 were made because of a prolonged GC visit, payment for each submission was the same. Mean reimbursement for other E&M services were: 99211-$17.25, 99212-$31.57, 99213-$61.00, 99214-$101.65, 99202-$59.96, 99241-$67.79, 99242-$113.17, 99243-$146.25, $99244-$243.68, 99245-$249.38.

Conclusions: Most private insurance carriers are paying for GC-only visits at our center. Although these visits do not require physician presence, reimbursement for physician-attended E&M consultations is much higher for a comparable period of time. Individual centers will need to determine what approach to provision of GC services will best suit their own circumstances, and what personnel are needed to deliver those services.

131

Towards recommendations for genetic counselling. *H. Kaariainen[1,2], E. Rantanen[2], M. Hietala[2], U. Kristoffersson[3], I. Nippert[4], J. Schmidtke[5], J. Sequeiros[6].* 1) Dept of Molec Med, National Public Health Institute, Helsinki, Finland; 2) Dept Med Genet, University of Turku, Finland; 3) Dept Clin Genet, University Hospital of Lund, Sweden; 4) Dept Hum Genet, Westfaelische Wilhelms-University Muenster, Germany; 5) Inst Hum Genet, Hannover Medical School, Germany; 6) ICBAS and IBMC, University of Porto, Portugal.

As genetic tests are increasingly offered across the borders, EuroGentest, a NoE aiming at improving the quality of testing, also aims at harmonizing the quality of genetic counselling. To achieve this, we analyzed European and global guidelines and policies related to genetic counselling, as well as some relevant American and other documents. The most prominent topics (mentioned in 30/56 of the documents) were considered to form the ideal of genetic counselling. This consisted of (1) appropriately trained professionals, who understand well genetics and its ethical implications; (2) relevant and objective information; (3) assurance of counselee's understanding; (4) psychological support; (5) informed consent; (6) confidentiality; (7) considering familial implications; (8) dealing properly with potential discrimination; and (9) assuring autonomous decision-making. We also investigated regulations and practices related to genetic counselling in European countries by an electronic survey among the National Societies of Human Genetics in 29 countries and contact persons in the 9 countries where a Society could not be traced. There is legislation related to counselling in 13 and guidelines in 21 countries, 70% of respondents hoped for more regulation. The topics most often covered in the regulations were counselling in the context of prenatal testing, informed consent, confidentiality, training of the counsellors, and non-directiveness. The seldom-covered topics were counselling in the context of predisposition testing for multifactorial diseases, duty to recontact the patient afterwards, and counselling persons from ethnic minorities. Based on this data, as well as two expert workshops and consultation rounds among human genetic societies, we are finalizing European recommendations for genetic counselling related to genetic testing.

132

Telegenetic Use in the United States: Results of 2007 NCC Telegenetics Workgroup Survey. *H.C. Andersson[1], B. Butler[2], J. Benkendorf[3,4], B. Bowdish[5], M. Watson[3,4].* 1) Hayward Genetics Ctr, Tulane Univ Medical Ctr, New Orleans, LA; 2) Genetic Counseling, Univ. Arkansas Med Sciences; 3) National Coordinating Center for National Coordinating Center for Regional Genetics and NBS Collaboratives; 4) American College of Medical Genetics; 5) Digital Union, LLC.

As supply of genetic specialists falls short of demand, a mechanism for increasing access to services is telecommunications. Telemedicine makes clinical services available at a distance, allows real-time conferencing of multiple parties, and online education. As technology has improved in quality, become more user-friendly, and decreased in cost, no information exists about current use of telecommunications in: 1) providing clinical genetic services; 2) meeting for research and administration; 3) providing genetics education; and 4) laboratory planning. To understand current telegenetic use, we developed a web-based survey and emailed it to ~1350 ACMG members and to public health communities through the Regional Genetics and NBS Collaboratives. After 2.5 weeks (of the 4-week survey period), > 390 surveys were completed. Respondents were genetic counselors (32%), MD geneticists (29%), PhD geneticists (14%) and various other genetic professionals in academic medical centers/university hospitals (52%), state health departments (10%), private community hospitals (7%), commercials laboratories (6%) and other health related sites. 44% of respondents had used telegenetics to provide some genetic services or education. Of the 57% who responded they never used telegenetics, 79% indicated they see telegenetics useful for their activities but only 16 % had plans for telegenetics. The greatest barriers identified by nonusers were unavailability of technology (54%), high cost (22%), and institutional barriers (20%). Some telegenetics users have systems that cross state lines (45%) and even national boundaries (17%). Survey results include data on encryption protocols, legal barriers, funding mechanisms, and specific telegenetic use, such as technical equipment, frequency of use, and barriers to sustainability. The results of this survey will be used to tailor recommendations for collaboration, education, public policy and funding.

133

Predictive Testing for Multiple Genetic Variants in Common Diseases: A different ELSI landscape from testing for traditional genetic diseases. *A.C.J.W. Janssens[1], M. Gwinn[2], C.M. van Duijn[3], M.J. Khoury[2].* 1) Department of Public Health, Erasmus MC, Rotterdam, Netherlands; 2) National Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA; 3) Department of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, Netherlands.

Unraveling the genetic origins of multifactorial diseases is expected to lead to personalized medicine, in which prevention and treatment are based on tests for multiple genetic variants (genomic profiles). Balancing the enthusiasm for this development is concern about ethical, legal, and social implications (ELSI) of genomic medicine. These implications may not be the same as for genetic testing in monogenic disorders. We conducted a simulation study to evaluate the predictive value and inheritance patterns of genomic profiles. We simulated genomic profiles and disease status for 1 million persons. Profiles included 40 genetic variants. Frequencies of risk genotypes varied in separate scenarios from 1% to 50% and odds ratios from 1.1 to 3.0. Population disease risk was 10%. Results were compared with genetic tests for Huntington's disease and hereditary cancers and their implications for the discourse on ethical, legal and social issues were considered. While genetic tests for monogenic disorders typically have two outcome results (high and low risk), genomic profiling yields a continuum of possible risk estimates, with minimal risk differences between profiles. When each variant in the profile segregates independently, the probability of inheriting the same at-risk profile is very low. It should be anticipated that genetic variants may decrease the risk of some diseases and at the same time increase the risk of others. The wide variation in genetic profiles and their interpretation should reduce the potential for discrimination and stigmatization and could affect privacy issues for family members (e.g., the right not to know). Our simulation studies show that the predictive value and inheritance of genomic profiles differ fundamentally from those of single, high-penetrance genetic variants. These differences have implications for the discourse on ethical, legal, and social issues of genomic profiling.

134

High throughput testing for common and recurrent mutations responsible for Mendelian diseases. *J.W. Belmont[1], R. Chen[1,2], L. Nazareth[2], D. Stockton[3], W. Craigen[1], C. Shaw[1], A. Beaudet[1], J. Lupski[1], R. Gibbs[1,2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 3) Children's Hospital of Michigan, Detroit, MI.

Clinical testing for common and recurrent mutations can be used for disease diagnosis, presymptomatic diagnosis, carrier testing, and disease risk analysis. We wished to examine the technical feasibility of using a high throughput genotyping platform to test large numbers of disease-causing mutations. We selected 114 disease genes and used information from a variety of databases and from the published literature to select 1238 validated common/recurrent mutations. Single nucleotide substitutions leading to nonsense, missense, splice site alterations, and small indels were included. We employed Molecular Inversion Probe chemistry (Affymetrix) which allows 4-color interrogation of single base positions in a highly parallel assay format. Indel assays were designed so that there were separate assays for the expected junctional base positions. To aid in QC, each single assay was duplicated within the entire multiplex assay. We genotyped all 270 HapMap subjects and an additional set of 96 European American samples. A subset of samples were genotyped in duplicate. These results demonstrated the general feasibility of genotype calling when the variant position is rare in the sample. The results also demonstrated the feasibility of indel genotyping assays on this platform. Overall, there was 85% assay conversion. Selecting the subset of assays that gave 95% completion the duplicate sample concordance was >99.8% and the on-chip duplicate assay concordance was 98.9%. A total of 197 mutations were confirmed in our subject sample with individuals bearing 0-6 different mutations. These results suggest the possibility of using a single assay to test for very large numbers of known disease causing mutations. Many detailed questions concerning the distribution of deleterious mutations in individuals and populations, assay quality standards and ethical implementation of such testing must be addressed in future research on this concept.

135

Model for disclosure of research genetic testing results. *S. Adam[1], D. Avard[4], P. Birch[1], P. Eydoux[3], B. Knoppers[4], S. Langlois[1], M.A. Marra[2], J. Samuel[4], J.M. Friedman[1].* 1) Dept Med Genet, Univ BC; 2) Genome Sci Ctr, BC Cancer Agency; 3) Dept Path & Lab Med, Univ BC. (1-3 Vancouver, BC); 4) Centre de recherche en droit public, Univ of Montreal, Que.

Genetic research in children raises many ethical concerns, especially if clinical implications of the results are difficult to interpret. We have recently conducted a study of array genomic hybridization (AGH) in children with mental retardation (MR) of unknown etiology despite clinical genetics evaluation and conventional cytogenetic analysis. Although the families (and their referring clinicians) were informed that our research results may not be clinically interpretable, identifying a precise cause for the child's MR was almost always the major reason for study participation. We found potentially pathogenic but previously unreported de novo submicroscopic CNVs in approximately 15% of the first 200 patients. In response to repeated requests to provide our research results to the families, we developed a model for disclosure of research AGH results that integrates the research, clinical and laboratory teams. Our disclosure process involves the collection of fresh blood samples and confirmation of the research result by a clinical laboratory using an established clinical method. Typically, locus-specific FISH is done with a probe chosen on the basis of the AGH findings. Thereafter, the research team consults with the family's geneticist and genetic counsellor regarding the limitations and interpretation of the findings. Considerations include the occurrence of similar CNVs or cytogenetic abnormalities in reported cases and available databases, the presence of apparently benign CNVs in the region, the genetic content, and genotype-phenotype correlations. The degree of uncertainty associated with interpretation of the CNV as pathogenic for the child's MR is explicitly considered. The family is then offered genetic counselling by the clinical geneticist and genetic counsellor who enrolled the child in the study. As clinical applications of new genetic technologies are developed, a comprehensive model for disclosure of research results to families participating in clinical studies will be critical.

136

The challenge of counseling families with results of unclear clinical significance by array CGH as illustrated by duplications of the BCR gene region at 22q11.23. *J. Coppinger[1], D. McDonald-McGinn[2], E. Zackai[2], K. Shane[3], J.F. Atkin[3], R. Leland[4], K. Schmidt[5], H. Feldman[5], W. Cohen[5], J. Phalin[6], B. Powell[6], B.C. Ballif[1], B.A. Bejani[1,7,8], T. Shaikh[2], S. Saitta[2], L.G. Shaffer[1].* 1) Signature Genomic Laboratories, LLC, Spokane, WA; 2) Children's Hospital of Philadelphia, PA; 3) Columbus Children's Hospital, The Ohio State University, Columbus, Ohio; 4) Cheyenne Children's Clinic, Cheyenne WY; 5) Children's Hospital of Pittsburgh, PA; 6) Children's Hospital Central California, Madera, CA; 7) Sacred Heart Medical Center, Spokane, WA; 8) Health Research and Education Center, Washington State University, Spokane, WA.

Deletions of the BCR locus at 22q11.23 have recently been described in individuals with mental retardation and congenital anomalies. Because these deletions are mediated by low-copy repeats, which are located distal to the 22q11.21 DiGeorge/VCF microdeletion region, duplications of the BCR locus are expected to occur with equal frequency. We have processed over 13,000 clinical array CGH cases and have detected 11 duplications of 22q11.23. In seven cases, the duplication was also detected in a parent, two of whom reportedly have learning problems or developmental delay. Of the remaining four cases, one is *de novo*, and three await parental studies. The *de novo* case has an ~368 kb duplication. Four cases (three familial, one awaiting parental studies) have a duplication of an overlapping ~677 kb region, and six cases (two familial/normal parents, two familial/delayed parent, and two awaiting parental studies) have a duplication of an overlapping ~848 kb region. Medical records, available for seven patients, reveal shared characteristics but also several examples of contradicting clinical features (e.g. macrocephaly vs. microcephaly, upslanting vs. downslanting palpebral fissures). The variable phenotypes and preponderance of familial cases necessitate further studies. Increased clinical use of array CGH will result in more frequent genetic counseling dilemmas. Using the 22q11.23 duplication cases as a model, we present counseling strategies for array CGH results of unclear clinical relevance.

137

Identification of susceptibility genes for Myocardial Infarction following the combined analysis of two large genome scans in German and UK samples. *P. Deloukas on behalf of CARDIOGENICS.* Human Genetics, Wellcome Trust Sanger Inst, Cambridge, United Kingdom.

CARDIOGENICS (EU F6 programme) has combined the data generated by the Wellcome Trust Case Control Consortium (WTCCC) which tested 500,000 SNPs (Affymetrix) in 2000 British Caucasian samples with coronary artery disease (CAD; BHF collection with family history; 70% MI cases)and 3000 controls (1500 each from the 1958 Birth Cohort and the UK Blood Services collection), and the German MI (GMI) study which scanned 875 MI cases with family history and 1644 controls (MONICA/KORA Augsburg survey) with the same SNP array. Analysis by the WTCCC at the level of single marker tests (469,557 pass QC) yielded six loci with significant association signals ($p < 10\text{-}5$) for CAD. The strongest signal was in the CDKN2A/2B locus on 9p21.3 (p 1.79E-14 for the trend test - OR 1.47). The same locus has an independent signal in T2D (WTCCC, DGI, FUSION). SNPs with $p<10\text{-}3$ for association to CAD in the WTCCC were assessed for the false positive report probability (FPRP; modification of method by Wacholder et al.) and those with FPRP <0.5 were further tested for replication in the GMI study. The CDKN2A/2B locus and two further ones on 2q36.3 and 6q25.1 replicated with nominal p values <10-3. A combined analysis of the two scans revealed four additional loci that had not exceeded the 10-5 threshold in WTCCC analysis. In total, we identified 11 loci with significant evidence for association corresponding to SNPs in both intra- and intergenic regions, no evidence of a coding SNP as yet. We conducted a fine mapping experiment in three of the loci interrogating 20 (9p21.3), 31 (6q25.1) and 30 (5q21.1) tagSNP that capture all known common variants in dbSNP for the respective loci. We identified two variants with additional signal to the lead SNP rs1333049 in the UK sample. The CDKN2A/2B locus harbours a non-coding RNA transcript with expression in many tissues including heart. Combined with the single marker and haplotype analyses performed on all loci we selected a total of 40 SNPs for replication in 8000 cases and 8000 controls. Our findings will be presented at the meeting.

138

A Sequence Variant Adjacent to *CDKN2A* and *CDKN2B* Affects the Risk of Atherosclerosis in Several Vascular Beds. *A. Helgadottir[1], K.P. Magnusson[1], S. Gretarsdottir[1], G. Thorleifsson[1], A. Manolescu[1], K. Kostulas[2], R. Pola[3], B. Lindblad[4], G. Tromp[5], N. Sakalihasan[6], R.E. Ferrell[7], J. Hillert[2], J. Powell[8], H. Kuivaniemi[5], E. Valdimarsson[9], S.E. Matthiasson[9], G. Thorgeirsson[9], J.R. Gulcher[1], A. Kong[1], K. Stefansson[1].* 1) deCODE Genetics, Reykjavik, Iceland; 2) Karolinska University Hospital, Huddinge, Sweden; 3) A. Gemelli University Hospital, Rome, Italy; 4) University Hospital MAS, Malmö, Sweden; 5) CMMG, Wayne State University,Detroit, MI; 6) University of Liege, Belgium; 7) Department of Human Genetics, University of Pittsburg School of Public Health, Pittsburg, PA; 8) Imperial College, London, UK; 9) Landspitali, University Hospital Reykjavik, Iceland.

Atherosclerotic cardiovascular disease is the leading cause of death worldwide. We have previously described a highly significant association ($P = 1.2\times10^{-20}$) between myocardial infarction (MI)/coronary artery disease (CAD) and a common sequence variant on 9p21. Approximately 21 percent of the Caucasian population is homozygous for this variant and they have an estimated 1.64-fold greater MI risk than non-carriers. The corresponding risk is 2.02-fold for early onset MI. The variant is located adjacent to the *CDKN2A* and *CDKN2B* genes, which have a critical role in regulating cell proliferation, cell aging/senescence, and apoptosis, that are all important features of atherogenesis. To explore the effect of this sequence variant on other atherosclerosis related phenotypes we have extended the association analysis to include subjects with peripheral arterial disease, large vessel disease stroke, and abdominal aortic aneurysm. For each of these phenotypes the association to the variant at 9p21 was analyzed in two to five different Caucasian case-control samples. All tested atherosclerotic phenotypes show significant association with similar effect as previously described for MI/CAD. The risk variant did not show association to T2D. This is intriguing because we and others have identified variants in an adjacent LD block beyond the recombination hotspot, showing significant association to T2D, but not to other atherosclerotic phenotypes without diabetes.

139

Genome-wide Scan for Coronary Artery Disease Genes using 500,668 markers. *A.F.R. Stewart, R. McPherson, L. Chen, K. Williams, N. Kavaslar, J. Rutberg, H. Doelle, G. Ewart, G.A. Wells, R. Roberts.* Univ Ottawa Heart Inst, Ottawa, ON, Canada.

Coronary artery disease (CAD) is the leading cause of death in the western world. Genetics account for approximately 50% of CAD risk, but CAD is polygenic, meaning that any single gene variant is neither necessary nor sufficient to fully account for CAD risk. With the exception of rare genetic variants with major effects on LDL cholesterol concentrations such as the LDLR or PCSK9 genes, candidate gene studies have provided little information on variability in CAD risk. Genome wide association studies using high density single nucleotide polymorphism (SNP) genotyping are providing a more fruitful approach to the study of complex diseases. Here, we report on the Ottawa Heart Genomics Study, the first genome-wide association study using 500,668 SNPs to identify novel risk and protective loci for CAD in 997 cases with early onset disease and 1054 elderly asymptomatic controls sampled from the Caucasian population in the Ottawa region. We have identified 1,411 risk SNPs (minor allele associated with CAD) and 810 protective SNPs (minor allele associated with controls). Clusters of SNPs within genes identified DIAPH3, GPC6 and ACTN4 as risk loci and NBEA, ABO, and GNG12 as protective loci, among others. Large intergenic clusters were detected at 2q22.1, 5q33.2 and one previously reported by us at 9p21.3. Since there is sufficient power to detect the presence of causative polymorphisms of moderate effect when 1000 individuals are sampled, 500 cases and 500 controls were selected at random for analysis. Of the significant SNPs identified, 21% were also significant in the independent sample of the remaining 497 cases and 554 controls. Nearly all of these loci were novel and had not been previously associated with CAD. The entire dataset is made public so that further investigations in larger case/control cohorts can validate or reject these novel loci.

140

***VNN1*, A Novel Gene for Cardiovascular Disease Risk.** *J.E. Curran[1], M.P. Johnson[1], H.H.H. Göring[1], T.D. Dyer[1], J.C. Charlesworth[1], S.A. Cole[1], J.B. Jowett[2,3], L.J. Abraham[4], D.L. Rainwater[1], M.C. Mahaney[1], L. Almasy[1], J.W. MacCluer[1], A.H. Kissebah[5], G.R. Collier[3], E.K. Moses[1], J. Blangero[1,3].* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) International Diabetes Institute, Caulfield, Vic; 3) ChemGenex Pharmaceuticals, Geelong, Vic; 4) University of Western Australia, Perth, WA; 5) Medical College of Wisconsin, Wisconsin, WI.

Our study describes an integrative approach to gene discovery utilizing large-scale transcriptional profiling to identify novel *cis*-acting genes that correlate with HDL cholesterol levels, a risk factor for CVD. Lymphocyte-based RNA samples from 1240 individuals in the San Antonio Family Heart Study were used to obtain genome-wide transcriptional profiles. Statistical analysis identified over 400 *cis*-regulated transcripts whose expression levels significantly correlated with HDL-C levels. One gene, *VNN1*, showed strong support for both *cis*-regulation ($p=1.2\times10^{-11}$) and correlation with HDL-C levels ($p=5.7\times10^{-9}$). *VNN1* expression levels were also significantly correlated with triglycerides (p=0.002), ApoA1 (p=0.0003), ApoA2 (p=0.0014) and LDL peak diameter (8.7×10^{-5}). Given the evidence for *cis*-effects, we resequenced 2kb of the promoter in 96 founders, identifying 22 SNPs. Genotyping in the cohort revealed 5 variants highly correlated with *VNN1* expression: -587 ($p=7\times10^{-85}$), -137 ($p=6\times10^{-83}$), -612 ($p=2\times10^{-36}$), -708 ($p=3\times10^{-34}$) and -667 ($p=8\times10^{-7}$). The variants at sites -667 ($p=3\times10^{-5}$) and -137 ($p=4\times10^{-4}$) also showed strong associations with HDL-C levels. Molecular analyses of the -137 variant using EMSA supported a functional role for this SNP. To identify genes causally downstream of *VNN1*, we tested for the *trans*-effects of *VNN1* promoter variants on the transcriptional profiles. *VNN1* variants were associated with expression levels of several lipid metabolism/CVD-risk genes including *LPL* (p=0.03), *LCAT* (p=0.03), *LRP3* (p=0.018), *ACAT2* (p=0.001) and *IL10* (p=0.001). These results support a significant role for *VNN1* in HDL-C variation, and show the value of transcriptional profiling for identifying genes reflecting causal relationships with complex phenotypes.

141

Identification and replication of FAM5C polymorphisms associated with myocardial infarction. *J.J. Connelly[1], A.B. Hale[1], S. Gadson[1], J.F. Doss[1], X. Lou[1], D.R. Crosslin[1], S.H. Shah[1,2], D.C. Crossman[3], C.B. Granger[1], V. Mooser[4], C.J.H. Jones[5], J.M. Vance[6], P.J. Goldschmidt-Clermont[6], W.E. Kraus[2], E.R. Hauser[1], S.G. Gregory[1].* 1) Department of Medicine and Center for Human Genetics, Duke University, Durham, NC; 2) Department of Medicine and Division of Cardiology, Duke University Medical Center, Durham, NC; 3) University of Sheffield, Sheffield, United Kingdom; 4) Genetics Research, GlaxoSmithKline, Collegeville, PA; 5) University of Wales College of Medicine, Cardiff, United Kingdom; 6) Miller School of Medicine, University of Miami, Miami, FL.

We previously identified a 40 Mb region of linkage on chromosome 1q in our early onset coronary artery disease (CAD) genome-wide linkage scan (GENECARD) with modest evidence for linkage (n=420, LOD 0.95). When the data are stratified by acute coronary syndrome (ACS), this modest maximum in the overall group became a well-defined LOD peak (maximum LOD of 2.17, D1S1589/D1S518). This peak overlaps a recently identified inflammatory biomarker (MCP-1) linkage region from the Framingham Heart Study (maximum LOD of 4.27, D1S1589) and a region of linkage to metabolic syndrome from the IRAS study (maximum LOD of 2.59, D1S1589/D1S518). The overlap of genetic screens in independent data sets provides evidence for the existence of a gene or genes for CAD in this region. We conducted a peak-wide association screen (457 SNPs) of the region 1 LOD score down from the peak marker (168-198 Mb) on chromosome 1. We identified polymorphisms within the family with sequence similarity 5, member C gene (FAM5C) that show genetic linkage and are associated with ACS (rs1891586, maximum LOD=1.54, p=0.027). We have confirmed the association with an independent case-control dataset (CATHGEN, p=0.023) and have identified strong association (p=0.0003) between FAM5C and myocardial infarction. In addition, several risk alleles of FAM5C show association with atherosclerotic burden (rs11581737, rs480692, rs12732902, odds ratio 2.5-5.0). FAM5C is known to promote proliferation, migration and invasion of pituitary tumors; a phenotype relevant to the cellular changes associated with the formation of an atherosclerotic plaque.

142

ACTA2 mutations cause diverse and diffuse vascular diseases, including aortic aneurysms, premature coronary artery disease and Moyamoya disease. *D. Guo[1], H. Pannu[1], V. Tran-fadulu[1], C. Papke[1], N. Avidan[1], S. Bourgeois[1], R. Yu[2], A. Estrera[1], H. Safi[1], P. Tung[1], L. Buja[1], S. Scherer[3], C. Raman[1], S. Shete[2], D. Milewicz[1].* 1) Univ Texas/Houston Medical School, Houston, TX; 2) Univ Texas/MD Anderson Cancer Center, Houston, TX; 3) Baylor College of Medicine, Houston, TX.

ACTA2 encodes smooth muscle cell (SMC) alpha-actin, a contractile protein that is the most abundant protein in vascular SMCs. A large family with autosomal dominant inheritance of thoracic aortic aneurysm and dissection (TAAD) was use to map and identify a mutation in ACTA2 (R149C) as the cause of TAAD. All individuals examined with ACTA2 mutations also had marked livedo reticularis (LR, rash due to dermal capillary occlusion); segregation of LR alone with the ACTA2 mutation yielded a LOD of 5.85. Six ACTA2 + members did not have TAAD, but rather premature coronary artery disease (CAD < 55 yrs). Sequencing of ACTA2 in 97 TAAD families identified mutations in 14 families that segregated with TAAD and premature CAD (R149C was present in 4 families); no ACTA2 SNPs were found in 196 controls. Three unrelated families with R258C/H ACTA2 mutations had 6 members with strokes < 30 yrs, with 4 diagnosed with Moyamoya disease (MMD, strokes due to carotid artery occlusion). Six families had unique ACTA2 mutations. ACTA2 mutations segregated with TAAD (LOD score 4.14) and premature CAD (combined TAAD and CAD, LOD score 8.5). Also, ACTA2 + family members were more likely to have premature CAD than ACTA2 - members ($p < 10\text{-}4$). Aortic pathology showed medial degeneration and the unique finding of focal occlusion of capillaries due to SMC hyperplasia. SMCs from ACTA2 mutation patients had little to no alpha-actin filaments compared to control SMCs. Therefore, ACTA2 mutations cause TAAD, premature CAD and strokes, MMD, and LR. The diversity of vascular diseases imparted by a mutation in a single gene alters our understanding and approach to identifying the genetic basis of vascular diseases.

143

Variants on 4q25 confer risk of atrial fibrillation. *D. Gudbjartsson[1], D. Arnar[2], A. Helgadottir[1], S. Gretarsdottir[1], G. Thorleifsson[1], G. Thorgeirsson[2], K. Kostulas[3], J. Hillert[3], R. Ma[4], M.C.Y. Ng[4], J. Rosand[5], P. Ellinor[6], H. Holm[1], J. Gulcher[1], U. Thorsteinsdottir[1], A. Kong[1], K. Stefansson[1].* 1) Statistics, deCODE Genetics, Reykjavik, Iceland; 2) Division of Cardiology, Department of Medicine, Landspitali University Hospital, Reykjavik, Iceland; 3) Department of Neurology, Karolinska Institutet at Karolinska University Hospital, Huddinge, Sweden; 4) Department of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, Hong Kong; 5) Department of Neurology, Massachusetts General Hospital Hospital and Harvard Medical School, Boston; 6) Cardiology Division and Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, Boston.

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in man and is characterized by chaotic electrical activity of the atria. It affects one in ten individuals over eighty, causes significant morbidity, and is an independent predictor of mortality. We performed a genome-wide association scan on an Icelandic AF sample with 316,000 SNPs on an Illumina BeadChip. The genome-wide scan was followed by replication studies in European populations from Iceland, Sweden, the U.S. and Norway and a strong association between two sequence variants on chromosome 4q25 to AF was confirmed ($P < 10^{-40}$ and $P < 10^{-10}$). Approximately 35% of individuals of European descent have at least one of the variants and the risk of AF increases by 1.72 and 1.39 per copy. We also tested the variants in a Chinese population from Hong Kong and replicated the association to the stronger variant. The stronger variant is carried by 75% of individuals in the Chinese sample and the risk of AF is increased by 1.42 per copy. A stronger association was observed in individuals with typical atrial flutter (AFl), individuals with lone AF and individuals with early onset of disease. The variants are in the same LD block, adjacent to PITX2, which is known to play a critical role in left-right asymmetry of the heart.

144

Association mapping of the five quantitative ECG traits RR, P, PQ, QRS AND QT in a 500K genomewide scan: confirmation of the NOS1AP association to QT and identification of a spectrum of additional QTLs. *A. Pfeufer[1,2], M. Akyol[1,2], M.F. Sinner[2,3], S. Perz[2], C. Gieger[2,3], B.M. Beckmann[3], T. Illig[2], H.E. Wichmann[2,3], S. Kaab[3], T. Meitinger[1,2].* 1) TU Munich, Germany; 2) GSF National Research Center, Neuherberg, Germany; 3) LMU University of Munich, Germany.

Background: We have investigated five quantitative electrocardiographic (ECG) traits by genomewide association (GWA), namely the RR interval (a measure of heart rate), the P wave duration (a measure of atrial excitation and repolarization), the PQ interval (a measure of AV conduction), the QRS interval (a measure of ventricular excitation) and the QT-interval (a measure of ventricular repolarization). All traits are indicators of physiologic as well as pathologic states in cardiac electrophysiology and are known endophenotypes for predisposition to arrhythmias and cardiac sudden death. Aim: To map the spectrum of QTLs for these traits we undertook a genome-wide association scan in n=1,664 individuals using Affymetrix 500k arrays. Probands were participants of the follow-up (F3) of the population based KORA S3 survey from Augsburg, Southern Germany. Association was calculated under additive, dominant and recessive models. Results: We considered SNPs with CR>98%, p(HWE)>1e-5 and with n≥30 individuals responsible for an association signal p≤1e-6 suitable for follow up genotyping. The known QTL for QT interval at the NOS1AP gene gave the strongest genomewide association signal. 60 SNPs throughout a 500kb genomic region were associated with significance levels down to 1e-7.5. In addition we identified 12 additional putative QTLs, 2 for RR, 1 for PQ, 7 for QRS and 2 for QT. Conclusions: The QTL at the NOS1AP gene was confirmed as the single most significant signal for QT interval from a 500k-genomewide scan. Its well identifiable signal is due to its high allele frequency (MAF= 0.35), 500kb long LD relationship and relatively strong effect size. The newly identified QTLs for QT and other ECG traits display a spectrum of different effect sizes, allele frequencies and are currently undergoing replication testing in larger population based samples.

145

Genetic association of the epithelial sodium channel γ-subunit with 25-year follow-up blood pressures in Utah pedigrees - a replication study. *C.J. Büsst[1], K.J. Scurrah[1,2], J.A. Ellis[1,3], Y. Xin[4], E.A. Brinton[4], P.N. Hopkins[4], S.C. Hunt[4], S.B. Harrap[1].* 1) Department of Physiology, University of Melbourne, Melbourne, Australia; 2) Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Melbourne, Australia; 3) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; 4) Cardiovascular Genetics, University of Utah, Salt Lake City, UT.

The γ-subunit of the epithelial sodium channel (γ-ENaC), encoded by *SCNN1G*, has been implicated in the Mendelian diseases Liddle's syndrome and pseudohypoaldosteronism type 1, and is the rate limiting step of sodium reabsorption in the kidney. *SCNN1G* is located on a region of chromosome 16 that has been linked to systolic blood pressure (SBP) by a number of independent studies, including the Victorian Family Heart Study (VFHS). At last year's meeting we reported the findings from our association analysis of SBP in VFHS subjects from the upper and lower deciles of the SBP distribution, in which we detected association of 4 *SCNN1G* SNPs (rs13331086, rs11074553, rs4299163 and rs5740) and a number of haplotypes with SBP. To replicate these findings, we genotyped six of the SNPs previously typed in the VFHS in 1971 relatives from 68 large Utah pedigrees selected for high risk of cardiovascular disease. Of these, 675 have returned to date for a 25-year follow-up exam. FBAT was used to test for association of individual SNPs and haplotypes while controlling for related observations in families. After adjusting for age, sex and body mass index, we detected significant associations for rs13331086 with DBP at 25-year follow up (p=0.002) and for change in DBP from baseline to 25-year follow up (p=0.003). Haplotypes of rs4299163 and rs5740 also revealed association with change in DBP from baseline to 25-year follow up (p=0.013). Preliminary results for analysis of DBP in the VFHS are consistent with DBP findings in the Utah pedigrees. In conclusion, the *SCNN1G* gene is significantly associated with BP in the Utah pedigrees at 25-year follow up. These results appear to replicate our finding that γ-ENaC variants contribute to BP variation in the general Caucasian population.

146

Whole-genome association study in the Old Order Amish identifies *STK39* as a novel hypertension susceptibility gene. *Y. Wang, P.F. McArdle, E. Rampersaud, H. Shen, X. Shi, N.I. Steinle, B.D. Mitchell, A.R. Shuldiner, Y-P.C. Chang.* Division of Endocrinology, Univ Maryland, Baltimore, MD.

Hypertension (HTN) is a major risk factor for cardiovascular and renal diseases. However the specific genes that confer predisposition to HTN remain elusive. We conducted a genome-wide association study of systolic blood pressure (SBP) and diastolic blood pressure (DBP) by analyzing 93,087 SNPs in 551 subjects from the Amish Family Diabetes Study (AFDS). Twelve of the SNPs most strongly associated with SBP and DBP were located within the gene *STK39* in 2 overlapping linkage disequilibrium blocks. One representative SNP from each block was then genotyped in an expanded set of 743 nondiabetic AFDS subjects. Both SNPs showed strong association with BP traits (p-value $<10^{-8}$ for SBP and 10^{-5} for DBP). The at-risk allele was associated with an estimated 5 and 2 mmHg increases in SBP and DBP, respectively. As an independent replication, we then analyzed SNPs from the two associated blocks in a third independent set of Amish (n=868) that were younger and healthier than the AFDS population. Again, we detected strong association, in the same direction as seen in AFDS, between these SNPs and baseline SBP levels, as well as with other more sensitive measures of vascular function, including SBP response to cold pressor test (p-values $< 10^{-5}$). In addition, analyses of BP traits by the BROAD Institute (http://www.broad.mit.edu/diabetes/scandinavs/metatraits.html) also demonstrates significant association between the same *STK39* SNPs and SBP level and HTN status ($p<0.02$). *STK39* encodes serine threonine kinase 39 and is known to stimulate the activity of Na^+-K^+-$2Cl^-$ co-transporter (NKCC1) in the distal nephron. Furthermore, mutations in *WNK1* and *WNK4*, which phosphorylate and activate NKCC1 through *STK39*, can cause a monogenic form of HTN. In summary, evidence from analyzing the Old Order Amish as well as more outbred populations showed that variants in *STK39* are strongly associated with BP levels. Some associated SNPs are located within highly conserved putative regulatory elements and are excellent candidates for functional analyses of this novel HTN susceptibility gene.

147

Functional characterization of a sensory organ-specific miRNA cluster. *P.D. Witmer[1,2], S. Xu[3], J.T. Mendell[1], S. Fisher[1], D. Valle[1].* 1) McKusick-Nathans Institute of Genetic Medicine; 2) Predoctoral Training Program in Human Genetics, Johns Hopkins Univ, School of Medicine, Baltimore, MD; 3) Department of Ophthalmology/Neurological Sciences, Rush Univ Medical Ctr, Chicago, IL.

Using a custom array to profile miRNA expression in adult mouse tissues, we identified a group of miRNAs whose expression is apparently limited to retina. Among these, we identified a polycistronic, paralogous miRNA cluster including *miR-96*, *miR-182* and *miR-183* on mouse chromosome 6qA3 with conservation of synteny to human chr7q32.2. Reported studies and our own results strongly suggest that expression of this cluster is restricted to sensory neurons of the retina, inner ear, olfactory epithelium, taste buds and dorsal root ganglia. Sequence conservation extends to zebrafish, where expression is also detected in the lateral line, a mechanosensory organ. Our hypothesis that members of the *miR-183/96/182* cluster play important roles in sensory neural biology is further supported by analysis of their predicted targets, which includes many genes required for the development and/or function of various sensory organs. We performed *in vitro* functional studies that showed that *MITF*, which is required for the establishment and maintenance of retinal pigmented epithelium, is a direct target of *miR-96* and *miR-182*, suggesting that these miRNAs may contribute to neuroretinal identity. Additionally, we performed *in vivo* functional studies utilizing morpholino-directed knockdown of expression of the *miR-183/96/182* cluster in zebrafish and produced larvae with abnormal balance, swimming abnormalities and an attenuated response to vibrational stimuli. Embryos (5 dpf) treated with DASPEI, a dye that labels sensory hair cells, showed decreased staining or absence of lateral line neuromasts as compared to uninjected controls. Together, our results suggest a phenotype similar to those reported for zebrafish *circler* mutants with known vestibular defects. We conclude that members of the *miR-183/96/182* cluster are important for sensory neuron development and/or function. Additional studies are underway to characterize the nature of the sensory deficits in these fish and relate these observations to human phenotypes.

148

Misregulation of small noncoding regulatory RNAs by the loss of MeCP2 in a mouse model of Rett Syndrome. *K. Szulwach[1], X. Li[2], S. Mathias[3], X. Zhao[2], P. Jin[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept of Neuroscience, University of New Mexico, Albuquerque, NM; 3) Division of Biocomputing, University of New Mexico School of Medicine, Albuquerque, NM.

Rett Syndrome (RTT) is a neurodevelopmental disorder mainly caused by mutations in X-linked gene methyl-CpG-binding protein 2 (MECP2) and primarily affects females. Identification of the genes regulated by MeCP2, particularly in the context of neurodevelopment, is critical for understanding the molecular pathogenesis of RTT. MicroRNAs (miRNAs) are small (18-24nt) noncoding regulatory RNA able to suppress translation from protein coding transcripts without necessarily having an effect on steady state mRNA levels. MeCP2 mediated regulation of miRNA expression, therefore, provides an alternative mechanism by which MeCP2 deficiency could alter expression of proteins without affecting mRNA transcription itself. Toward this end, we have examined the possibility that MeCP2 might regulate a subset of small noncoding regulatory RNAs in neurogenesis. Using TaqMan based miRNA profiling and Solexa 1G sequencing based expression profiling we have assayed expression of small noncoding RNAs in proliferating and differentiated adult neural stem cells (aNSCs) derived from wildtype and MeCP2 knockout mice. We have identified and verified increased expression of at least two miRNAs, mmu-mir-137 and mmu-mir-301, in the absence of MeCP2. Furthermore, we have observed direct binding of MeCP2 to the genomic regions proximal to mmu-mir-137 in wildtype aNSCs using chromatin immunoprecipitation, suggesting that MeCP2 could directly regulate the expression of miRNAs. Furthermore, using Solexa 1G sequencing based expression profiling, we have obtained over 30,000 unique small RNA sequences corresponding to more than 1.5 million individual small RNA derived from both wildtype and MeCP2 KO proliferating aNSCs. We have identified previously unannotated small noncoding RNAs with altered expression in the absence of MeCP2. Our results suggest that MeCP2 could regulate a subset of small noncoding regulatory RNAs and that dysregulation of these small RNAs could contribute to the pathogenesis of RTT.

149

Prader-Willi syndrome is caused by paternal deficiency for the HBII-85 C/D box snoRNA cluster. *T. Sahoo[1], D. del Gaudio[1], J.R. German[1], M. Shinawi[1], S.U. Peters[1], R. Person[1], A. Garnica[2], S.W. Cheung[1], A.L. Beaudet[1].* 1) Dept Human & Molec Gen, Baylor Col Medicine, Houston, TX; 2) St. Francis Hospital, Tulsa, OK.

Prader-Willi syndrome (PWS) is a neurobehavioral disorder manifested by infantile hypotonia, feeding difficulties in infancy, followed by morbid obesity secondary to hyperphagia. It is caused by lack of paternally expressed genes within the human chromosome region 15q11-q13. The small nuclear ribonucleoprotein polypeptide N (*SNRPN*) and necdin (*NDN*) genes have been considered as PWS candidate genes. PWS patients harboring balanced chromosomal translocations with breakpoints within *SNRPN* have provided significant evidence that the snoRNA HBII-85 cluster is likely to play a major role in the PWS- phenotype. Here we report the identification and characterization of a de novo microdeletion within 15q11.2 in a child expressing a typical PWS phenotype. The patient exhibits all the 8 major diagnostic criteria including neonatal and infantile central hypotonia, feeding problems in infancy, excessive or rapid weight gain after 12 months, characteristic facial features, hypogonadism, hyperphagia/food foraging, and deletion 15q11-q13. He also exhibits mild mental retardation and meets criteria for a diagnosis of autism. A combination of high-resolution deletion analysis, breakpoint mapping and expression studies identified a loss of ~174 kb leading to the complete loss of the HBII-85 snoRNA cluster and partial loss of the HBII-52 cluster. Based on expression analysis in lymphoblasts for *SNRPN* (Exon1-3), HBII-13, AK094315, AB061718, and *UBE3A*, this interstitial deletion does not negatively impact the physical integrity or expression of other imprinted genes in its vicinity. Combined with previously reported data, this case provides strong evidence that paternal deficiency of the snoRNA HBII-85 cluster causes most or all of the phenotypic features of PWS. This interpretation would represent the first well documented example of a human phenotype caused by deficiency of a snoRNA, a subclass of regulatory noncoding RNAs.

150

SnoRNA Pwcr1/MBII-85 deletion mouse model for Prader-Willi syndrome shows growth retardation, hyperphagia and altered metabolism. *F. Ding[1], HH. Li[1], S. Zhang[2], N. Solomon[3], S. Camper[3], E. Mignot[2], U. Francke[1].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Department of Psychiatry & Behavioral Science, Stanford University; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia, distinct facial features, short stature due to abnormal growth hormone secretion, hypogonadism, obesity due to hyperphagia, developmental delay, insensitivity to pain and other neuropsychological features. It is caused by the lack of paternal expression of imprinted genes on 15q11.2. Our previous work in human and mice suggested that the lack of expression of a small C/D box nucleolar RNA (snoRNA), PWCR1/HBII-85, contributes to the PWS phenotype (Schüle et al BMC Med. Genet. 6:18, 2005; Ding et al. Mamm. Genome 16:424, 2005). To test this hypothesis, we created a new mouse model with a ~150kb deletion of the *Pwcr1/MBII-85* snoRNA cluster. In contrast to the neonatal lethality observed in previous mouse models, our mutant mice survive to adulthood with normal fertility. Apparently normal at birth, they have severe growth retardation before weaning, with moderate growth retardation afterwards. Liver Igf1 mRNA is decreased in 4 and 8 week old mutants. Histological and immunohistochemical studies of pituitary organogenesis and structure revealed no gross abnormalities. Adult male mutants and wild-type littermates were subjected to metabolic and cognitive/behavior tests. Similar to PWS individuals, the mutant mice show hyperphagia and increased anxiety. Unlike human with PWS, they have normal response to thermal pain and are not hypotonic. The mutants outperformed controls initially in rotarod tests, but failed to improve upon training, indicative of a motor learning deficiency. Despite their hyperphagia, the mutants are leaner on regular and high-fat diet. They have less body fat content and are more sensitive to insulin. The cause of this surprising result is revealed by their altered fuel usage. This is the first snoRNA deletion animal model, and the phenotypes indicate an essential role of Pwcr1/MBII-85 snoRNA in neurodevelopment, growth and metabolism.

151

MicroRNAs influence gene expression phenotypes of ataxia telangiectasia carriers. *V.G. Cheung, D.A. Smirnov.* Pediatrics & Genetics, ARC 516G, Univ Pennsylvania/ Child Hosp, Philadelphia, PA.

Ataxia Telangiectasia (AT) is an autosomal recessive disorder caused by mutations in the *ATM* gene. Previously, we showed that the gene expression phenotype of AT carriers is distinct from non-carriers and also from carriers of a similar disorder, Nijmegen Breakage Syndrome. Even though patients with an autosomal recessive disease like AT are rare, carriers are not. Therefore, carriers for recessive mutations can contribute significantly to phenotypic diversity.

The goal of this study is to characterize gene expression phenotypes of AT carriers and to study the underlying mechanism. We chose gene expression as the phenotype since many diseases are due to altered expression levels of genes. Knowing that ATM plays a key role in radiation response, we compared the radiation-induced gene expression responses of non-carriers, AT carriers and AT patients. We found 22 gene expression phenotypes that behaved in a "recessive" manner, where the radiation-induced expression response of carriers is similar to that of non-carriers, but differs significantly from AT patients ($P<0.01$). In addition, we found 29 gene expression phenotypes that behaved in a "dominant" manner, where the expression response of AT carriers differed significantly from that of non-carriers ($P<0.01$), but not from AT patients. To account for these patterns, we examined microRNAs that regulate gene expression. When we compared these among the three AT genotypes, we observed 15 microRNAs with recessive pattern and 7 with dominant pattern. Finally, we showed that the gene expression patterns can be explained by microRNAs that regulate the genes. For example, the dominant expression pattern of *TNFSF4* mRNA is due to the dominant pattern of expression of miRNA-125b that regulates its expression.

To our knowledge, this is the first report that implicates the role of microRNA in a human Mendelian disorder and offers a mechanism that accounts for the phenotypic manifestation in carriers of recessive mutations.

152

Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by over-expression of α-synuclein. *G. Wang[1], J. van der Walt[2], G. Mayhew[1], Y. Li[2], S. Züchner[1], W.K. Scott[1], E. Martin[1], J.M. Vance[1].* 1) Institute for Human Genomics, University of Miami , Miami, Fl; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC 27710.

Parkinson disease (PD) is a common neurodegenerative disorder caused by environmental and genetic factors. We have previously shown linkage of PD to chromosome 8p. Subsequently, fibroblast growth factor 20 (FGF20) at 8p21.3-22 was identified as a risk factor in several association studies. To identify the risk-conferring polymorphism in FGF20 we performed genetic and functional analysis of single nucleotide polymorphisms within the gene. In a sample of 729 nuclear families with 1089 affected and 1165 unaffected individuals, the strongest evidence of association came from rs12720208 in the 3' UTR of FGF20. We show in several functional assays that the risk allele for rs12720208 disrupts a binding site for microRNA-433, increasing translation of FGF20 in vitro and in vivo. In a cell-based system and in PD brains, this increase in translation of FGF20 is correlated with increased α-synuclein expression, which has previously been shown to cause PD through both over-expression and point mutations. We suggest a novel mechanism of action for PD risk in which the modulation of the susceptibility gene's translation by common variations interfere with the regulation mechanisms of microRNA. We propose this is likely to be a common mechanism of genetic modulation of individual susceptibility to complex disease.

153

Enrichment and variability of PIWI-interacting RNAs (piRNAs) in segmental duplications and copy number variants (CNVs) suggest a functional role in the integrity of the genome. *L. Armengol[1], M. Caceres[1], A. Brunet[1], X. Estivill[1,2,3].* 1) Genetic Causes of Disease Group, Genes and Disease Program, and CIBERESP, Center for Genomic Regulation (CRG-UPF), Barcelona, Catalonia, Spain; 2) Genetics Unit, Department of Health and Experimental Life Sciences, Pompeu Fabra University (UPF), Barcelona, Catalonia, Spain; 3) National Genotyping Center (CeGen) Barcelona Genotyping Node, CRG, Barcelona, Catalonia, Spain.

PIWI-interacting RNAs (piRNAs) are a novel class of small (30 nt) noncoding RNAs identified in mammalian germline cells and constitute the most abundant known class of genes with over 32,000 elements in humans. We have examined piRNAs' organization in the human genome and have found that currently known human piRNAs map to 70,736 sites and are structured in about 400 clusters, containing each at least 10 piRNAs. A large proportion of the piRNA loci (about 65%) are located within repeat sequences, mainly LTRs and LINE sequences, and over 50% contain repeated units of a single piRNA, 71 being composed of tandem copies of a unique piRNAs. Surprisingly, over 25% of total piRNAs are located in regions that contain segmental duplications (SDs) and about 37% are within copy number variant (CNVs) regions. In addition, 233 (58%) and 220 (55%) piRNA clusters are within SDs and CNVs, respectively. Similarly, a significant subset of SDs (43%), especially those with the highest level of nucleotide identity, contains piRNAs. Finally, we have confirmed experimentally that the genomic sequences in which piRNAs are embedded in vary in copy number in humans. Since SDs and CNVs account for 5% and 12% of the human genome sequence, respectively, the significant enrichment of piRNAs is suggestive of a functional role of these elements. Interestingly, we have found a similar relationship for piRNAs and SDs in the mouse genome. This association provides the first link between SDs and CNVs with elements that could have a putative functional role in the integrity of the genome. Supported by EU AnEUploidy grant and by Catalan Government.

154

Exhaustive analysis of non-coding DNA around *phox2b* reveals most biologically important sequences are not detected by sequence conservation. *D.M. McGaughey[1], R.M. Vinton[1], J. Huynh[1], A. Al-Saif[1], M.A. Beer[1,3], A.S. McCallion[1,2].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; 3) Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Multi-species sequence conservation is one of the principal tools for the prediction of functional non-coding elements. However, it is unclear whether conservation-based approaches systematically overlook regulatory sequences and, if so, with what frequency. We have addressed this question directly through the construction of a tiling path comprising 33 amplicons across a genomic interval of 40.7 kb, encompassing the *phox2b* neurogenic transcription factor whose exons total 3.1 kb. All sequences therein, excluding the *phox2b* coding region, were functionally evaluated for enhancer activity via zebrafish transgenesis. 20 amplicons could be aligned with other species (Fugu / Tetraodon / Human / Mouse), of which 13 displayed enhancer activity in tissues overlapping endogenous *phox2b* expression. Of significant interest, four of the remaining 13 non-aligned amplicons also demonstrated tissue-specific expression. Analyses of this interval using established and novel algorithms resulted in the following observations. First, the established sequence conservation algorithms detected only 30-53% of the identified functional sequence elements. Second, functional and non-functional sequences in this interval could be discriminated using a de novo motif identification algorithm. Third, we identify functional non-coding sequences conserved between *phox2b* and the non-orthologous human *CART1* locus inferring their common ancestry, a theme we show is iterated at many other loci. Collectively, these data suggest that non-coding conservation between orthologous loci is frequently neither necessary nor sufficient to predict sequence functionality. Consequently efforts to decipher a regulatory vocabulary or search for regulatory variants associated with disease will be significantly impacted.

155

Polysome fractionation suggests that a fraction of Txfrags is translated. *S. Nikolaev[1], S. Deutsch[1], R. Genolet[2], L. Parand[1], B. Conne[1], P. Descombes[3], J-D. Vassalli[1], J. Curran[2], S.E. Antonarakis[1].* 1) Genetic Medicine and Dev., University of Geneva, Switzerland; 2) Microbiology and Molecular Medicine, University of Geneva, Switzerland; 3) NCCR Genomics platform, University of Geneva, Switzerland.

Recent studies have shown extensive transcriptional activity across the human genome, a substantial fraction of which is not associated with any functional annotation (Txfrags). However, very little is known regarding the post-transcriptional processes that operate in different classes of RNA molecules. To characterize the translational behavior of transcriptional units in the the entire non-repetitive human chromosome 21 and the ENCODE region ENm001, we separated RNA molecules of 3 cell lines (GM06990, HelaS3 and SKNAS) in a sucrose gradient according to their association with ribosomes. Pools of fractions representing translated RNA (associated with 2 or more ribosomes) and total RNA were hybridized to a 22 bp-resolution custom-made genomic tiling array. Positive signals were extracted using a conservative algorithm requiring at least 3 consecutive probes being above the 99% confidence threshold. We observed that 3.5 - 6% of HSA21 is transcribed in each cell line, and a total of 8% is transcribed in at least 1 cell line. On average 62% or the transcribed regions correspond to annotated regions (mRNAs + ESTs), whereas the remaining 38% do not overlap with any previous annotation (Txfrags). In addition, 85% of Refseq exons were detected in at least one cell line, suggesting that the arrays had a good sensitivity to detect transcription. To estimate the translation level for each exon (or transcriptional unit), we calculated the ratio of expression between polyribosomal and total RNA. We observed a wide distribution of ratios in all cell types. As expected, Refseq exons are significantly more translated than Txfrags; however, there was a large overlap between the 2 distributions suggesting that many transfrags are likely to be translated. Additional analyses are ongoing. Our study provides an initial functional characterization of Txfrags and underscores that they are unlikely to be mere transcriptional noise.

156

Insights into the noncoding RNA transcriptome in the mammalian brain. *S. Sunkin[1], T. Mercer[2], M. Dinger[2], M. Mehler[3], A. Jones[1], J. Mattick[2].* 1) Allen Institute for Brain Science, Seattle, WA; 2) Institute for Molecular Bioscience, University of Queensland, St Lucia, Australia; 3) Institute for Brain Disorders and Neural Regeneration, Albert Einstein College of Medicine, New York, NY.

Within the adult mouse brain, the Allen Brain Atlas has comprehensively mapped the spatial expression patterns of protein-coding mRNAs by in situ hybridization, but has yet to systematically assess the expression profiles of noncoding RNAs (ncRNAs). NcRNA transcripts include small RNAs as well as long ncRNAs. While ncRNAs possess little or no protein coding capacity, they have been increasingly recognized for their modulatory roles within mammalian genomes particularly in relation to physiological functions, nervous system development, and various disease states.

MicroRNAs (miRNAs) are important regulators of mRNA translation and stability, and play central roles in many developmental and regenerative processes. It is also becoming clear that other types of ncRNAs may also play important, but as yet undefined, roles in mammalian biology. These include antisense RNAs, which are prevalent in the nervous system, suggesting the importance of these transcripts in regulating gene expression within the brain.

In this study, we show that miRNAs exhibit diverse expression patterns in the adult mouse brain, from widespread to regionally enriched profiles. We also describe over 1,000 long ncRNAs that display various expression profiles in the brain, including near ubiquitous, regionally enriched, and cell class specific patterns. In addition, some transcripts appear to be restricted to particular subcellular compartments. The spatial expression patterns of these long ncRNAs were also evaluated in relation to their genomic context (cis-antisense, intronic, and bi-directional pairs) and the identity of the corresponding or adjacent protein-coding genes, many of which are important in neural development, constitutive adult neurogenesis, behavioral functions and in the etiology of disease states. This study provides the first large-scale insight into the extraordinary complexity of ncRNA expression in the brain.

157

Genome-wide association and platelet system biology studies to unravel the genetic architecture of coronary artery disease. *A.H. Goodall, The Bloodomics and WTCCC Consortia.* Cardiovascular Science, Univ of Leicester, Leicester, United Kingdom.

The recently completed WTCCC genotyping of 14,000 cases, representing 7 diseases, and 3,000 shared controls on the Affymetrix GeneChip 500K Array identified association signals for all diseases. For myocardial infarction (MI), 7 genomic regions were identified with robust P values but an additional large number of single SNPs produced signal. Previous studies have shown ~90% of these to be false-positive. To aid identification of true-positives we integrated the WTCCC-GWA MI data with the Bloodomics platelet systems biology study. Platelets play a pivotal role in MI. Platelet response varies between individuals. Family and replication studies indicate a large degree of genetic control. To uncover genes encoding regulatory nodes, we determined platelet response through the ADP and collagen pathway in 500 individuals. Resequencing in 48 CEPH DNA samples of the exons and flanking intronic sequence of 100 genes, selected from a priori knowledge of both pathways doubled the number of SNPs compared to dbSNP to 1949 and enriched for SNPs with a minor allele frequency ≤0.05. Genotyping of the 500 subjects for 1536 SNPs, tagging sequence variation at r2 >0.9 and capturing all 89 non-synonymous SNPs, identified 26 SNPs that were associated at P values <0.005 with a modification of the cellular response, in either the ADP (n=12) collagen (n=10) pathway, or both (n=4). Microarray studies with platelet RNA from individuals at the extremes of the functional distribution identified 69 transcripts for which abundance correlated with the magnitude of the platelet response. Approximately 50% of the 95 genes were adequately tagged in the WTCCC study and 16 contained SNPs that showed a significant difference in allele frequency between cases and controls. A replication study for the 16 genes in 8,000 MI cases and 8,000 controls is underway to unequivocally define genes that confer risk for MI. Studies of 6 genes with hitherto unknown function in platelets are underway, using morpholino-based silencing in Danio rerio and studies in human platelets, to determine their effect on atherothrombosis.

158

AAV mediated expression of myotubularin in muscle corrects the myotubular myopathy phenotype in a mouse model and suggests a function in membrane remodeling at the sarcolemma. *A. Buj-Bello[1,3], F. Fougerousse[2], Y. Schwab[1], N. Messaddeq[1], D. Spehner[1], P. Schultz[1], O. Danos[2], J. Laporte[1,3], A-M. Douar[2], J-L. Mandel[1,3].* 1) IGBMC, CNRS/INSERM/University of Strasbourg, 67404 Illkirch, France; 2) Genethon, CNRS UMR8115, 91000 Evry, France; 3) Genetique Humaine, College de France.

X-linked myotubular myopathy (XLMTM) is a severe congenital disease due to mutations affecting the phosphoinositide phosphatase myotubularin (MTM1 gene), and characterized by small skeletal muscle fibers with frequent occurence of central nuclei. The pathophysiology of the disease is still poorly understood and specific treatment is unavailable. We have constructed a recombinant serotype 1 adeno-associated virus (rAAV2/1) vector expressing myotubularin and injected it into skeletal muscle to analyze the subcellular localization of myotubularin in myofibers and test its therapeutic potential in a faithful XLMTM mouse model (Mtm1 conditional KO). We show that a substantial proportion of myotubularin associates to the sarcolemma and I band, including triads. Transgene expression in Mtm1 KO muscle halts the progression of the histological phenotype, leading to a large increase in muscle weight and myofiber area and to a decrease in the percentage of fibers with internal nuclei. Mislocalization of other organelles such as mitochondria was also corrected. A single injection in Mtm1 KO muscles leads to a full rescue of the contractile force in the injected muscle. Overexpression of myotubularin in wild-type muscle causes myofiber vacuolation and accumulation of packed membrane saccules (myelin-like) close to the sarcolemma. This suggests that myotubularin is involved in plasma membrane remodeling and/or homeostasis, like the two other known genes implicated in autosomal forms of centronuclear myopathy. Such a role fits also with the implication of MTM1 paralogs MTMR2 and MTMR13 in forms of recessive demyelinating CMT (CMT4B). This study has provided insights into the function of myotubularin in muscle and a proof of principle that viral-mediated MTM1 gene delivery may be an effective therapeutic approach for patients with myotubular myopathy.

159

The first report of a *de novo* heterozygous missense *DISP1* mutation in a patient with congenital diaphragmatic hernia (CDH) and additional malformations. *S. Kantarci[1,2], F. O'Neill[1], M.K. Russell[1], K.M. Noonan[1,2], R. Pieretti-Vanmarcke[1,2], L. Mitova[3], J. Wilson[2,3], P. Dickman[4], K. Yboa[5], P.K. Donahoe[1,2], B.R. Pober[1,2,3].* 1) Massachusetts General Hosp., Boston, MA; 2) Harvard Med. Sch., Boston, MA; 3) Children's Hosp., Boston, MA; 4) Phoenix Children's Hosp., Phoenix, AZ; 5) Columbia Univ., New York, NY.

Background: Congenital diaphragmatic hernia (CDH) is a common birth defect with high mortality and morbidity. Although the etiology in many cases is unknown, recent reports suggest that genetic aberrations, including microdeletions and gene mutations, cause or contribute to CDH. We chose to sequence a novel candidate gene, dispatched homolog 1 (Drosophila) [*DISP1*], given its location in the chromosome 1q41 CDH- hotspot region as well as its role in the Sonic Hedgehog (SHH) pathway.

Methods: In 24 patients with CDH plus additional anomalies, including 5 patients with Fryns syndrome, we sequenced the 8 exon *DISP1*. We also used the multiple ligation-dependent probe amplification (MLPA) technique to screen for exon deletions/duplications. Sequence variants not found in SNP databases were genotyped in parents and in 96 controls.

Results: A missense heterozygous *DISP1* mutation, c.4412C>G, was identified in a multiply malformed male patient with left-sided Bochdalek hernia, facial dysmorphism, cleft lip/palate, VSD, developmental delay and additional musculoskeletal anomalies. This mutation, changing an evolutionarily conserved alanine to glycine at position 1471 (A1471G), was predicted to be deleterious by PolyPhen and SIFT. After confirming paternity, we showed that neither parents nor controls, carried this mutation. We did not detect any other *DISP1* point mutations or exon deletion/duplication in the 24 patients screened.

Conclusion: This is the first *DISP1* human mutation identified in a patient with CDH. *DISP1* is an attractive candidate gene as its protein product is required for SHH signaling, a pathway possibly important for normal diaphragm and lung development.

160

Genetic interaction of Bardet-Biedl syndrome genes and implication for polydactyly utilizing zebrafish model system. *M. Tayeh[1,2,3], H-S. Yen[1,2,3], J. Beck[1,3], C. Searby[1,3], H. Griesbach[4], E. Stone[3, 5], D. Slusarski[4], V. Sheffield[1,3].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Genetics Program, Univ Iowa, Iowa City, IA; 3) Howard Hughes Medical institute; 4) Dept Biology, Univ Iowa, Iowa City, IA; 5) Dept Ophthalmology, Univ Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder, characterized by obesity, retinal degeneration, postaxial polydactyly, cognitive impairment, hypogenitalism, renal and cardiovascular anomalies. Multiple BBS genes have been identified and mutations in each of these genes result in a similar phenotype. In zebrafish, we previously demonstrated that knockdown of any of the zebrafish bbs orthologues (bbs1-11) using antisense morpholinos (MO) display similar phenotypes including disruption of Kupffer's vesicle (KV), premature loss of KV cilia, organ laterality defects and delayed intracellular retrograde transport. We identified genetic interactions between a subset of BBS genes, using a pair-wise co-injection of low-dose bbs MOs. Low-dose knockdown of two genetically interacting bbs genes alters KV and retrograde transport. Since MO-induced phenotypes can be rescued by its wild-type RNA, we evaluated functional redundancy between bbs genes by testing if knockdown of one bbs gene can be rescued by RNA of a different bbs gene. As none of the RNAs was able to rescue different bbs MO-induced phenotypes, our data argue against functional redundancy but rather suggest a required stoichiometry within a multi-subunit complex. Of note, human polydactyly is a cardinal feature of BBS not reproduced in Bbs-mouse models. Since several bbs genes are expressed in the zebrafish fin bud, we evaluated Sonic hedgehog (SHH) expression due to its conserved role in the tetrapod limb patterning. Manipulation of SHH expression alters digit identity and in bbs morphant fin buds, we observe altered shh expression domains as well as subsequent changes in the fin skeletal elements. Consistent with the genetic interactions data, low-dose knockdown of two genetically interacting bbs genes alters shh expression and skeletal elements. Thus, SHH expression and skeletal patterning provide a model for BBS polydactyly. .

161

Growth retardation, hyperactivity, abnormal anxiety-related responses, and impaired neuromuscular and sensorineural coordination in a mouse model overexpressing *Rai1*. *S. Girirajan[1], N. Patel[2,3], R.E. Slager[4], M.E. Tokarz[5], M. Bucan[6], J.L. Wiley[5], S.H. Elsea[1,2].* 1) Department of Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Department of Pediatrics, Virginia Commonwealth University, Richmond, VA; 3) Department of Chemistry, University of the West of England, Bristol, U.K; 4) Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE; 5) Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA; 6) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA.

Gene dosage or copy number studies has taken a pivotal role in the discovery of complex morphogenetic and behavioral pathways in humans. Retinoic acid induced 1 (*RAI1*) when deleted or mutated results in Smith-Magenis syndrome (SMS), and duplication of 17p11.2, including *RAI1*, results in the dup(17)p11.2 syndrome. In order to assess the dosage sensitivity of *Rai1*, a BAC transgenic mouse overexpressing *Rai1* was created. Transgenesis was confirmed by copy number assessment and expression analyses for *Rai1*. Phenotypic consequences of *Rai1* overexpression were evaluated using both qualitative (modified SHIRPA) and quantitative (functional observational battery) methodologies for physical, neurological, and behavioral paradigms in transgenic and non-transgenic mice. Our analyses show that the *Rai1* transgenic mice have postnatal growth retardation which normalizes by adulthood. Further, the transgenic mice have increased exploratory motor activity, hyperactivity, and decreased anxiety-related rearing behavior. *Rai1* transgenic mice also have an altered gait with short strides and long sways, impaired ability on cage-top hang test, decreased forelimb grip strength, and a dominant social behavior. Analyses of homozygous transgenic mice with increased *Rai1* copy number and expression showed a dosage-dependent progression of severity of the phenotypes, including extreme growth retardation, severe neurological deficits, and increased hyperactivity. These studies indicate a definitive role for *Rai1*, in a dosage-dependent manner, in development, behavioral-modification, and neuromuscular and sensorineural coordination.

162

Hypomorphic mutations in the syndromic encephalocoele gene MKS1 perturb gastrulation movements and cause Bardet-Biedl syndrome. *C. Leitch[1], J.L. Badano[1], N.A. Zaghloul[1], C. Stotzel[2], B. Drehman[1], M. Al-Fadhel[4], R.A. Lewis[3], W. Eyaid[4], H. Dollfus[2], P.L. Beales[5], N. Katsanis[1].* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Laboratoire de Génétique Médicale EA 3439, Faculté de Médecine de Strasbourg, Université Louis Pasteur, Strasbourg, France; 3) 3Departments of Molecular and Human Genetics, Ophthalmology, Pediatrics, and Medicine, Baylor College of Medicine, Houston, TX 77030, USA; 4) Department of Pediatrics, King Fahad Hospital, Riyadh 11426, Saudi Arabia; 5) Molecular Medicine Unit, Institute of Child Health, University College London, London WC1N 1EH, UK.

Meckel-Gruber syndrome (MKS) is a genetically heterogeneous, neonatal lethal malformation and the most common form of syndromic neural tube defects (NTDs). To date, two MKS genes have been identified, MKS1 and MKS3, whose protein products are predicted to be involved in ciliary function. Here we show that mutations in MKS1 both cause Bardet-Biedl syndrome (BBS) and also have a potential epistatic effect on mutations in known BBS loci, since five of six families with MKS1 and BBS mutations manifested seizures, a feature that is not a typical component of either syndrome. Functional studies in zebrafish showed that mks1 is necessary for convergence and extension (CE) and that it interacts genetically with known bbs genes to modulate the severity of CE defects. Finally, in contrast to the exclusively null alleles found to date in all MKS1 patients, BBS-causing genotypes lead to reduced, but not extinguished, protein function, suggesting that BBS and MKS, although clinically distinct, are allelic forms of the same molecular disorder.

163

Mutations in insulin-like factor 3 receptor are associated with osteoporosis. *A. Ferlin[1], A. Pepe[1], L. Gianesello[1], A. Garolla[1], S. Feng[2], R. Morello[3], A.I. Agoulnik[2], C. Foresta[1].* 1) University of Padova, Department of Histology, Microbiology and Medical Biotechnologies, Centre for Male Gamete Cryopreservation, Padova, Italy; 2) Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77030, USA; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

Background: Insulin-like factor 3 (INSL3) is produced primarily by testicular Leydig cells. It acts by binding to its specific G-protein coupled receptor RXFP2 (Relaxin family peptide 2) and is involved in testicular descent during fetal development. The physiological role of INSL3 in adults is not known. The aim of the study was to verify whether reduced INSL3 activity could cause or contribute to some signs of hypogonadism, such as reduced bone density, currently attributed to testosterone deficiency. This was possible by the availability of the largest series of men with mutations in the RXFP2 gene. Methods: Extensive clinical investigation, including bone densitometry by DEXA, was performed on 25 young men (age 27-41) with the T222P mutation in the RXFP2 gene. Expression analysis of INSL3 and RXFP2 on human bone biopsy and human and mouse osteoblast cell cultures was performed by RT-PCR and immunohistochemistry. Real-time cAMP imaging analysis was performed on these cells. Lumbar spine of Rxfp2-deficient mice was studied by histomorphometric analysis. Results: Sixteen out of 25 young men with RXFP2 mutations have significantly reduced bone density. No other apparent cause of osteoporosis was evident in these subjects, whose testosterone plasma concentrations were in the normal range. Expression analyses showed the presence of RXFP2 in human and mouse osteoblasts. Stimulation of these cells with INSL3 produced a dose- and time-dependent increase in cAMP, confirming the functionality of the RXFP2/INSL3 receptor-ligand complex. Consistent with the human phenotype, bone histomorphometric analysis of Rxfp2-/- mice showed decreased bone volume. Conclusions: This study suggests a role for INSL3/RXFP2 signaling in bone metabolism and link RXFP2 gene mutations with human osteoporosis.

164

Mutations in NIMA-related kinase NEK8 causes nephronophthisis in humans and affects ciliary and centrosomal localization. *E. Otto[1], M. Trapp[2], U. Schultheiss[1], L. Quarmby[2], F. Hildebrandt[1].* 1) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

Nephronophthisis (NPHP), an autosomal recessive kidney disease, is the most frequent genetic cause of chronic renal failure in the first 3 decades of life. Mutations in 8 genes (*NPHP1-8*) have been identified and homologous mouse models for *NPHP2* and *NPHP3* have been described. Another mouse model of a recessive cystic kidney disease is the *jck* mouse, which is caused by a missense mutation G448V, in a highly conserved RCC1 (regulator of chromosome condensation) domain in Nek8. Under the hypothesis that mutations in *NEK8* might cause NPHP in humans, we performed mutational analysis in a worldwide cohort of 188 patients with NPHP by direct sequencing. We identified 3 different amino-acid changes L330F, H425Y, and A497P, which were absent from at least 80 healthy control individuals. All three mutations are within the RCC1 domain of Nek8, and the mutation H425Y is positioned within the same RCC1 repeat as the murine *jck* mutation. To test their functional significance, we introduced these mutations into full length mouse *Nek8 (mNek8)* GFP-tagged cDNA constructs. In transient overexpression experiments using inner-medullary-collecting-duct (IMCD-3) cells, sub-cellular localization of mutant Nek8 was investigated and compared to wild-type Nek8 expression. All mutant forms of Nek8 showed defects in ciliary localization to varying degrees. The murine mNek8 mutant H431Y (human H425Y) was completely absent from cilia and showed decreased localization to centrosomes. Overexpression of these mutants did not affect overall ciliogenesis, mitosis, or centriole number. Our finding that *Nek8*, when mutated, causes nephronophthisis type 9 strengthens the link between proteins mutated in cystic kidney disease and their localization at cilia and centrosomes.

165

Bioinformatics approach to identification of genes involved in multiple pituitary hormone deficiency: homeotic selector *Ash1l*. *S. Camper[1], N. Solomon[1], A. Mortensen[1], M. Brinkmeier[1], J. MacDonald[1], D. Ghosh[1], P. Carninci[2], Y. Hayashizaki[2], R. Lyons[1].* 1) Dept. Human Genetics, Univ. Michigan, Ann Arbor, MI 48109, USA; 2) Riken Genomic Sciences Center, Yokohama, Kanagawa 230-0045, Japan.

Mouse studies have advanced understanding of the underlying mechanisms pituitary hormone deficiency, often leading to the discovery of lesions in human patients. Mutations in the transcription factors POU1F1 and PROP1 cause pituitary hormone deficits. The progenitor cells in *Prop1* mutant pituitaries fail to migrate to form the anterior lobe, and poor vascularization and enhanced apoptosis are evident. These features contrast with those of *Pou1f1* mutants, which include normal vascularization and pituitaries that are indistinguishable from those of their normal littermates at birth. These differences support the idea that *Prop1* controls the expression of genes besides *Pou1f1* that are important for migration, survival, and differentiation of pituitary cells. These genes are candidates for cases of human hormone deficiencies of unknown etiology. We took several approaches to identify such genes, including construction of full-length cDNA libraries and microarray analysis of gene expression. Comparison of pituitary transcripts from newborn *Prop1* and *Pou1f1* mutants and their littermates revealed Wnt-frizzled signaling, organ morphogenesis, and anterior-posterior patterning processes were significantly different in *Prop1* mutants. The cDNA libraries from *Prop1* mutant and normal embryonic pituitaries contain over 40,000 sequences in a searchable database, implicating genes in some of the same processes, novel homeobox genes, and their regulators. We chose *Ash1l* for functional studies because absent, small, homeotic discs-1, a member of the trithorax gene family, is a critical regulator of homeotic selector genes in Drosophila. *Ash1l* homozygous mice exhibit severe growth insufficiency, anterior pituitary hypoplasia, poor survival, eye and craniofacial anomalies, balance problems, and fragile skeletal structure. This suggests that our bioinformatics approach will continue to contribute to understanding the genes that control pituitary organogenesis.

166

Loss of function of the *ACTN3* gene alters muscle metabolism and has been selectively favored during recent human evolution. *D.G. MacArthur[1], J.M. Raftery[1], G.A. Huttley[2], J.T. Seto[1], K.G.R. Quinlan[1], S. Easteal[2], N. Yang[1], K.N. North[1].* 1) Institute for Neuromuscular Research, Childrens Hospital at Westmead, Westmead, NSW, Australia; 2) John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia.

The protein α-actinin-3, encoded by the *ACTN3* gene, is a highly conserved component of fast muscle fibres. Remarkably, a nonsense variant in the human *ACTN3* gene (R577X) results in complete deficiency of α-actinin-3 in ~16% of the global human population. This variant is rare in Africans but approaches a frequency of 50% in Eurasian groups. We have previously shown that R577X is associated with human performance, with the XX null genotype being under-represented among elite sprint athletes, over-represented among elite endurance athletes, and associated with poorer muscle strength and sprint performance in non-athlete cohorts.

Given the effects of the 577X allele on muscle function and its high frequency in non-Africans we investigated whether this allele has been subject to natural selection in European and East Asian populations. Sequencing revealed low haplotypic diversity specifically associated with the 577X allele in both populations. In addition, analysis of HapMap data revealed a region of complete homozygosity associated with the 577X allele spanning more than 350 kb in Europeans, further than that associated with any of 17,000 frequency-matched control SNPs. The presence of low diversity and extended homozygosity associated with the 577X allele suggests that this allele reached its high frequency in a recent, rapid expansion, almost certainly driven by natural selection.

To characterise the mechanisms by which R577X affects muscle function we have generated a knockout mouse model of α-actinin-3 deficiency. Knockout mice have reduced grip strength and increased endurance capacity, consistent with the phenotype of 577XX humans. The muscle of knockout mice displays a marked metabolic shift towards the more efficient oxidative pathway. We propose that the effects of α-actinin-3 deficiency on muscle metabolism underlie recent positive selection on the 577X allele in humans.

167

A genome-wide autism association study identifies a common variant with sex-dependent effects at the neurexin-superfamily member *CNTNAP2*. *D.E. Arking[1], D.J. Cutler[1], C.W. Brune[2], T.M. Teslovich[1], K. West[1], M. Ikeda[1], A. Rea[1], M. Guy[1], S. Lin[1], E.H. Cook Jr.[2], A. Chakravarti[1].* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Institute for Juvenile Research, University of Illinois at Chicago, Chicago, IL.

Autism is a common childhood neuropsychiatric disorder that, despite high heritability, has largely eluded efforts to identify genetic variants underlying its etiology. We performed a two-stage genetic study, in which genome-wide mapping was validated by replication in an independent sample. In stage I, we screened 72 multiplex families, corresponding to 78 sib-pairs and 145 parent-child trios, using Affymetrix 500K arrays. Genome-wide linkage analysis revealed a single significant peak at 7q35 (lod = 3.4). A parallel genome-wide association analysis using TDT was performed for both single SNPs and haplotypes; however, no genome-wide significant results were observed. In contrast, TDT under the chromosome 7q35 linkage peak revealed a single SNP significantly associated with autism after correcting for the number of SNPs tested under the linkage peak by permutation ($P < 0.006$). This SNP is common (MAF = 0.36) and resides in an intron of the contactin-associated protein-like 2 (*CNTNAP2*) gene, which encodes a member of the neurexin family. To validate this initial finding, we genotyped an independent sample of 1,295 parent-child trios, and again observed over-transmission with the same allele ($P < 0.005$). Given the marked sex-difference in the incidence of autism, we examined transmission stratified by parental gender and by offspring gender. The overall transmission frequency (0.55) is significantly greater from mothers (0.61) than from fathers (0.53) in the combined sample and this parent-of-origin difference is significant ($P < 0.001$). This genetic effect is largely observed in affected males, although the rarity of affected females implies that this may be due to reduced power in females. In summary, we identified a common polymorphism in *CNTNAP2* that is significantly associated with autism susceptibility, and displays a parent-of-origin and gender effect recapitulating the inheritance of autism.

168

Identification of OXTR and MAFF deletions within independent autism families by whole genome tilepath microarray analysis. *S.G. Gregory[1], J.J. Connelly[1], S. Donnelly[3], R. Abramson[1], H. Wright[2], M. Cuccaro[3], J.P. Hussman[4], J.R. Gilbert[3], M.A. Pericak-Vance[3].* 1) Duke Center for Human Genetics, Durham, NC; 2) Dept of Neuropsychiatry, SOM-USC, Columbia; 3) Miami Institute for Human Genomics, University of Miami, Miami, FL; 4) The Hussman Foundation, Elliott City, MD.

Autistic disorder (AutD) is a neurodevelopmental disorder characterized by disturbances in social, communicative, and behavioral functioning. It has been established that at least 5% of individuals with idiopathic autism contain chromosomal rearrangements, suggesting that genomic loss or gain could underlie the development of AutD. Here we describe use of genome-wide tilepath microarrays, at 100kb resolution, to identify regions of chromosomal rearrangement within 119 unrelated probands (78% male, 22% female) from our unique multiplex AutD families. We identified 23 regions of genomic gain or loss (average 0.8 Mb) contained within one or more of the 119 AutD probands. Sixteen of these regions contain known copy number variants (CNVs) and segmental duplications, while seven regions did not. Six of the 23 regions localize to previous areas of possible genetic linkage, including 3p25.3 within an AutD male that contains the oxytocin receptor gene (OXTR). We also identified a small (250kb) deletion in 22q13.1 in an unrelated female AutD proband that contains the transcription factor MAFF, which has previously been shown to regulate OXTR. Quantitative RT-PCR analysis of OXTR and MAFF deletions in the parents and affected siblings of the probands show that the two deletions are familial and do not segregate with the disorder. However, given the coincidence of independent deletions within the oxytocin signaling pathway within unrelated affected individuals, we are carrying out detailed bisulfite sequencing to establish the methylation status of both genes which have previously been shown to be differentially methylated. Here we present the results of this analysis which further suggests the involvement oxytocin signaling pathway in the etiology of autism spectrum disorders and identifies MAFF as a new candidate gene in the development of autism.

169

A comprehensive association study of 106 candidate genes for Attention Deficit Hyperactivity Disorder. *B.S. Maher[1], B. Devlin[2], R.E. Ferrell[2], G.P. Kirillova[2], H. Chilcoat[3], E.L. Murrelle[3], R.E. Tarter[2], M.M. Vanyukov[2].* 1) Dept Psychiatry/MCV, Virginia Inst Psych/Behav Gen, Richmond, VA; 2) University of Pittsburgh, Pittsburgh, PA; 3) GSK, RTP, NC.

Attention deficit hyperactivity disorder (ADHD) is among the most common and heritable psychiatric disorders of childhood. We performed a comprehensive scan of several ADHD liability candidate gene systems, comprising 106 genes, using a 1536 SNP custom Illumina bead array in 313 Caucasian nuclear pedigrees. We used an iterative approach to candidate gene/SNP selection, first identifying SNPs involved in a series of candidate gene systems. Genes were ranked via consensus conference. The next steps focused on inclusion of functional SNPs and LD-coverage of the top ranking genes. All HapMap SNPs were selected in each of the candidate genes and submitted to Illumina for Quality Scoring (QS). SNPs returning a QS < 1 were deleted from the candidate list. The complete list of QS=1 SNPs for the top ranking candidate genes was submitted to the H-Clust algorithm for SNP selection. H-Clust identified 1500 SNPs that provided and average r2 coverage of .615 (based on HapMap) of the 106 highest-ranking candidate genes. In addition, all known non-synonymous common SNPs in each of the genes was selected for genotyping. Family-based association testing, accounting for parental phenotypes, of quantitatively-defined ADHD liability (factor analytically derived inattention (In) and hyperactivity-impulsiveness(HI)) was performed in PLINK. Multiple test correction was performed using FDR. Several genes yielded multiple significant results. Most notably SLC6A2, contained three SNPs (rs1948773: p = 0.0003; rs36009: p=0.0007; rs192303: p=.0.008) and COMT contained 2 SNPs (rs174675: p=0.0002; rs4485648: p=0.0002) that were significant for the HI and In phenotypes respectively.

170

Integration of novel statistical and biological methods identifies a causal SNP for schizophrenia in NOS1AP. *L. Brzustowicz[1], N.S. Wratten[1], H. Memoli[1], Y. Huang[3], M.A. Azaro[1], J. Messenger[1], J.E. Hayter[1], E.W.C. Chow[4], A.S. Bassett[4], S. Buyske[1,2], V.J. Vieland[3].* 1) Depts of Genetics and; 2) Statistics, Rutgers Univ, Piscataway, NJ; 3) Center for Quantitative and Computational Biology, Columbus Children's Research Institute, Columbus, OH; 4) Clinical Genetics Research Program, CAMH and Dept of Psychiatry, Univ of Toronto, ON.

We have previously shown linkage between 1q23 and schizophrenia and linkage disequilibrium (LD) with markers in NOS1AP in a set of European-Canadian families. We have also reported increased expression in schizophrenia of NOS1AP in postmortem samples from BA46, suggesting a regulatory mutation. Here we apply novel statistical methods and additional experiments to isolate at least one causal allele within the gene. Using the Posterior Probability of Linkage Disequilibrium (PPLD) to measure both the probability that a SNP is in LD with schizophrenia in these families as well as to estimate the extent of trait-marker LD, we are evaluating >130 SNPs (tagSNPs and additional SNPs from conserved regions) from the 300 kb genomic extent of the gene and flanking 5' and 3' regions. We have completed genotyping and analysis of 60 SNPs. Since causative mutations should show trait-marker D'=1, we prioritized all SNPs from this set with evidence of LD (PPLD >5%) and D' estimates of 1. This left just 4 of the 60 SNPs for functional testing. These 4 SNPs show strong LD with one another (all pairwise D' values >0.8). Three of these SNPs have been tested to date for regulatory function with a luciferase reporter assay, cloning the allelic variants into a vector with a luciferase gene and a NOS1AP promoter and transfecting into two neuronal cell lines with confirmed native NOS1AP expression. rs12742393 (PPLD= 43%, D'=1) demonstrated significant allelic expression differences in both SK-N-MC (p=.0002) and PFSK-1 (p<.0001). The allele associated with higher expression in this assay is also associated with higher expression in postmortem brain tissue and with schizophrenia in our family sample, implicating this allele in NOS1AP misexpression in schizophrenia. These studies bring us a step closer to understanding the causal genetic variants for schizophrenia.

171

High Risk Cohort Specific Variants in DISC1 are Identified and Associated with Schizophrenia with an Estimated Attributable Risk of 2%. *W. Song[1], J. Feng[1], W. Li[1], J. Longmate[1], L. Heston[2], S. Sommer[1].* 1) Dept Molecular Genetics, City of Hope, Duarte, CA; 2) Department of Psychiatry, University of Washington, Seattle, WA.

The association of DISC1 gene mutations with schizophrenia is controversial. In a large Scottish pedigree, a balanced translocation t(1;11)(q42.1;q14.3) disrupting the DISC1 gene segregates with major mental illness, including schizophrenia and unipolar depression. A frame-shift C-terminal deletion was reported in another Scottish family, but subsequently found in two controls. In addition, a few common structural variants have variably been associated with less than a two-fold increased risk for schizophrenia, but no large scale case control genotyping analysis has been performed. We have analyzed the regions of likely functional significance in the DISC1 gene with DOVAM-S and direct sequencing in 288 patients with schizophrenia (274 Caucasian, 14 African-American) and 288 Caucasian controls (5 megabases total). Six Cohort-Specific missense variants were found in patients with schizophrenia (p=0.01), and not in 6000 control samples, respectively (p<0.0001). In addition, two common missense variants were found in cases with significant excess ($p \leq 0.05$). We conclude that uncommon structural variants in DISC1 impart a high relative risk of schizophrenia with an attributable risk of 2%, while certain common structural variants increase risk slightly.

172

Genome-scan with a quantitative phenotype identifies new genes for the susceptibility to schizophrenia. *F. Macciardi[1], J. Turner[2], D. Keator[2], L. Geronazzo[1], J. Fallon[2], S.G. Potkin[2].* 1) Dept Sci & Biomedical Technol, Univ Milano, Milano, MILANO, Italy; 2) University of California, Irvine, USA.

Introduction: Genome-wide scans are now feasible given new chip technology and genome coverage. Methods: Rather than beginning with a candidate gene and looking for an associated neural phenotype our approach begins with an imaging phenotype (related to left hemisphere DLPFC activation), and then determines the variability in genes that contribute to it. The discovery sample (n=28) was genotyped on the Illumina Human1 (109,365 gene-centered SNPs) and subjects in the verification sample (n= 173) on the HumanHap300 (317,503 HapMap tagging SNPs). Results: Two genes, were identified by having at least one SNP whose QT analysis was significant at p < 10-8, with an empirical p-value of 10-6 by permutation. Four of the five top significant SNPs were on one gene (p's 10-8 to 10-6; empirical by permutation 10-6). A circuitry analysis revealed a consistent association between both genes and the prefrontal and dorsal neocortical circuits relevant to schizophrenia deficits. To establish if our method of gene discovery could successfully identify genes related to schizophrenia, we tested these SNP related to these two genes in an independent case-control study collected by the FBIRN consortium (www.nbirn.net). Fifteen SNPs in these genes are significant (< 0.05 to 10-5). Conclusions: Using a novel genome-wide screening strategy with brain activation as the quantitative phenotype we have discovered and verified the association of two genes with schizophrenia. Imaging-derived neural phenotypes are continuous, quantitative, richer than symptom-based diagnoses, and provide considerably more statistical power reflecting the greater penetrance of genetic effects at this more biologically proximate level.

173

Replicated analyses suggest a network of dopaminergic genes confer risk for schizophrenia. *M.E. Talkowski[1], M. Bamne[1], H. Mansour[1], K. Chowdari[1], J. Wood[1], L. McClain[1], G. Kirov[2], M.C. O'Donovan[2], M. Owen[2], B. Devlin[1], V.L. Nimgaonkar[1].* 1) Human Genetics and Psychiatry, University of Pittsburgh, Pittsburgh, PA; 2) Psychological Medicine, Cardiff University, Cardiff, UK.

Dopaminergic hyperactivity has been hypothesized in schizophrenia (SZ) genesis. We previously evaluated 18 dopamine (DA) related genes and detected associations with SLC6A3 (DAT), DRD3, SLC18A2 (VMAT2), and COMT. We report here comprehensive follow-up analyses.

Association Studies: We genotyped 69 tag SNPs at these four genes in two Caucasian samples: 1) 478 cases/501 controls from the US, 2) 659 trios from Bulgaria. In the US sample, we found significant associations at all four genes ($p<0.05$). Epistasis was observed between locus pairs in 17 of 117 total tests (interaction $p < 0.05$). Nearly half of the significant interactions (41.2%) included either rs3756450 (5' near promoter) or rs464049 (intron 4) at SLC6A3. In the Bulgarian cohort, significant associations were again detected with both of these SNPs (rs3756450, p=0.035; rs464049, p=0.017), and 5 of 17 epistatic interactions were replicated. Epistasis was most prominent between SLC6A3*COMT loci (interaction $p<0.005$ in both samples). The joint distribution of test statistics from both samples identified associations with 12 SNPs ($p_{joint}<0.05$).

Function: In silico experiments suggested changes in the binding pattern of transcription factors within the genomic region near rs3756450. We investigated rs3756450 and rs464049 using EMSA and observed band shifts at these SNPs. Intriguing allele-specific differences were found at rs3756450. Dual luciferase promoter assays also indicated allele-wise differences at rs3756450 in luciferase promoter activity ($p<0.0001$). The promoter activity exceeded observed differences of 6 other SNPs investigated within this 2.8kb region 5' to the DAT promoter.

Conclusions: We find replicable interactions between four key DA genes in SZ pathogenesis, centered on the dopamine transporter. Functional analyses suggest novel, allele specific effects for rs3756450 upstream of the SLC6A3 promoter. Futher analyses are warranted.

174

Polymorphisms in the SNAP25 gene are associated with early-onset bipolar affective disorder. *S. Jamain[1, 2], B. Etain[1, 2, 3], A. Dumaine[1, 2], F. Mathieu[1, 2], F. Chevalier[1, 2], J. Deshommes[1, 2], C. Henry[4], J.P. Kahn[5], F. Bellivier[1, 2, 3], M. Leboyer[1, 2, 3].* 1) INSERM U 841, IMRB, dept of Genetics, Psychiatry Genetics, Creteil, F-94000, France; 2) University Paris 12, Faculty of Medicine, IFR10, Creteil, F-94000, France; 3) AP-HP, Henri Mondor-Albert Chenevier Group, Department of Psychiatry, Creteil, F-94000, France; 4) Department of Psychiatry, Charles Perrens Hospital, Bordeaux, France; 5) Psychiatry and Clinical Psychology Department, CHU de Nancy, Jeanne-d'Arc Hospital, 54200 Toul, France.

The gene encoding the synaptosomal associated protein-25 kDa (SNAP25) has been associated to attention-deficit hyperactivity disorder (ADHD) in several independent studies. This gene is located on chromosome 20p12, in a region that we recently reported to be more frequently shared in early-onset bipolar affective disorder (BPAD) families, which is known to be a frequent ADHD comorbid condition. As an altered level of SNAP25 has been reported in bipolar patient's brains, we assumed that ADHD and early-onset BPAD may share common susceptibility variants in SNAP25. To test this hypothesis, we genotyped 7 polymorphisms along the SNAP25 gene in 197 patients with early-onset BPAD, 202 patients with late-onset BPAD and 136 unaffected subjects. Among patients, 154 were also assessed for ADHD symptoms. Early-onset BPAD was associated with two variants of SNAP25, one located in the promoter region (p=0.005) and another in the intron 7 of the gene (p=0.04). These associations were not explained by comorbid ADHD. Haplotype analysis showed the strongest association for a 4-markers haplotype in the 5' region of the gene (p=0.002), whereas the haplotype previously associated to ADHD was located in the 3'UTR. Altogether, these results suggest SNAP25 may be divided in two blocks of haplotypes, one located in the 5' part of the gene and containing a susceptibility variant for early-onset BPAD, and a second located in the 3'UTR and leading to ADHD vulnerability. This result may explain the high comorbidity rate between the two disorders.

175

Psychotherapeutic mechanisms of change: the role of genes in depression treatment outcome. *A. Kotte[1, 2, 3], J.R. McQuaid[1,3], J.R. Kelsoe[1,3].* 1) Psychiatry, University of California, San Diego, San Diego, CA; 2) Psychology, San Diego State University, San Diego, CA; 3) Veterans' Affairs Healthcare System, La Jolla, CA.

Introduction. Recent evidence suggests that genetic variation in the coding of BDNF (NTRK2) receptors and serotonin receptors (HTR2A) is associated with antidepressant (AD) response for major depression treatment. While no studies have examined whether these polymorphisms and their interactions predict response to psychotherapy, research using fMRI techniques has provided a model for the neurological basis of psychotherapy. Specifically, fMRI studies present evidence for neurobiological changes after treatment with cognitive behavioral therapy (CBT) similar to neurological changes after treatment with SSRI's suggesting commonalities in the biological mechanisms of psych- and pharmacotherapy. The current study investigated associations of the NTRK2 and HTR2A genotype with cognitive behavioral therapy (CBT) response.Methods. One hundred fifty one male veterans who completed a 16-week group CBT for unipolar depression in the past five years were initially contacted for this study. Sixty-five agreed to participate providing permission for review of their charts to assess treatment response. In addition, a 60 cc sample of blood was drawn for genotyping. Results. A repeated-measures ANOVA found a significant linear main effect for NTRK2, $F(1,65)= 4$, $p=.05$ and a significant linear interaction for NTRK2 and HTR2A, $F(2,59)=3.8$, $p = .03$. For the main effect, AA NTRK2 homozygosity did better than the GG and AG genotype (BDI change: 26.7 to 18.8). For the interaction effect, those with a NTRK2 GG and HTR2A GG genotype did significantly better (BDI change: 21 to 15.2) than those with a combination of the HTR2A AA and AG and NTRK2 GG genotype (BDI change: 25 to 22.4). Discussion. Findings are the first to suggest that NTRK2-HTR2A genotype interactions predict response to CBT in the unipolar depression population. These results support a model where psycho- and pharmacotherapy share similar neurobiological mechanisms of action. However, our current results are preliminary and need to be interpreted cautiously.

176

The Development of a Broad-Based ADME Panel for use in Pharmacogenomic Studies and Drug Development. *A.M.K. Brown[1], Y. Renaud[1], I. Mongrain[1], N. Gaudreault[1], C. Ross[2], C. Taylor Lawley[3], R. Shen[3], C.H. Lin[3], J-C. Tardif[1], M.S. Phillips[1].* 1) Genome Quebec & Montreal Heart Institute Pharmacogenomics Centre, Montreal, QC, Canada; 2) University of British Columbia, Vancouver, BC, Canada; 3) Illumina, Inc. San Diego, CA.

While the mechanisms of action and the therapeutic uses of many drugs may differ, they are all "metabolized" by a well known set of genes and pathways that can influence their pharmacokinetic responses. In order to address the inter-individual variability observed in these genes and pathways, we have set out to create a genotyping assay that encompasses the majority of the known variation present in key genes involved in the Absorption, Distribution, Metabolism and Excretion (ADME) of many therapeutic agents. We have incorporated into the panel previously published and predicted functional markers, as well as tag SNPs that account for blocks of Linkage Disequilibrium (LD) across all HapMap populations. The consensus candidate gene list was composed with input from both academia and the pharmaceutical industry. These genes can be grouped into four categories: Phase I and II metabolism enzymes, responsible for the modification of functional groups and the conjugation with endogenous moieties respectively; transporters, responsible for the uptake and excretion of drugs in and out of cells; and modifiers, that can either alter the expression of other ADME genes or affect the biochemistry of ADME enzymes. Many of the genes described above have been difficult to assay successfully in the past due to underlying polymorphisms and regions of homology. In collaboration with Illumina, we have used novel design strategies to overcome the genomic interference, resulting in optimized and validated assays. To date, the panel will support two of our current studies; one involving the toxicity of lipid lowering and novel anti-atherosclerotic agents, and a second involving cisplatin and anthracycline adverse reactions in children. Moreover, our panel has broad applicability to any study or clinical trial that would benefit from the evaluation of an extensive list of ADME genes.

177

Refining the molecular and clinical definitions for JP-HHT syndrome. *C.J. Gallione[1], C.L. Clericuzio[2], T.P. Leedom[1], J.C. Fahl[2], J.M. Drautz[2], J.D. Waldman[2], K. Henderson[3], M.J. Beis[4], M. Ludman[5], T. Berk[6], M.K. Maisenbacher[7], C.A. Williams[7], Z. Fan[8], A.S. Aylsworth[8], J. Garvie[9], M.E. Faughnan[10], R.I. White[3], D.A. Marchuk[1].* 1) Duke Univ Medical Center, Durham, NC; 2) Univ of New Mexico School of Medicine, Albuquerque, NM; 3) Yale Univ School of Medicine, New Haven, CT; 4) IWK Health Centre, Halifax, Canada; 5) Dalhousie Univ Faculty of Medicine, Halifax, Canada; 6) Mount Sinai Hospital, Toronto, Canada; 7) Univ of Florida College of Medicine, Gainesville, FL; 8) Univ of North Carolina at Chapel Hill, Chapel Hill, NC; 9) St. Joseph Hospital Medical Center, Phoenix, AZ; 10) St. Michael's Hospital, Univ of Toronto, Toronto, Canada.

Mutations in *SMAD4* are known to be a cause of the gastrointestinal cancer disorder Juvenile Polyposis (JP). We recently described individuals and families where *SMAD4* mutations co-segregate with a combined phenotype of JP and the inherited vascular disorder, Hereditary Hemorrhagic Telangiectasia (JP-HHT syndrome). The initial series of patients all met clinical criteria for both JP and HHT and showed a pattern of *SMAD4* mutations that clustered in the exons encoding the MH2 domain of the protein. Presented here are molecular and clinical findings from additional individuals that challenge both the apparent genotype:phenotype correlation and the clinical spectrum of the syndrome. We show that mutations in the MH1 domain of SMAD4 can lead to the vascular phenotype associated with JP-HHT, arguing that any mutation in *SMAD4* places the carrier at risk for JP-HHT syndrome. There is a high rate of *de novo* JP-HHT cases, and in a number of patients, the epistaxis and muco-cutaneous telangiectases are less pronounced than in typical HHT, suggesting that the Curaçao criteria for HHT diagnosis should be relaxed or modified for JP-HHT patients. The emerging clinical picture of JP-HHT is that of a young individual with JP who presents with visceral arteriovenous malformations, particularly in the lung, with no family history of either JP or HHT. Molecular diagnosis and careful monitoring for the full spectrum of JP-HHT symptoms in all *SMAD4* mutation carriers is critical to the clinical care of these patients.

178

Sporadic Venous Malformation is Caused by Somatic Mutations in TIE2. *V. Wouters[1], N. Limaye[1], M. Uebelhoer[1], J.B. Mulliken[3], L.M. Boon[1,2], M. Vikkula[1].* 1) Human Molecular Genetics, Christian de Duve Inst, Brussels, Belgium; 2) 2Center for Vascular Anomalies, Cliniques Universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium; 3) Vascular Anomalies Center, Children's Hospital, Boston, USA.

Venous malformations (VM) are the most frequent vascular malformations referred to vascular anomaly centers. An autosomal dominant familial trait, glomuvenous malformation (GVM), representing about 5% of venous lesions, is caused by premature truncation mutations in the glomulin gene, whereas another autosomal dominant form, termed cutaneomucosal venous malformation (VMCM), representing about 1% of venous lesions, is caused by gain-of-function mutations in the TIE2 gene. Recently, we identified several novel mutations in the TIE2 gene in VMCM patients, as well as, for the first time, a somatic second-hit deletion (see abstract N. Limaye et al.). The aetiology of sporadic VM, which represents more than 95% of venous lesions, has however remained unknown. Here we show that sporadic VMs are caused by somatic mutations in TIE2. We identified seven missense mutations in VM tissue DNA, which were however absent in blood DNA from these patients, and in tissue DNA from 89 controls. All the mutations, which were predicted by bioinformatic analysis to have deleterious effects of varying severity, were found to result in a strong in vitro ligand-independent increase in phosphorylation of TIE2. In some patients, we observed two mutations acting in cis. Such combinations on the same allele induced even higher phosphorylation levels of the receptor. Furthermore, we identified additional non-synonymous changes in TIE2 at the cDNA level, suggesting a somatic "second-hit" hypothesis to explain the localized nature of these lesions, as well as the presence of mechanisms that perhaps attempt to repair the dysfunctional allele. In conclusion, these data identify the etiopathogenic cause of sporadic VMs, thereby pinpointing the TIE2 signaling pathway for the development of novel therapeutic strategies, such as small molecule inhibitors. (miikka.vikkula@uclouvain.be).

179

Phenotypic features associated with TGFBR1 and TGFBR2 mutations in familial thoracic aortic aneurysms and dissections. *H. Pannu[1], V. Tran-Fadulu[1], M.C. Willing[2], A. Muilenberg[2], C. Ahn[1], D.M. Milewicz[1].* 1) Department of Internal Medicine, The University of Texas Medical School at Houston, Houston, TX; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

Mutations in the transforming growth factor β receptor type I and II genes (TGFBR1 and TGFBR2) cause thoracic aortic aneurysms and dissections (TAAD), but the full spectrum of the clinical disease is not fully delineated. Mutations in these genes may present as severe aortic disease in children with associated syndromic features, as in Loeys-Dietz syndrome (LDS), or as adult onset TAAD with an absence of syndromic features (FTAAD). Although there are large families reported with TGFBR2 mutations, no multigeneration families with TGFBR1 mutations have been reported. We report 4 multigeneration families with FTAAD due to TGFBR1 mutations (G312S, H315R, L486S, and R487W). To define the extent and progression of vascular disease associated with TGFBR1 and TGFBR2 mutations, we compared the clinical features of 29 affected individuals from 4 families with TGFBR1 mutations to 79 affected individuals from 5 families with TGFBR2 mutations (R460C, R460H). TGFBR1 mutation carriers presented with vascular disease at a younger age (30 years) than those with TGFBR2 mutations (44.8 years, p=0.0002). An effect of gender on vascular disease presentation and survival was evident with TGFBR1 but not TGFBR2 mutations. Women with TGFBR1 mutations presented more frequently with diffuse vascular disease than women with TGFBR2 mutations (p= 0.002). Men with TGFBR1/TGFBR2 mutations presented primarily with aortic disease. Women with TGFBR1 mutations had later onset and survived longer than men with TGFBR1 mutations, (p=0.006, p<0.05, respectively). The gender difference in age of onset in TGFBR2 mutation carriers approached significance (p=0.056), with no difference in survival. Three TGFBR2 mutation carriers had type A dissections at diameter below 5.0 cm (the guideline for repair) while 8 patients with TGFBR1 mutations had type A dissections, none at diameter below 5.0 cm. These data suggest gender based differences in vascular disease presentation, age of onset and survival between TGFBR1 and TGFBR2 mutation carriers.

180

Hutchinson-Gilford Progeria Syndrome(HGPS): Comprehensive characterization of 15 children. *M.A. Merideth[1,2], W.J. Introne[1], L.B. Gordon[3], M.B. Perry[4], S.B. Clauss[5], V. Sachdev[6], C.K. Zalewski[7], C.C. Brewer[7], J. Kim[7,8], J.C. Graf[4], A.C.M Smith[1,8], L.H. Gerber[9], J.A. Yanovski[10], D.L. Domingo[11], T.C. Hart[11], F.S. Collins[1], E.G. Nabel[6], R.O. Cannon[6], W.A. Gahl[1,2].* 1) NHGRI, NIH, Bethesda, MD; 2) Intramural ORD, NIH, Bethesda, MD; 3) Brown Univ, Providence, RI; 4) CC, NIH, Bethesda, MD; 5) CNMC,Washington, DC; 6) NHLBI, NIH, Bethesda, MD; 7) NIDCD, NIH, Bethesda, MD; 8) Georgetown UMC, Washington, DC; 9) GMU, Fairfax, VA; 10) NICHD, NIH, Bethesda, MD; 11) NIDCR, NIH, Bethesda, MD.

Hutchinson-Gilford Progeria Syndrome(HGPS), a sporadic autosomal dominant premature aging syndrome, has an incidence of 1/4-8 million. The cause is an abnormal lamin A protein(progerin), produced by a cryptic splice donor site activated by a GGC>GGT change in codon 608 of exon 11 of LMNA. HGPS is a multisystemic disease, uniformly fatal at an average age of 13y, with mortality primarily caused by cardiovascular disease. Progerin disrupts the nuclear scaffold and interferes with transcription; it also accumulates with age in normal cells, supporting HGPS as a model for studying the normal aging process. Fifteen children with HGPS, aged 1-17y, were investigated at the NIH between Feb 2005 and May 2006. Our studies confirmed the universal presence of sclerotic skin changes, bone abnormalities, joint contractures, alopecia, growth impairment, and decreased body fat; CV and CNS complications also occurred. New clinical findings included prolonged prothrombin times, elevated platelet counts and serum phosphorus levels, dental and oral soft tissue abnormalities, and a low-frequency conductive hearing loss. Bone density improved with age until 7y; % body fat decreased with age. Growth impairment was not due to inadequate nutrition, impaired insulin action, or growth hormone(GH) deficiency. GH treatment increased height growth by 10% and weight growth by 50%. Increased BP was common, and arterial studies identified diminished arterial distensibility, and increased carotid intima-medial thickness and augmentation indices. This comprehensive evaluation of the HGPS phenotype defines potential outcome parameters for therapeutic interventions, which may also apply to the normal aging process.

181

Phenotypic subclassification amongst individuals with cohesin-related Cornelia de Lange Syndrome: *SMC1A*, *SMC3* and *NIPBL* specific features. *D. Yaeger[1], M.A. Deardorff[1], M. Kaur[1], L.G. Jackson[2], I.D. Krantz[1].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Drexel University School of Medicine, Philadelphia, PA.

The Cornelia de Lange syndrome (CdLS) is a dominantly inherited multisystem developmental disorder with characteristic facial features, hirsutism, abnormalities of the upper extremities, growth and cognitive retardation. Mutations in the cohesin regulator *NIPBL* account for 60% of cases and in the cohesin structural proteins *SMC1A* and *SMC3*, another 5%. Severe CdLS is easily recognized, yet mild cases require an appreciation for the phenotypic variability to make an accurate diagnosis, especially as the spectrum continues to expand when inclusive of individuals with *SMC1A* and *SMC3* mutations. While ascertained as CdLS, most of the *SMC1A* and *SMC3* mutation-positive individuals have a distinct phenotype when compared to the more classic CdLS phenotype associated with *NIPBL* mutations. This bias in ascertainment of our study population suggests that a significant cohort of individuals with *SMC1A*/*SMC3* mutations may be undetected amongst populations of individuals with mental retardation. Here we summarize features of 18 individuals confirmed to have a *SMC1A* or *SMC3* mutation and delineate phenotypic differences that will help in classification and targeted molecular analysis. Unlike *NIPBL*-related individuals, birth weight and length are often in the normal range and several individuals had normal growth as they aged. None of the individuals had severe retardation commonly seen amongst individuals with *NIPBL* mutations. Strikingly, no medically significant structural anomalies were present in the *SMC1A*/*SMC3* cohort. Differences in facial morphology included a nose lacking a depressed nasal bridge and upturned tip, substituted with a more elongated and tubular shape; full eyebrows with prominent synophrys without a typical tented arch shape; and normally shaped ears without helical anomalies. As more individuals are identified with *SMC1A* and *SMC3* mutations, recognition of subtle phenotypic variations within the CdLS spectrum will allow for more efficient screening protocols for those that fall within it.

182

It's in your hands - a combined clinical, molecular and developmental approach to the diagnosis of radial ray defects. *R.A Newbury-Ecob[1], A. Sharif[2], M. Logan[3].* 1) Clinical Genetics, St Michaels Hospital , Bristol, United Kingdom; 2) Molecular Genetics, City Hospital, Hucknall Rd, Nottingham, United Kingdom; 3) National Institute for Medical Research, The Ridgeway, Mill Hill, London, United Kingdom.

Abnormalities of the upper limb are the second commonest congenital malformation. The last decade has seen the identification of a number of genes which cause syndromic radial ray defects (TBX5, SALL4, SALL1, RECQ4, FANC, del 1q21) allowing more accurate diagnosis and genetic counselling as well as delineation of the associated phenotypes. Knowledge of the role of these genes in normal and abnormal development comes from studies in various model systems. We have recently reviewed clinical information provided for over 100 cases presenting with radial ray defects for a diagnostic opinion and molecular genetic testing. Diagnostic criteria developed from clinical studies were shown to be highly predictive of a positive mutation result for patients with HOS, Okihiro and TAR) and allowed directing of further investigation. Phenotypic features such as the presence of additional malformations were significant as was the precise pattern of abnormality. In addition it is possible to draw on studies of the interactions and expression patterns in normal and knockout mice (e.g. SALL4, TBX5) which show correlation to the human phenotypes to aid diagnosis. With increasing pressure to justify the validity of diagnostic molecular genetic testing, a tailored approach to diagnosis in patients with radial ray defects utilising predominantly clinical assessment is presented.

183

Delineation of star syndrome (syndactyly, telecanthus, anogenital, and renal anomalies). *S. Unger[1,2], D. Böhm[3], W. Borozdin[1,3], B. Steiner[4], T. Schmitt Mechelke[5], K. Borowski[6], K. Keppler-Noreuil[6], G. Mortier[7], R. Sandford[8], B. Zabel[1,2], A. Superti-Furga[2], J. Kohlhase[3].* 1) Inst Human Genetics, Univ Freiburg, Freiburg, Germany; 2) Centre for Pediatrics and Adolescent Medicine,Univ Freiburg, Freiburg, Germany; 3) Center for Human Genetics Freiburg, Freiburg, Germany; 4) Institute for Medical Genetics, University of Zürich, Zürich, Switzerland; 5) Division of Neuropaediatrics, Children's Hospital Lucerne, Switzerland; 6) Division of Medical Genetics, University of Iowa, Iowa City, Iowa, USA; 7) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 8) Department of Medical Genetics, University of Cambridge, Cambridge, UK.

In 1996, Green et al. reported a mother and daughter with toe syndactyly and anogenital and renal malformations. Here we report four unrelated children with an identical constellation of malformations and an update on the original family. All four new cases came to attention because of anal atresia and pronounced lateral cutaneous syndactyly of the feet. Renal/urinary tract anomalies and abnormalities of the external genitalia were also present in every patient. Minor heart malformations (ASD and pulmonary artery stenosis), reproductive organ malformations (duplication of the vagina and/or uterus), and craniosynostosis were seen in some. All had strikingly similar dysmorphic features including telecanthus and lop ears. Chromosome analysis was normal in all. *SALL1* and *SALL4* were analyzed for mutations and deletions as mutations in these genes have been associated with anal atresia in Townes-Brocks and Okihiro syndromes, respectively, but no mutations were found. Also, as the children had fifth finger clinodactyly, the *MYCN* gene was analyzed to exclude Feingold syndrome and no mutations/ deletions were found. We hypothesize that this pattern of anomalies represents a distinct, possibly dominant syndrome and this was confirmed by detection of deleterious de novo mutations in a novel candidate gene in all four cases as well as detection of a mutation in the same gene in the patients reported by Green. The clinical and the molecular data (currently in preparation for submission) will be presented.

184

***SPECC1L*, a Novel Cytoskeletal Protein, is Haploinsufficient in a Patient with Bilateral Oblique Facial Clefts, Ocular Hypoplasia and Club Feet.** *I. Saadi[1], F.S. Alkuraya[1], J.J. Lund[1], A. Turbe-Doan[1], T.W. Glover[3], R. Erickson[2], R.L. Maas[1].* 1) Medicine, Brigham & Womens Hospital, Boston, MA; 2) Human Genetics, University of Michigan, Ann Arbor, MI; 3) Pediatrics, Genetics Section, The University of Arizona College of Medicine, Tucson, AZ.

Oblique facial clefts (OFC) and cleft lip and palate (CL/P) are complex birth defects that result from perturbation of fusion between the different facial processes during early embryonic development. As part of the Developmental Genome Anatomy Project, we have studied *de novo* balanced translocation cases with clefting phenotypes to discover genes involved in CL/P that may be impossible to determine by other means. We ascertained a patient with bilateral oblique facial clefts, ocular hypoplasia and club foot deformity and a *de novo* balanced chromosomal translocation 46,XX,t(1;22)(q21;q12). By FISH and Southern analyses, the 22q breakpoint was found to directly disrupt intron 14 of *SPECC1L* while the 1q breakpoint did not disrupt any gene. *SPECC1L* encodes a large protein predicted to have a single calponin homology domain (CHD) and 3 coiled coil domains (CCD). Whole mount *in situ* hybridization confirmed *Specc1l* expression in the 1st and 2nd branchial arches, the eyes and the hind limbs during embryogenesis. Interestingly, in transfected cells, Specc1l protein showed a spindle-like filamentous expression pattern, which co-localized with a subset of β-tubulin microtubules. Moreover, the tubulin-polymerization blocking agent, nocodazole, abolished the filamentous expression. Partially truncated constructs that lack the N-terminal CCD or the CHD also failed to show the filamentous pattern. Taken together, these data indicate SPECC1L to be a microtubule-associated protein (MAP) likely involved in cytokinesis and spindle formation, functions that are currently being tested. We are determining the cellular phenotype following shRNA knockdown of *SPECC1L* in cultured cells and in haploinsufficient patient lymphoblasts. The genetic etiology of OFC has remained largely elusive. The identification of a novel MAP involved in OFC will facilitate our understanding of this complex disorder.

185

Severe mutations of ARX are associated with an abnormal phenotype in most heterozygous females but not in mothers of affected children. *J. Sudi[1], M. Kato[2], G. Mancini[4], A. Toutain[3], S. Das[1], S. Christian[1], W. Dobyns[1].* 1) Dept. of Human Genetics, The University of Chicago, Chicago, IL; 2) Dept. of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan; 3) Service de Génétique et Service de Neuropédiatrie, Centre Hospitalier Universitaire de Tours, Tours, France; 4) Dept. of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands.

ARX (Aristaless-related homeobox) is a homeobox-containing gene involved in CNS, islet cell and testes development. In males, mutations in ARX have been associated with X-linked lissencephaly with abnormal genitalia (XLAG), infantile spasms and X-linked mental retardation with dyskinesia and epilepsy. We have identified 30 families with severe mutations of ARX including 26 reported (Kato et al., 2004) and 4 novel mutations, and here we present data on 22 heterozygous females including 12 ascertained as mothers of affected genotypic males. All affected males in these families had XLAG. Among the mothers, we found normal intelligence in 12/12, seizures in 0/8, and agenesis of the corpus callosum (ACC) in 3/6. Among the 10 other heterozygous females including 3 probands, we found normal development in 3/10 and variable mental retardation in 7/10. Seizures occurred in 5/7 females including infantile spasms, generalized convulsive and absence seizures. MRI demonstrated ACC in 7/9. We performed X inactivation (Xi) studies by methylation-specific PCR (Kubota et al., 1999) to assess the contribution of Xi to phenotypic variability. Xi was normal in 4 mothers and 3 other females, and skewed in one girl (89:11) with further studies underway. However, we found high normal Xi ratios above 70:30 in 3 mothers and 2 other females. Interestingly, one normal mother and her abnormal daughter were skewed in opposite directions (77:23 and 24:76), supporting the hypothesis that mild skewing may also influence the phenotype. We also detected post-zygotic mosaicism in 3 mothers but none of the other females. These data will significantly alter genetic counseling for ARX-associated disorders, and have broad implications for other X-linked diseases.

186

Clinical, cellular and neuropathological consequences of *AP1S2* mutations: delineation of a novel recognizable X-linked mental retardation syndrome, MESCH-X. *G. Borck[1], A. Molla Herman[5], N. Boddaert[2], F. Encha-Razavi[3], A. Philippe[1], L. Robel[4], F. Brunelle[2], A. Benmerah[5], A. Munnich[1], L. Colleaux[1].* 1) INSERM U781 and Medical Genetics; 2) Pediatric Radiology; 3) Fetal Pathology; 4) Child Psychiatry, Hôpital Necker-Enfants Malades, Paris, France; 5) INSERM U567, Institut Cochin, Paris, France.

X-linked mental retardation (XLMR) is a clinically and genetically heterogeneous condition. Recently, mutations in the *AP1S2* gene, encoding the σ1B subunit of the heterotetrameric clathrin-associated adaptor complex AP-1, have been reported in three XLMR families. Here, we report four patients belonging to two unrelated families in which *AP1S2* truncating mutations segregate. Besides previously reported clinical features such as hypotonia, delayed walking, aggressive behavior, small head circumference and speech delay, we found that autism spectrum disorder, calcifications of the basal ganglia appearing during childhood and highly elevated protein levels in cerebrospinal fluid (CSF) are also part of the disease spectrum. Based on these observations, we propose that *AP1S2* mutations are responsible for a clinically recognizable disease that we have named MESCH-X syndrome (mental retardation, elevated CSF protein, speech delay, cerebral calcifications and hypotonia in infancy, X-linked).

Mice deficient for AP-1 subunits γ and μ1A are embryonic lethal, demonstrating that AP-1 is essential for development. By contrast, no major alteration of the stability, subcellular localization and function of the AP-1 complex was observed in patient fibroblasts. Functional analyses suggested that, in these cells, the absence of σ1B protein can be compensated by another σ1 subunit. Interestingly, while neither macro-nor microscopic structural defects were observed in the brain of an affected fetus harboring a p.R52X mutation, preliminary results suggest decreased cerebellar staining of synaptophysin, a protein that interacts with the AP-1 γ subunit. Our results suggest that the observed phenotype is the consequence of a subtle and brain-specific defect of AP-1 dependent intracellular protein traffic.

187

Mutant mitochondrial genes in *Drosophila*: a model for mitochondrial dysfunction and disease. *B.H. Graham[1], Z. Li[1], E.P. Alesii[1], C.V. Ly[1], P. Verstreken[2], H.J. Bellen[1], W.J. Craigen[1].* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Laboratory of Neuronal Communication, K.U.Leuven, Leuven, Belgium.

Drosophila melanogaster has been established as a powerful genetic model system for many human diseases. To develop models of mitochondrial dysfunction and disease in *Drosophila*, P element alleles of nuclear-encoded mitochondrial genes are being examined as hypomorphic mutants as well as being utilized as mutagenic tools through imprecise P element excision. The first mutant to be examined is the fly functional ortholog (*porin*) of the Voltage-Dependent Anion Channel (VDAC). VDAC is the predominant pore-forming protein of the mitochondrial outer membrane. Analysis of flies homozygous for a P element imprecise-excision allele of *porin* reveals abnormal phenotypes including male infertility, neuromuscular dysfunction manifested by increased sensitivity to mechanical stress ("bang" sensitivity), and by synaptic abnormalities including a deficiency of mitochondria in pre-synaptic termini of neuromuscular junctions. In order to better understand VDAC's functional roles, a genetic screen to identify suppressors of increased bang sensitivity and male infertility has been performed. A series of deletions covering approximately 38% of the genome have been crossed into a homozygous mutant *porin* background and assessed for suppression of these phenotypes. From this pilot screen, several deficiencies that suppress bang sensitivity and/or male infertility have been identified. Interestingly, the strongest suppressor of bang sensitivity in the *porin* mutant background also suppresses increased bang sensitivity observed in P element mutants of two predicted orthologs of human mitochondrial disease genes: *SDHB* and *ATPAF2*. The analysis of *porin* mutant phenotypes validates *Drosophila* as a model for mitochondrial dysfunction that is relevant to mammals. The identification of suppressor loci for mutant mitochondrial phenotypes in *Drosophila* will provide new insights into mitochondrial function as well as potentially identify novel candidate therapeutic targets for mitochondrial diseases.

188

Drosophila NnaD mutant flies model mouse purkinje cell degeneration (pcd) and implicate mitochondrial dysfunction in Nna recessive phenotypes. *S.M. Jackson[1], G. Dunn[1], S.L. Baccam[1], L.J. Pallanck[2], A.R. La Spada[1].* 1) Dept Laboratory Medicine, Univ Washington, Seattle, WA; 2) Dept Genome Sci, Univ Washington, Seattle, WA.

The Purkinje cell degeneration (pcd) mouse is a unique recessive model of neurodegeneration, as pcd mice undergo dramatic postnatal degeneration of Purkinje cells and retinal photoreceptors, yielding a phenotype of ataxia, blindness, and male sterility. The causal gene for pcd is Nna1, encoding a protein (Nna1) that contains putative carboxypeptidase and ATP/GTP binding domains. How loss of Nna1 leads to neurodegeneration remains unclear. Analysis of Drosophila genome sequence revealed a single orthologue of mouse Nna1 (CG32627; NnaD) located on the Drosophila X chromosome. Overall, NnaD is 48% similar and 25% identical to Nna1; however, conservation is much higher in the carboxypeptidase domain (42% similar and 59% identical). To define the normal function of Nna1 and understand the implications of Nna1 loss-of-function, we studied NnaD with a Drosphila strain carrying a P-element insertion ($NnaD^{PL90}$) at the NnaD locus, a mutation that reduces NnaD expression to 10% of normal. Most $NnaD^{PL90}$ hemizygotes die during the larval stage of development, although ~30-40% survive to adulthood. $NnaD^{PL90}$ males have reduced lifespans (17d vs 40d), are sterile, and go blind. The sterility of $NnaD^{PL90}$ hemizygotes results from defective spermatid individualization, and was associated with apoptotic activation, as evidenced by increased activity of the executioner caspase Drice. Examination of $NnaD^{PL90}$ mutants revealed blindness due to progressive retinal degeneration. Ultrastructural analysis of degenerating retinae showed strikingly abnormal mitochondrial morphologies. All phenotypes of $NnaD^{PL90}$ mutants were rescued by ectopic expression of NnaD, confirming that these phenotypes derive from loss of NnaD. Our findings suggest that loss of NnaD in Drosophila recapitulates pcd phenotypes seen in mice lacking Nna1, and that loss of NnaD - Nna1 produces disease pathology by affecting mitochondrial function, pinpointing mitochondria as a likely target for other cerebellar and retinal degenerative processes.

189

Mitochondrial *ADCK3*, an ancestral prokaryotic kinase involved in Coenzyme Q biosynthesis, is mutant in a new form of recessive ataxia. *C. Lagier-Tourenne[1], M. Tazir[2], C. Quinzii[3], L. López[3], C. Busso[4], N. Drouot[1], M. Assoum[1], S. Makri[2], L. Pacha[2], T. Benhassine[2], M. Anheim[5], S. Schmucker[1], D. Lynch[6], F. Plewniak[1], C. Tranchant[5], O. Poch[1], J.L. Mandel[1], M. Barros[4], M. Hirano[3], M. Koenig[1].* 1) IGBMC, CNRS/INSERM/ULP, Illkirch, France; 2) Service de Neurologie, Centre Hospitalier Universitaire Mustapha, Alger, Algeria; 3) Department of Neurology, Columbia University College of Physicians and Surgeons, New York, NY, United States; 4) Departamento de Microbiologia, Universidade de São Paulo, São Paulo, SP, Brasil; 5) Department of Neurology, Hospital of Strasbourg, Strasbourg, France; 6) Children's Hospital, Philadelphia, PA, United States.

A SNP-based genome-wide scan in a large consanguineous family allowed us to identify a new locus for autosomal recessive ataxia at chromosome 1q41. We found deleterious mutations in *ADCK3* gene in 7 patients from 4 families. All patients have childhood-onset cerebellar ataxia with slow progression and few additional signs. The yeast homologue of *ADCK3* encodes for a mitochondrial protein and is mutated in the ubiquinone (or Coenzyme Q) deficient S. cerevisiae strain *coq8*. Two mutations identified in patients result in protein truncation. Three non-truncating mutations were introduced into the yeast *COQ8* gene and resulted in growth failure on selective respiratory medium, confirming the deleterious nature of these mutations. Likewise, we found low Coenzyme Q in muscle of one patient and impaired ubiquinone synthesis in fibroblasts of 2 out of 3 patients. Although its biochemical function in ubiquinone biosynthesis is unknown, COQ8 most likely has an indirect role, because COQ8/ADCK3 belongs to a small family of ancestral prokaryotic kinases. Coenzyme Q10 deficiency was previously identified in severe encephalopathy-nephrotic syndromes with defects in the biosynthetic pathway and, surprisingly, in ataxia-oculomotor apraxia 1 which is caused by a defective nuclear DNA repair protein. The identification of *ADCK3* mutations emphasizes the role of Coenzyme Q10 in the physiopathology of degenerative ataxias and raises the possibility of supplementation therapy.

190

Mitochondrial Dysfunction and Glutathione Depletion in a Murine Model of muto Methylmalonic Acidemia. *C. Venditti[1], R. Chandler[1], S. Shanske[2], P. Zerfas[3], T. Cowan[4], G. Enns[4], V. Hoffman[3], S. DiMauro[2].* 1) NHGRI/NIH, Bethesda, MD; 2) Columbia U, NY, NY; 3) DVM/NIH, Bethesda, MD; 4) Stanford U, Palo Alto, CA.

Methylmalonic acidemia (MMA) is commonly caused by defective activity of the enzyme methylmalonyl-CoA mutase (MUT). Patients with severe forms of MMA display a clinical phenotype of intermittent metabolic instability and multi-systemic secondary complications of unclear etiology. Mitochondrial dysfunction and oxidative stress have not been studied as pathogenetic causes. A methylmalonyl-CoA mutase (Mut) mouse model, generated by targeted deletion, was modified by outcrossing (C57BL6x129SvEV) carriers to other strains of inbred mice, followed by intercrossing to generate affected mice. Some Mut -/- G2 animals, which escaped the uniform neonatal lethality of the parental strain, were further studied. Mitochondrial function was assessed by electron microscopy and electron respiratory chain (RC) enzyme assays. Glutathione was measured in tissue extracts by a direct recycling assay or in whole blood using an LC-MS/MS technique. Protein markers of oxidative stress were examined by Western analysis. The G2 Mut -/- survivor mice had massive elevations of pathometabolites as well as poor growth and fragility but survived past weaning. Target tissues from these mice displayed a reproducible electron microscopic mitochondrial phenotype. Liver extracts showed RC dysfunction with a severe decrease in cytochrome c oxidase activity in affected mice. Glutathione pools were reduced in liver and whole blood of mutants. Western analysis suggested that Mn-SOD was upregulated in the liver of some Mut-/- survivors. This new murine model of severe but stable muto MMA, developed by background modification of an existing null allele, strongly demonstrates that modifier(s) of the lethal phenotype of MMA exist and might partly explain the clinical heterogeneity of the disorder. Furthermore, these studies document that RC dysfunction and glutathione depletion are inherent features of methylmalonic acidemia. Our results suggest treatment strategies, in mice and patients, directed towards improving mitochondrial function, repleting glutathione pools and protecting from oxidative stress.

191

Mitochondrial dysfunction caused by germline mutations in succinate dehydrogenase subunit genes in Cowden and Cowden-like syndromes. *K. Zbuk[1,2], A. Patocs[1,2], G. Lobo[1,2], T. Sadler[1,2], J. Stein[1,2,3], K. Waite[1,2,3], C. Eng[1,2,3].* 1) Genomic Medicine Institute; 2) Lerner Research Institute; 3) Taussig Cancer Center, Cleveland Clinic, Cleveland, OH.

The majority of patients with Cowden syndrome (CS) harbor germline mutations of the tumor suppressor *PTEN* . However, approximately 15% remain *PTEN* mutation negative, despite increasingly comprehensive analysis including deletion/rearrangment analysis and *PTEN* promoter mutation analysis. Additionally, a large number of patients are assessed for cancer risk who exhibit some features of CS, but who do not meet diagnostic criteria for CS. Referred to as CS-like, >90% of these patients do not have a detectable *PTEN* mutation. It is evident that patients with CS/CS-like phenotypes share certain clinical features with syndromes associated with germline mutations of genes encoding succinate dehydrogenase (SDH) and fumarate hydratase (FH), both part of the mitochondrial respiratory chain. This observation leads to the hypothesis that mitochondria dysfunction could be involved in the pathogenesis of CS. We screened lymphoblastoid cell lines from 128 *PTEN* mutation negative patients with CS or CS-like phenotypes, using manganese superoxide dismutase (MnSOD) protein expression levels as a marker of mitochondrial respiratory dysfunction. 32 patients had increased MnSOD expression levels. These patients were screened for germline *SDHB*, *SDHC* and *SDHD* mutations, and 4 (12.5%) patients were found to have a mutation, none of which were seen in 350 population based controls. Similar to what is commonly observed in patients with germline *PTEN* mutations, lymphoblastoid cell lines from these patients demonstrated activation of the anti-apoptotic pathways protein kinase B (Akt pathway) and mitogen activated protein kinase (MAPK). Finally, we demonstrate an alteration of mitochondrial function, but not *SDH* mutations in 3 of 8 (37.5 %) patients with CS and *PTEN* mutations. These findings suggest that CS syndrome is associated with mitochondrial dysfunction, and that this dysfunction can occur by different molecular mechanisms.

192

Global transcript profiles of adipose tissue in weight-discordant MZ twin pairs: Pathways behind acquired obesity. *J. Naukkarinen[1], K.H. Pietiläinen[2,3,4], A. Rissanen[2], J. Saharinen[1], P. Ellonen[1], H. Yki-Järvinen[3], M. Oresic[5], J. Kaprio[4], L. Peltonen[1,6].* 1) Dept Molecular Medicine, National Public Health Inst., Finland; 2) Obesity Research unit, HUCH, Helsinki, Finland; 3) Dept of Medicine, Division of Diabetes, HUCH, Helsinki, Finland; 4) Finnish Twin Cohort Study, Dept of Public Health, University of Helsinki, Finland; 5) VTT Technical Research Centre of Finland, Espoo, Finland; 6) The Broad Institute, MIT, Cambridge, MA, USA.

The metabolic consequences of obesity arise from a complex interaction of genes and environment, the contributions of which are difficult to discern. We aimed to expose biological pathways affected by acquired obesity using a unique study design of deeply phenotyped MZ twin pairs discordant for recent onset of obesity (n=14 pairs, age 25 years, 15.2 kg mean weight difference). This design facilitates identification of obesity-induced changes in biological pathways independent of genetic background. Body composition was carefully assessed using DEXA, MRI and spectroscopy, and insulin sensitivity by the euglycemic clamp technique. Transcript profiles of abdominal subcutaneous fat was done by Affymetrix U133 Plus 2.0 arrays. Lipidomics and amino acid measurements in serum and adipose tissue were done by liquid chromatography/mass spectrometry. The obese co-twins' subcutaneous fat revealed marked reductions in the mtDNA copy number. Our novel pathway analysis of transcript profiles reveal significant downregulation of mitochondrial branched-chain amino acid (BCAA) catabolism and upregulation of inflammatory pathways. The pathway changes correlate with liver fat accumulation, insulin resistance and hyperinsulinemia. Additional support was obtained from parallel changes in serum levels of insulin secretion-enhancing BCAAs and proinflammatory lipid species in serum and fat. The data provide compelling evidence for the inflammatory character of acquired obesity associated with significant defects in BCAA catabolism. These aberrations are closely associated with ectopic fat accumulation and insulin resistance, hallmarks of obesity from early on in young healthy adults.

193

Prenyldiphosphate synthase (PDSS1) and OH-benzoate prenyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders. *J. Mollet[1], I. Giurgea[1], D. Schlemmer[1], G. Dallner[2], D. Chretien[1], A. Delahodde[3], D. Bacq[4], P. de Lonlay[1], A. Munnich[1], A. Rötig[1].* 1) INSERM U781 and Department of Genetics, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France; 2) Department of Molecular Medicine and Surgery, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden; 3) Institut de Génétique et Microbiologie, UMR 8621 CNRS, Université Paris-Sud, Orsay, France; 4) Centre National de Génotypage, 2 rue Gaston Crémieux, 91057 Evry, France.

Coenzyme Q10 (CoQ10) plays a pivotal role in oxidative phosphorylation (OXPHOS), as it distributes electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. We have identified two novel inborn errors of CoQ10 biosynthesis in two distinct families. In both cases, enzymologic studies showed that quinone-dependent OXPHOS activities were in the range of lowest control values, while OXPHOS enzyme activities were normal. CoQ10 deficiency was confirmed by restoration of normal OXPHOS activities after addition of quinone. A genome-wide search for homozygosity in family 1 identified a region of chromosome 10 encompassing the prenyldiphosphate synthase gene (PDSS1) which encodes the human ortholog of the yeast COQ1 gene, a key enzyme of CoQ10 synthesis. Sequencing PDSS1 identified a homozygous nucleotide substitution modifying a conserved amino acid of the protein (D308E). In the second family, direct sequencing of the OH-benzoate prenyltransferase gene, the human ortholog of the yeast COQ2 gene, identified a single base pair frameshift deletion resulting in a premature stop codon (c.1198delT, N401fsX415). Transformation of yeast Δcoq1 and Δcoq2 strains by mutant yeast COQ1 and mutant human COQ2 genes, respectively, resulted in defective growth on respiratory medium showing that these mutations are indeed the cause of OXPHOS deficiency.

194

Cell biology, genetics and genomics; a powerful liaison to match genetic to phenotypic variation: the example of Reactive Oxygen Species. *H. Attar[1], K. Bedard[2], H. Prokisch[3], T. Meitinger[3], D. Mehta[3], E. Wichmann[3], ET. Dermitzakis[4], KH. Krause[2], SE. Antonarakis[1].* 1) Genetic Medicine, Geneva University, Geneva, Switzerland; 2) Rehabilitation and Geriatrics, Geneva University Hospital, Switzerland; 3) GSF Research Center, Human Genetics and Epidemiology, Neuherberg, Germany; 4) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Natural variation in DNA sequence contributes to individual differences in complex quantitative traits. To date, few cellular traits have been studied that are more closely related to clinical manifestations. Here, we investigate the production of reactive oxygen species (ROS), a complex cellular phenotype involved in a number of human disorders including trisomy 21. We assessed individual variation for ROS production in EBV-transformed B-lymphoblastoid cell lines (LCL) with a fluorescent AmplexRed assay to identify the genetic architecture and potential regulatory loci. We found substantial individual variation in ROS production and a heritability of 45% (10 CEPH families). We identified 2 genome-wide significant linkage signals on loci of Hsa12 and Hsa15. To further refine our search for contributing variation, we performed a genome-wide association analysis for HapMap individuals (N=60). Results confirmed previously detected linkage signals; in addition 8 new significantly associated loci were detected (2.2 million SNP markers, $P < 1.00\ e^{-8}$). Given the limited size of the HapMap population, we repeated a genome-wide association in an independent sample of LCLs of healthy German individuals (KORA project). Analysis of 200 LCLs confirmed the locus on Hsa15 and replicated two previously associated loci on Hsa4 and Hsa6 (550K Affymetrix SNP markers, $P < 1.00\ e^{-8}$). Furthermore, genome-wide gene expression variation of 47'000 transcripts from the HapMap population was correlated to ROS variation. Several genes close to linked and associated loci were among the highest correlations, providing additional biological evidence for the involvement of detected loci in regulation of ROS production. Cellular phenotypes could be used as proxies for complex disorders, and the approach described here may contribute to genetic dissection of these traits.

195

Mutation of *RRM2B*, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. *A. Bourdon[1], L. Minai[1], V. Serre[1,2], J-P. Jais[3], E. Sarzi[1], S. Aubert[1], D. Chrétien[1], P. de Lonlay[1], V. Paquis-Flucklinger[4], H. Arakawa[5], Y. Nakamura[5], A. Munnich[1], A. Rötig[1].* 1) U781, INSERM, PARIS,France; 2) Université Paris 7, PARIS, France; 3) Service de biostatistique et informatique médicale, PARIS, France; 4) Département de génétique médicale, NICE, France; 5) Human Genome Center, Institute of Medical Science, TOKYO, Japan.

Mitochondrial DNA (mtDNA) depletion syndrome (MDS; MIM 251880) is a prevalent cause of oxidative phosphorylation disorders characterized by a reduction in mtDNA copy number. The hitherto recognized disease mechanisms alter either mtDNA replication (POLG) or the salvage pathway of mitochondrial deoxyribonucleotides 5'-triphosphate (dNTPs) for mtDNA synthesis (DGUOK, TK2, SUCLA2). A last gene, MPV17, has no known function. Yet, the majority of cases remain unexplained. Studying seven cases of profound mtDNA depletion (1-2% residual mtDNA in muscle) in four unrelated families, we have found nonsense, missense, splice-site mutations and in-frame deletions of the p53R2 gene encoding the cytosolic p53-inducible ribonucleotide reductase small subunit. Accordingly, severe mtDNA depletion was found in various tissues of the p53R2-/- mouse. The mtDNA depletion triggered by p53R2 mutations in both human and mouse suggests that p53R2 has a crucial role in dNTPs supply for mtDNA synthesis.

196

Preimplantation diagnosis for mitochondrial DNA disorders: contribution to understanding mitochondrial DNA segregation during early human embryonic development. *J. Steffann[1], N. Gigarel[1], N. Frydman[2], P. Burlet[1], V. Kerbrat[3], G. Tachdjian[2], J.P. Bonnefont[1], R. Frydman[3], A. Munnich[1].* 1) Genetics Department, Necker Hospital, Paris, France; 2) Reproductive Medecine, Beclere Hospital, Clamart, France; 3) Obstetrics and Gynecology, Beclere Hospital, Clamart, France.

Mitochondrial DNA (mtDNA) mutations cause a wide range of serious genetic diseases with maternal inheritance. Due to the high transmission risk and the absence of efficient therapy in these disorders, "at risk" couples often ask for prenatal and/or preimplantation diagnosis (PGD). However, little is known about the factors that might determine the mutant loads (heteroplasmy) in a child of a carrier mother. Studies in animals and humans of pathogenic mtDNA mutations have suggested that a genetic bottleneck during oogenesis can affect the segregation of mtDNA sequence variants. We recently performed the first PGD for the NARP (Neurogenic weakness, Ataxia, Retinitis Pigmentosa) mtDNA mutation and an extremely skewed mtDNA segregation was observed supporting the hypothesis of a tight bottleneck during oogenesis. We performed 2 PGD for 2 women at risk of transmitting the MELAS m.3243A>G mutation, responsible for Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes, and the ND3 m.10197G>A mutation responsible for Leigh syndrome, respectively. Mutant loads were assessed in two oocytes and six embryos at risk of carrying either MELAS or ND3 mutations, by using PCR tests enabling single-cell quantification of heteroplasmy. Unlike NARP embryos, the majority of these oocytes and embryos were heteroplasmic (only one embryo was homoplasmic for the ND3 mutation). At this stage, comparative analysis of heteroplasmy in different blastomeres from non-transferable embryos (5 affected or arrested embryos) did not show any variation of the mutant DNA rate between cells from a given embryo. Only one embryo, carrying less than 10% of ND3 mutation was transferred but no pregnancy ensued. These results emphasize the wide variation of the bottleneck size, arguing that the nature of the mtDNA variant and/or individual factors might influence these variations.

197

SNP array mapping of 20p deletions: genotypes, phenotypes and copy number variation. *N.B. Spinner, A.J. Greco, B.T. Thiel, J. Glessner, P. Munoz, X. Gai, D.A. Piccoli, S.F.A. Grant, H. Hakonarson, I.D. Krantz, B.M. Kamath.* Dept Pediatrics, The Children's Hosp, of Phila, Phila, PA.

We analyzed 21 patients with deletions of 20p using the Illumina Human Hap550 SNP array to 1) establish genotype/phenotype correlations, 2) identify breakpoints and 3) investigate the use of the HumanHap550 platform for analysis of chromosome deletions. Deletions of 20p are relatively rare, although those that include JAG1 at 20p12 occur in 5% of patients with Alagille syndrome (AGS). The 21 patients had deletions of 20p identified by cytogenetics (N=6), molecular cytogenetics (N=11) or MLPA (N=4). Nineteen patients had clinical features of AGS, and these deletions included the JAG1 gene. Deletions ranged from 100 Kb to 14.62 Mb, and all of the breakpoints were unique. Seven patients had deletions sized between 100kb and 2.83 Mb, and all had normal development, with no clinical anomalies outside of those associated with Alagille syndrome. Apparently, haploinsufficiency for the 10 genes (excluding JAG1) within this region does not cause phenotypic anomalies. Eleven patients had deletions between 3 and 8.3 Mb, and of these, 8/11 had developmental delay. Only one patient was deceased, secondary to complications of AGS. Few additional anomalies were seen in this group, but these included renal anomalies (N=4), conductive hearing loss (N=2) and bifid uvula (N=1). Three patients with large, proximal deletions had the most clinical abnormalities, which included CNS and endocrine anomalies and hearing loss. These 3 patients all had significant delays, and features of autism and/or "savant" characteristics". In addition to defining the 20p deletions, analysis using the HumanHap550 array identified 31 different genome-wide copy number variants (>20 SNPs), with 1-5 variants called per patient. Deletions of the short arm of chromosome 20 are relatively mild, with limited clinical anomalies. The use of SNP arrays provides accurate high -resolution definition of genomic abnormalities. Copy number variants that are unlikely to be pathogenic were identified in all patients, and careful cataloging of these is crucial to correct interpretation of these studies.

198

Somatic partial chromosome 11 duplication in patients with Proteus Syndrome. *K. Duffy[1], D. Bick[1,2], P. vanTuinen[1], S. Dugan[2], A. Yilmaz[3], C. Schwartz[4], W. Foulkes[3], M. Olivier[1].* 1) Medical College of Wisconsin, Milwaukee, WI; 2) Children's Hospital of Wisconsin, Milwaukee, WI; 3) McGill University, Montreal, Canada; 4) J.C. Self Research Institute, Greenwood, SC.

Proteus syndrome (PS) is a rare sporadic disorder characterized by variable, progressive, asymmetric malformations and overgrowth in a variety of tissues. Here, we present evidence of a somatic duplication within chromosome band 11p15.2 as the cause of this disorder. We examined a fibroblast cell line derived from a surgically removed epidermal nevus from a 4-year old female patient diagnosed with PS. Copy number variant analysis using the Affymetrix GeneChip® Human Mapping 100K Set identified a partial duplication of chromosome 11 unique to the cell line from the affected tissue. The duplication was localized to 11p15.2, resulting in a partial trisomy spanning over 800 kb. The duplicated region was verified using five quantitative PCR assays (TaqMan) from within the region when compared with two assays from locations outside the duplicated region identified as chromosomally normal. The same region was also duplicated in two additional DNA samples from fibroblast cell lines derived from affected tissue from two previously described PS patients, but not in DNA from blood of the initial PS patient, nor in DNA from the parents or other control DNA samples from unaffected individuals. The region duplicated in all cell lines contains seven known genes. RNA expression analysis was run in triplicate for each sample and revealed that only the gene expression of two of these seven genes, PDE3B and CALCB, was significantly up-regulated (p-values <0.0004) in all three PS cell lines when compared to four age- and gender-matched control fibroblast cell lines. These two genes have been implicated in the regulation of cell growth in a variety of tissues, and may explain the symptomatic overgrowth seen in adipose, epidermal, and bone tissue of patients with PS. This study, for the first time, identifies a somatic mutation in affected tissue from patients with PS and suggests that the effect of this partial chromosomal duplication is mediated through increased gene expression of individual genes.

199

The 1q41q42 Microdeletion Syndrome: Characterization of a New Genomic Disorder. *T.H. Shaikh[1], S. Saitta[1], D. Kostiner[2], M. MacDonald[3], J.W. Ellison[4], A.S. Aylsworth[5], L.G. Shaffer[6].* 1) Children's Hosp Philadelphia, Phila., PA; 2) Kaiser Permanente, Portland, OR; 3) Duke Univ., Durham, NC; 4) Mayo Clinic, Rochester, MN; 5) Univ. of N. Carolina, Chapel Hill, NC; 6) Signature Genomic Labs, Spokane, WA.

Recent developments in microarray technology have greatly improved our ability to detect microdeletions and microduplications in patients with congenital abnormalities. We have identified a new, recurrent microdeletion in 1q41q42 in 7 patients with overlapping phenotypic features using microarrays. These microdeletions were detected by BAC arrays and further characterized by high-resolution, SNP-based oligonucleotide arrays. The most common clinical features include mental retardation, profound speech delay, seizures and distinct dysmorphic features. This strongly suggests that the 1q41q42 microdeletions may represent a new genomic disorder. The deletions range between 2.6-9.1 Mb, with a smallest region of overlap (SRO) of ≈1.17 Mb. The SRO contains 5 known genes, 4 of which have known functions. One of the genes, *DISP1*, is involved in the sonic hedgehog (SHH) pathway which is crucial in early brain development. *DISP1* may play a role in the neurologic features associated with the microdeletion. Interestingly, two highly identical 44 Kb segmental duplications (SDs) were detected in close proximity of the SRO. SD-mediated rearrangements are a common feature of known genomic disorders like DiGeorge and Williams syndromes. The presence of SDs in this region suggests that microdeletions in 1q41q42 may be more prevalent and predicts the existence of a reciprocal microduplication syndrome. Additionally, two of the more severely affected patients with the microdeletion were diagnosed with Fryns syndrome. Preliminary analysis suggests that these Fryns deletions may extend into more distal regions of 1q42. The identification and analysis of additional cases will help delineate the critical region for the syndrome and the gene(s) responsible for the phenotypic features. Furthermore, high resolution array analysis and breakpoint mapping will allow the elucidation of the mechanisms underlying recurrent microdeletions in 1q41q42.

200

complex Segmental Duplication Superstructure found on Human Chromosome 17q. *D. Chen[1], V. Leppä[2], T. Miettinen[3], A. Palotie[3, 4], L. Peltonen[2, 4], J. Saarela[2].* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA, USA; 2) National Public Health Institute, Helsinki, Finland; 3) Finnish Genome Center, University of Helsinki, Helsinki, Finland; 4) The Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Human Chromosome 17 is enriched for a variety of human neoplasia, genetic diseases and polymorphic structural variations. The underlying cause of such observations is chromosomal instability. In has been proposed that chromosome 17 is significantly enriched with segmental duplications. A major mechanism resulting in chromosomal instability is the non-allelic homologous recombination between 2 highly similar genomic sequences. To characterize segmental duplication on chromosome 17, we conducted genome-wide sequence alignment using expressed transcripts mapped within the previously identified duplicated domains on 17q22-24. The analysis identified a segmental duplicated superstructure (SDS) on the 17q arm. The superstructure consisted of 13 discrete genomic domains and shared significant sequence homology. A similar structure was also found on the sytentic chimpanzee chromosome. In addition, the segmental duplicated structure is found to enrich with retrotransposable sequence element. Analysis of the genomic sequences of the duplication domains revealed the most highly copied sequences to be retrotransposable sequence elements. Twelve retrotransposable mRNAs and their sequence copies were found almost exclusively within the SDS, both in the human 17q and the chimpanzee 19q. The highest numbers of sequence alignments were observed with two transcripts, AK125814 and AK125932. Interestingly, the AK125814 transcript was found to be expressed in 5/6 healthy human and two chimpanzee PBMC samples. Sequencing of the rt-PCR products showed that AK125814 was preferentially expressed from one of the duplicated locations, which varied between individuals. The complex duplication architecture on 17q may predispose to chromosomal instability via NAHR and possibly lead to disease causing copy number variations. Furthermore, the structure served a template in which accelerated chromosomal evolution can occur.

201

Nonrecurrent MECP2 duplications in neurodevelopmentally delayed males reveal a prone rearrangement region in Xq28. *C. Carvalho[1], A. Patel[1], T. Sahoo[1], C. Bacino[1], S. Peacock[1], A. Pursley[1], S.W. Cheung[1], J.R. Lupski[1,2,3].* 1) Dept Molecular Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, BCM, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Recent reports suggest not only impaired or abolished gene function, but also increased MECP2 gene copy number, resulting in a developmental delay (DD)and mental retardation(MR)phenotype. Virtually nothing is known about the rearrangements and mechanisms associated with MECP2 copy number alterations. In order to investigate this issue, we designed a tiling path oligonucleotide microarray spanning 4 Mb around the MECP2 region on the Xq28. So far we have analyzed 12 males, each one carrying different duplication sizes, varying from ~ 2.6 Mb to ~ 289 Kb. All duplicated patients have their distal breakpoints inside or near LCR pairs with more than 99% of sequence similarity. Interestingly, ten (83%) out of 12 patients presented the distal breakpoint grouped into the same 233 Kb region, located 35 Kb upstream to MECP2 gene. That region is characterized by a complex architecture with two large (~37 Kb each) low-copy repeats in direct orientation, and two smaller (~11.3 Kb each) inverted repeats. Remarkably, previous reports have shown these inverted repeats are implicated in the deletion of the Emerin gene which causes Emery-Dreifuss muscular dystrophy (EMD) and also generates the inversion present in 33% of females of European descent. We were also able to sequence one patient breakpoint whose duplication in tandem spanned about 1.7 Mb. Its breakpoint junctions occurred amid an Alu sequence and presented just 1 bp of sequence homology between them. In conclusion, these data show that the MECP2 nonrecurrent duplication results from specific genomic architectural features causing susceptibility to such rearrangements. The presence of LCRs in the MECP2 vicinity may generate an unstable non-B DNA structure which induces double-strand break, most probably re-joined by non-homologous end joining (NHEJ). Our study provides evidence that MECP2 duplication events may be stimulated by LCRs and further supports an alternative role of genomic architecture in rearrangements responsible for genomic disorders.

202

BubR1 deficiency causes centrosome amplification in PCS (MVA) syndrome. *S. Matsuura[1], H. Izumi[1], Y. Matsumoto[1], T. Ikeuchi[2], H. Saya[3], T. Kajii[4].* 1) Dept. Rad. Biol., RIRBM, Hiroshima Univ., Hiroshima, Japan; 2) MRI, Tokyo Med. Dent. Univ., Tokyo, Japan; 3) IAMR, Keio Univ., Tokyo, Japan; 4) Hachioji, Tokyo, Japan.

Spindle attachment to the kinetochores is monitored by mitotic spindle checkpoint to ensure accurate chromosome segregation in mitosis. Spindle checkpoint operates by delaying the onset of anaphase until all chromosomes have established bipolar microtubule attachment. PCS (MVA) syndrome is a disorder with premature chromatid separation (PCS), mosaic variegated aneuploidy (MVA), Dandy-Walker complex and other anomalies, and a high risk of childhood cancer. Patients with the syndrome are known to have mutations of the BUB1B gene and reduction of its product BubR1, a component of mitotic checkpoint. We found that cells from the patients with the syndrome show amplification of the centrosomes, multipolar mitoses, reduced centrosomal localization of BubR1, and increased activities of Polo-like kinase 1 (Plk1). Normalization of BubR1 expression or reduction of Plk1 in these cells corrected these abnormalities. Induction of overexpression of Plk1 in HeLa cells resulted in centrosome amplification. In view of these findings, we propose that BubR1 operates to prevent centrosome amplification through negative regulation of Plk1.

203

The effect of chromosomal rearrangements on gene expression. *L.A.J. Harewood, F. Schütz, M. Delorenzi, A. Reymond.* Centre for Integrative Genomics, Genopode Building, Lausanne, Switzerland.

Balanced chromosomal rearrangements, such as reciprocal translocations, resulting in no apparent gain or loss of genetic material are frequently occurring human chromosomal aberrations. An example is the t(11;22)(q23;q11) rearrangement, which is the only known recurrent non-Robertsonian balanced translocation in humans. Carriers are phenotypically normal, but are at risk of having progeny with Emanuel syndrome. It is conceivable that these large chromatin rearrangements influence the transcription levels of genes mapping both near, and distant, to the chromosome breakpoints, even if these genes are present in normal copy numbers. To test this hypothesis, we compared the gene expression profiles of lymphoblastoid cell lines and skin fibroblasts from 13 cytogenetically normal individuals, to those of 9 balanced translocation carriers and 4 Emanuel syndrome patients. This final group of individuals were included as proof of principle for the technique and to determine whether links could be made between the genes with altered expression and the phenotype, thereby providing a deeper understanding of the clinical basis of the syndrome. Comparison of unbalanced individuals with controls revealed twice as many gene expression changes than the balanced/control comparison. The greater number of differences in unbalanced individuals was anticipated as they are partially aneuploid for both HSA11 and 22 and are phenotypically affected. Consistently, a statistically significant fraction of the differentially expressed genes mapped to these two chromosomes. Permutation tests showed, however, that despite being lower, the number of differentially expressed genes between the two groups with complete genome complements is statistically significantly higher than that observed when one compares control samples alone. This suggests that balanced rearrangements have a greater effect on gene expression than normal variation even though individuals are phenotypically normal. Interestingly, the genes that show modified expression cluster to a set of genomic regions, indicating that these may be under a common mechanism of expression modulation.

204

Cytogenetic approaches for identifying novel genes and regulatory elements associated with hearing loss. *K. Kocher[1], R. Williamson[1,2], K. Arnos[3], K. Crow[4], J. Reiss[4], C.C. Morton[1,2].* 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA; 3) Gallaudet University, Washington, DC; 4) Kaiser Permanente Northwest, Portland, OR.

Genetic linkage analysis has been a powerful method for deafness gene discovery. However, this approach only detects mutations when families with sufficient numbers of deaf members are available. Additionally, genes affected by non-genic regulatory mutations are difficult to identify with this method. As an alternative, we have ascertained individuals with hearing loss and apparently balanced chromosomal rearrangements to assess candidate deafness genes lying near the rearrangement breakpoints. To illustrate the utility of this approach, we present two cases. In the first case, mapping the breakpoints of a t(2;13)(p24;q21) in an individual with profound sensorineural deafness revealed a novel gene, *FLJ21820*, disrupted by the translocation. RNA *in situ* hybridization experiments showed that the gene is expressed in the spiral ganglion, stria vascularis, and Organ of Corti of the inner ear suggesting it may be a deafness gene. Biochemical assays to determine the function of *FLJ21820*, mutation screening of a panel of deaf individuals, and characterization of a mouse model are in progress to verify the role of this gene in the auditory system. For the second case, an inv(7)(q21.3q35) segregating with conductive hearing loss in a family with five affected members seems to disrupt tissue-specific expression of the nearby *DLX5/6* genes. Transgenic mouse experiments suggest that a 5 kb region of 7q21.3 deleted at the breakpoint contains an enhancer necessary for proper expression of *DLX5/6* in the developing middle ear. Gel shift assays and *in vitro* expression experiments are planned to identify proteins that bind and activate the enhancer. These findings confirm the value of cytogenetics for discovery of novel genes and regulatory elements essential for development of the auditory system.

205

A Gene Dosage Map of the Human Genome: A Map with Clinical Utility. *J.D. Cody, P.L. Heard, A.C. Crandall, E.M. Carter, D.E. Hale.* Dept Pediatrics, Univ Texas Health Sci Ctr, San Antonio, TX.

We have developed an annotated dosage map of chromosome 18 that depicts the regions with known clinical consequence as well as regions identified as dosage insensitive. Data from a variety of sources has shown that there are thousands of regions in the genome that are dosage insensitive i.e. copy number variations (CNV). In the clinical realm, array comparative genomic hybridization (aCGH) detects changes in copy number in patients. Whether or not those changes are CNVs or have clinical relevance is not easily determined. We saw the need for clinical aCGH results to be compared to genome and gene function data in order to make predictions about potential phenotypic consequences. Predictions about possible phenotypic consequences would be valuable in planning and implementing therapeutic options for patients. We have combined data from the UCSC Genome Browser, with data on individual genes from the literature and our own data on critical regions linked to particular phenotypes in people with chromosome 18 deletions and duplications. These data were compiled to create a dosage map of chromosome 18 in which we assigned regions and/or genes to one of 4 general dosage categories: Haplolethal (dosage critical - prenatal lethal) Haploinsufficient (dosage sensitive) Conditional Haploinsuifficient (dosage sensitive dependent on other genetic or environmental factors) Haplosufficient (dosage insensitive) This information, displayed as a Custom Track on the UCSC Genome Browser, is now predominantly small genomic regions. It will evolve from genomic regions to specific genes as more is learned about gene function. This map allows us to align patient aCHG deletion/duplication data alongside the critical region and CNV data to determine what phenotypes the individual patient is at risk of developing. As more interventions are developed for the phenotypic manifestations of chromosome abnormalities, such a map will be the link between diagnosis and treatment.

206

Mitotic reduction divisions in adult murine hepatocytes. *A.W. Duncan[1], N.K. Paulk[1], M.J. Finegold[2], M. Grompe[1].* 1) Oregon Stem Cell Center, Oregon Health and Science University, Portland, OR; 2) Texas Children's Hospital, Dept. of Pathology, Houston, TX.

Hepatocytes are unique among cell types as they are frequently polyploid. We propose that hepatocyte polyploidization is a dynamic and reversible process that promotes liver adaptation. We previously showed that mouse hepatocytes derived by cell fusion of transplanted bone marrow cells may proliferate and restore normal liver function in an animal model of metabolic liver disease, the fumarylacetoacetate hydrolase (*Fah*) knockout mouse. Chromosomal analysis revealed the presence of fusion-derived hepatocytes containing half of the expected DNA content. We hypothesized that a fraction of fusion-derived hepatocytes underwent a "mitotic reduction division," yielding daughter cells with half the chromosomes of parental cells.

To determine whether mitotic reduction divisions play a role in normal liver regeneration, transplantation studies were performed. Highly pure FACS-sorted octaploid hepatocytes from wild-type mice were transplanted into congenic *Fah* knockout mice. Livers from transplanted animals showed extensive repopulation. Consistent with the idea that polyploid hepatocytes undergo reduction division during regeneration, immunohistochemical analysis of donor-derived nodules revealed the loss of one or more donor markers. Moreover, FACS and cytogenetic analysis of repopulated livers revealed donor-derived diploid, tetraploid and octaploid hepatocytes. Together these data showed that octaploid hepatocytes give rise to diploid cells during liver regeneration, demonstrating that hepatocyte polyploidization is reversible by mitotic reduction divisions. During reduction divisions independent markers segregate randomly, and we therefore propose that such divisions promote adaptation to hepatotoxic stress. The independent segregation of chromosomes from polyploid cells results in genetically heterogeneous diploid daughter hepatocytes. Daughter cells may inherit a selective advantage, yielding either a subset of normal cells more resistant to hepatotoxins or transformed hepatocytes with tumorigenic properties.

207

Haplotype-Sharing Test as a tool to map genes for familial cardiomyopathy. *F. Gerbens[1], J.P. van Tintelen[1], P.A. van der Zwaag[1], L.G. Boven[1], J.J. van der Smagt[2], R.N. Hauer[3], R.M.W. Hofstra[1], G.J. te Meerman[1].* 1) Department of Genetics, University Medical Center Groningen, Groningen; 2) Department of Clinical Genetics, University Medical Center Utrecht, Utrecht; 3) Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands.

In Mendelian diseases, such as arrhythmogenic right ventricular and dilated cardiomyopathies (ARVC and DCM), chromosomal regions identical-by-descent (IBD) from a common founder can be ascertained both by linkage analysis and by haplotype-sharing methods. Finding shared haplotypes is greatly facilitated with the currently available high-density SNP arrays. However, determining which of the shared haplotypes is IBD and contains the disease-associated mutation, and which are identical-by-state (IBS) and are shared by chance, is difficult. However the probability for shared haplotypes to be IBD rather than IBS increases with an increasing number of SNPs. We hypothesized that the largest shared haplotype is the most likely region to hold the causative disease mutation. We designed the Haplotype-Sharing Test (HST) using SNP genotyping data from isolated patients and parent-offspring pairs and trios to identify the largest possibly shared haplotypes between patients that are members of a (large but unobserved) pedigree. We applied HST to: (A) three distantly related families each with at least one ARVC patient using 10K SNP arrays, and (B) a large family with 5 DCM patients using 250K SNP arrays. In pedigree A a haplotype run of 118 SNPs spanning 32 MB on chromosome 12p12.3-q13.13 was identified. This haplotype is substantially larger than any other area that is shared due to random effects. Screening of the PKP2 gene, located in this region, revealed a pathogenic splice mutation. In family B (DCM) the largest shared haplotype was 178 SNPs, spanning 3.5 MB on chromosome 15. Sequencing of potential candidate genes (a.o. TPM1) is pending. Identification of the causative mutation in pedigree A in the largest shared region shows that our hypothesis, though heuristic in character, was correct. More importantly, besides linkage analysis, HST is a powerful tool for identifying disease-causing genes in single and extended families.

208

LDOrbits: Feature Definition in Genome Wide Association Studies. *P. Croteau, J. Segal, Q. Nguyen-Huu, T. Keith, J. Raelson, P. Van Eerdewegh.* Genizon BioSciences, St-Laurent, QC, Canada.

In GWAS, where typically more than 300,000 SNPs are analyzed, two issues become critical: 1) distinguishing between truly independent signals (and their respective ranking across the genome) and signals that are correlated due to linkage disequilibrium (LD) and 2) the challenging problem of multiple testing. To reduce the problem of multiple testing and increase the power to detect real signals we have developed a method, LDOrbits, that defines sets of SNPs or "orbits". An orbit consists of the SNP with the highest signal and all genotyped SNPs that are in high LD. Various thresholds for LD, as measured by r^2, can be considered. In turn, we identify the highest signal among the remaining SNPs that are not part of any orbits and proceed recursively to define all orbits in the genome. This methodology ensures that two orbits are not correlated, up to the chosen r^2 threshold. We then test for genome wide significance of these p-values by random permutations of case-control status. Although disjoint in the LD space, orbits often consist of non-contiguous sets of SNPs and several such orbits can be interlaced. We therefore compared it to the Asymmetric Running Average (ARA), which is implicitly based on LD but creates disjoint sets of contiguous SNPs. The ARA is based on the single SNP nominal p-values and determines separately the left and right boundaries of the signal where the mean $-\log_{10}$ p-values falls below a set threshold. To measure the advantage of defining orbits or ARA features before testing for genome wide significance, rather than considering each SNP separately, we compare the threshold for significance by each method based on the distribution of order statistics, in a scan of 370,000 markers. Considering first order statistics would lead to identical results among methods, but since true signals will not necessarily rank the highest, it is important to consider lower ranks as well. Using the second order statistic and its 95th percentile, LDOrbits with an r^2 value of 0.2 and ARA increase the threshold for significance for p-values from 9.2×10^{-7} to 1.25×10^{-6}. Similar effects are observed for lower order statistics.

209

Whole Genome Linkage Disequilibrium Association Mapping of Binary Traits. *P. Scheet[1], M. Stephens[2], G.R. Abecasis[1].* 1) Dept. Biostatistics, Univ. of Michigan, Ann Arbor, MI; 2) Depts. Human Genetics and Statistics, Univ. of Chicago, Chicago, IL.

Genome wide association (GWA) studies are being used to successfully identify many common alleles that underlie complex disease susceptibility. Most of these studies rely on commercial genotyping panels with 300,000 to 600,000 SNPs. Rarer SNPs, which account for the bulk of genetic variants in human populations, are less well-represented than other variants (directly or through tagging) in these commercial genotyping panels. Haplotype-based approaches may be able to capture the impact of these SNPs on disease susceptibility better than the single marker association tests commonly used in GWA analysis. Unfortunately, traditional haplotype-based methods suffer either from the "curse of high dimensionality" due to the large number of unique haplotypes in a sample, or from reduced power due to considering haplotypes of a small number of SNPs only. Inherent in these approaches is the added complication of first estimating haplotypes. We introduce a method for mapping disease associated variants in case-control studies. Our method may be applied to unphased data directly, summarizing the evidence for association at any position while taking into account the information on all flanking SNPs. The method allows the information from adjacent markers to decrease gradually, mimicking linkage disequilibrium (LD) patterns in the region. To flexibly capture such patterns of LD, we use a hidden Markov model for genetic variation based on clustering the latent haplotypes over short regions. Parameters are estimated with an expectation-maximization algorithm. We then test for association between the trait and each haplotype cluster. On simulated data, our method offers an increase in absolute power of 34% over single-marker tests when the disease allele is rare (< 5%) and typically untyped. A preliminary analysis of a large case-control study of type 2 diabetes (FUSION) using 2,335 individuals identifies promising leads for further analysis. We are currently extending our method, implemented in the software package fastPHASE, to deal with quantitative traits, as well.

210

BIMBAM: Bayesian IMputation Based Association Mapping. *Y. Guan, M. Stephens.* Human Genetics, University of Chicago, Chicago, IL.

To briefly explain the rationale for imputation-based methods, consider the "tag-SNP"; design for association studies, where SNPs are first identified (eg by resequencing) in a panel of individuals, and then a subset of these SNPs ("tags") are typed in the study sample. The imputation-based approach exploits the fact that tag SNPs are often good predictors for the other (non-tag) SNPs, to first "impute" the genotypes of all individuals at all non-tag SNPs, and then assesses the strength of the association between the imputed genotypes and the phenotype. The idea is that this both improves power to detect associations, and interpretability of results (by assessing which SNPs, both tag and non-tag, are the best candidates for causally affecting the phenotype). Imputation-based methods should also be helpful in combining data from multiple studies that have typed different SNPs in the same region (eg genome-wide scans using different genotyping platforms). Here, the idea is to use known patterns of correlation among the two sets of markers (eg from the HapMap data) to impute genotypes at all markers in all individuals, allowing the data from both studies to be used when assessing correlation between phenotype and each marker. We distribute a software package BIMBAM (Bayesian IMputation Based Association Mapping). Bimbam computes both single-SNP Bayes Factors (BFs) for each SNP, and, optionally, multi-SNP BFs for combinations of SNPs. The latter allows one to assess the potential that multiple SNPs in a data sets are combining to influence phenotype, and is intended for use in small genomic regions (e.g. candidate genes). The imputation is performed using the algorithm used in fastPHASE. BIMBAM can handle quantitative phenotype, where BFs are computed using the prior D2 from Servin and Stephens (2007). Bayesian logit regression based approach has been developed to handle case/control phenotype and will be integrated into BIMBAM shortly.

211

Bayesian approaches for detecting association in case-control studies. *D. Vukcevic, P. Donnelly.* Department of Statistics, University of Oxford, Oxford, United Kingdom.

Following recent marked successes, it seems likely that genome-wide association studies will become a method of choice for understanding the genetics of common human diseases. Questions of how best to analyse such studies remain unresolved. A typical initial approach would be to apply classical frequentist statistical tests based on contingency tables or regression models, such as the Cochran-Armitage, or Trend, test. The strength of evidence at each SNP is then summarised by the p-value of the test. This approach suffers from at least two disadvantages. (1) Interpretation of the p-value is difficult without also knowing the power of the test. Since power depends on allele frequency, a p-value of a given magnitude represents much weaker evidence of association at a rare SNP than at a more common SNP (assuming similar effect sizes in each case). (2) There is confusion about how to handle multiple testing. Several studies have shown that Bayesian approaches can have advantages in terms of power and efficiency, and the Bayesian analogue of a p-value, called the Bayes Factor, is often easier to interpret. Here we use data from recently published large association studies and simulations to compare and contrast the use and interpretation of p-values and Bayes Factors as measures of evidence in genome-wide association studies.

212

Coverage and power for genetic association studies using near-complete variation data from candidate genes. *T.R. Bhangale, M.J. Rieder, D.A. Nickerson.* Department of Genome Sciences, University of Washington Seattle, Seattle, WA.

Recent studies show that the HapMap captures most of the common variation and that SNPs derived from HapMap on the commercial genome-wide arrays provide promising coverage and power for association studies. Most evaluations of coverage and power have been based on using the Phase II HapMap data and SNPs in the ten ENCODE regions as the reference. We have used a near complete variation data set from SeattleSNPs, generated by resequencing of 24 Yoruban (YRI) and 23 CEPH (CEU) individuals from the HapMap panel, across 76 candidate genes, to evaluate the performances of the Phase II HapMap, and SNPs on the latest commercial arrays. Only 44% (YRI) and 48% (CEU) of relatively high frequency SNPs (minor allele frequency or MAF $\geq$ 0.3) were found in the HapMap. HapMap SNPs also revealed differences in the coverage for the two populations. While 84% of common (MAF $\geq$ 0.05) SNPs were captured at r2 $\geq$ 0.8 in CEU, the coverage in YRI was 70%. These estimates are lower than previous estimates using the ENCODE regions. Coverage of the common variation in SeattleSNPs by the commercial arrays was also considerably lower than that using HapMap as the reference. For example, for the Illumina 650K array, the estimates were: 44% (YRI) and 74% (CEU) for SeattleSNPs and 68% (YRI) and 90% (CEU) for HapMap. By extending a recently described haplotype-sampling approach for power evaluation, using more flexible disease model specifications, we quantified power over a range of effect sizes and common allele frequencies. Overall, the power estimates for HapMap and the arrays were found to be lower in YRI compared to CEU. Despite the differences in the coverage values, at higher effect sizes and in additive models, the power estimates of most arrays were quite similar to those of HapMap in CEU. In YRI and at lower effect sizes, however, the arrays and the overall HapMap variation were found to provide considerably lower power at the sample sizes on the order of 1,000.

213

A comprehensive analysis of the HapMap for trans- and long-range cis- associated SNPs - potential inference errors for genome-wide association studies. *R.W. Lawrence, L.R. Cardon, E. Zeggini.* WTCHG, Oxford University, UK.

Recent advances in high-throughput genotyping and a better understanding of human genome sequence variation have now made genome-wide association scans (GWAS) possible. However, exhaustive screening of common variation is not yet feasible. Therefore, inferences about the localisation of disease variants have to be made on the basis of GWAS results. Incomplete surveys of local linkage disequilibrium (LD) architecture could conceivably lead to misinterpretation of findings. We have calculated LD between every SNP pair (with MAF≥5%) from all three HapMap phase II samples (CEU, YRI, and JPT/CHB combined). We observe that a number of SNPs have at least one strongly associated (r^2≥0.7) marker on a different chromosome or at a distance greater than 1Mb on the same chromosome. 1.0% and 1.1% of common SNPs (MAF≥5%) were found to be strongly correlated (r^2≥0.7) with another variant at a distance greater than 1Mb (CEU and YRI respectively). Although the reason behind these observations in not yet clear, SNPs that have little or no LD with neighbouring markers but display trans-chromosomal and/or long-range (>1Mb) LD could represent mis-mapped variants. SNPs with both local and long-range/trans-chromosomal LD could stem from distal segmental duplications. Although relatively rare (11,770 out of approximately 2 million SNPs from the CEU sample), trans-chromosomal associations could lead to inference errors in the downstream interpretation of GWAS results. Several SNPs displaying long-range/trans-chromosomal associations in the HapMap phase 2 CEU sample (1,492 and 3,010 SNPs with trans-chromosomal and >1Mb associations (r^2≥0.7), respectively) are present in current whole-genome SNP chip arrays (Affymetrix 500k and Illumina 550k) so there is direct relevance to current GWAS. We are developing a resource enabling researchers to quickly retrieve information on these long-range associations for any given common HapMap SNP. This will conceivably help gene-hunters localise strong signals emerging from disease association studies, plan targeted replication strategies and delineate appropriate intervals for fine-mapping and resequencing.

214

Highly cost efficient genome wide association studies using DNA pools and dense SNP arrays. *S. Macgregor[1], Z.Z. Zhao[2], A. Henders[2], N.G. Martin[1], G.W. Montgomery[2], P.M. Visscher[1].* 1) Genetic Epidemiology, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 2) Molecular Epidemiology, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

Recent advances in large scale genotyping have made genome-wide association (GWA) possible. GWA is one of the primary tools for the identification of loci contributing to susceptibility to complex common disease. However, the major limiting factor in many GWA studies is cost. Individually genotyping GWA samples is often prohibitively expensive, with genome scans of suitable size (hundreds/thousands of cases and controls, hundreds of thousands of markers) typically costing over US$1 million. Alternative approaches which reduce the genotyping cost are therefore highly desirable. We will demonstrate that DNA pooling offers a means of dramatically reducing the cost of GWA studies. Building on previous work on Affymetrix arrays, new methodology will be outlined for statistical analysis of data from the Illumina platform, including a novel quality control metric. The method is based upon contrasting case and control pools and hence does not require independent estimates of rates of unequal amplification of alleles. Illumina and Affymetrix arrays were applied to the same pools; Illumina arrays were found to offer an order of magnitude decrease in pooling error variance compared with Affymetrix arrays. With Illumina arrays concordance with individual genotyping data is excellent; in terms of effective sample size it is possible to extract >80% of the information available with individual genotyping. Guidance will be given on best study design for pooling based GWA studies. It will be shown that even after taking into account pooling error, one stage scans can be performed for >100 fold reduced cost compared with individual genotyping. With appropriately designed two stage studies, individual genotyping can provide confirmation of pooling results whilst still providing ~20 fold reduction in total cost compared with individual genotyping based alternatives. The large cost savings with Illumina based pooling imply that future studies need only be limited by the availability of samples and not cost of arrays.

215

Broad and fine scale recombination rate variation in humans. *G. Coop, W. Wen, C. Ober, J.K. Pritchard, M. Przeworski.* Department of Human Genetics, University of Chicago, Chicago, IL.

The crucial roles of recombination in ensuring proper disjunction and maintaining genome integrity suggest that genetic exchanges should be tightly regulated. Yet recent studies in humans have revealed substantial variation in the total number of recombination events among females. At a much finer scale, analyzes of linkage disequilibrium indicate that the recombination landscape of humans and their closest evolutionary relatives, chimpanzees, differ markedly. These observations hint at the existence of tremendous variation in recombination rates within and between primate species, but the extent of variation over different genomic scales and its determinants remain largely unknown. Here, we infer recombination events in 422 male and 422 female meioses from a set of 500k genome-wide SNPs collected in 725 related Hutterites (a founder population of European descent). The refinement of crossover events offered by this dense marker set offers unparalleled opportunities to study fine scale rates of recombination in a pedigree. We find very strong support for the hotspot model of recombination of rate variation based on sperm typing and linkage disequilibrium (LD) studies. Specifically, we estimate that 60% of crossover events in both males and females occur in hotspots inferred from LD data. Strikingly, we further find significant variation among individuals in this proportion, suggesting differences among humans in the genome-wide use of recombination hotspots. The density of SNPs in our study also allows characterization of recombination rate variation over larger genetic scales, as well as association mapping of recombination phenotypes. More generally, this study illustrates how the imminent availability of dense genotyping data in large pedigrees will yield important insights into the recombination process and its effects on fertility.

216

Post genome-wide association challenges at the complex-disease associated locus *CD25* on chromosome 10p15. *C.E. Lowe, J.D. Cooper, J.A. Todd.* Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, University of Cambridge, UK.

Large-scale, genome-wide association studies of complex traits have led recently to the identification and confirmation of several new disease loci, a turning point in the genetic analysis of multifactorial disease. However, the next steps in the investigation of these loci are more challenging and uncertain. The aim is to correlate the most disease-associated variant(s) in the disease-associated region with phenotypes relating to the expression and/or function of genes in the region. However, as much as 30% of the common variation is unknown, any of which could be the causal variant(s). In order to evaluate the association of the interleukin-2 receptor gene (*IL2RA* or *CD25*) region on chromosome 10p15 with type 1 diabetes (T1D), we resequenced the entire 180 kb region (total 5.7 Mb), identified 737 polymorphisms, including 468 SNPs at MAF ≥ 0.05, of which 95 were novel. We genotyped 307 SNPs in 2,965 cases and 2,548 controls, and followed up 12 SNPs in 5,312 cases and 6,855 controls. Logistic regression identified two overlapping regions, covering 40 kb of intron 1 and the 5' of *CD25*, that were independently associated with T1D. The combined OR for the most associated SNPs, rs41295061 (susceptibility allele frequency, SAF=0.90) and rs11594656 (SAF=0.75), from these two regions was 2.04 (95% CI=1.70-2.45; P= 1.92×10^{-28}). Multiallelic and copy number polymorphisms have yet to be investigated. Nevertheless, we tested the two "current best SNPs" for association with the plasma concentration of the immune activation marker soluble CD25 in 1,357 T1D cases. Both rs41295061 and rs11594656 were associated with this biomarker (P = 1.88×10^{-8} and 2.15×10^{-23}, respectively), indicating that the association of chromosome 10p15 with T1D involves the function of *CD25*. None of the 11 SNPs from the two T1D-associated regions alter coding sequence or any known regulatory element in or near *CD25*, highlighting the complexity that disease-associated variants could have obscure roles in the regulation of gene expression.

217

Genome-wide Mapping of Allele-specific Protein-DNA Interactions in Human Cells. *N.D. Maynard[1], T.H. Kim[1], J. Chen[2], J.B. Fan[2], B. Ren[1].* 1) Cellular & Molecular Medicine, LICR - UCSD, La Jolla, CA; 2) Illumina, Inc., San Diego, CA, USA.

Recent investigations have reported differences in allele transcript levels of a large number of human genes. However, little is known about the mechanisms governing the majority of these differentially expressed alleles. Mapping of transcription factors and proteins involved in chromatin architecture to specific alleles should provide insight into the mechanisms involved in allele-specific expression. Here we combine chromatin immunoprecipitation (chIP) with SNP arrays (300K SNPs) to detect protein binding to the different alleles in human fetal fibroblasts (IMR90). We show significant variation in allele-specific binding of RNAP and insulator binding protein CTCF to approximately 4.2% and 7.9% of enriched heterozygous SNPs sampled, respectively. Our results confirmed allele-specific binding of RNAP and CTCF to the IGF2/H19 locus, as well as RNAP binding to other known imprinted loci. The methodology described here provides a window into understanding transcription by looking at binding of transcription machinery or markers to DNA in an allele-specific manner. This new level of information should lead to a greater understanding of the factors that play a role in allele-specific expression.

218

CTCF binding in *cis* regulates CAG/CTG instability at the spinocerebellar ataxia type 7 (SCA7) locus. *K.A. Hagerman[1], R.T. Libby[2], V.V. Pineda[2], R. Lau[1], J.D. Cleary[1], B.L. Sopher[2], D.H. Cho[3], S. Baccam[2], S.J. Tapscott[2,3], G.N. Filippova[3], C.E. Pearson[1], A.R. La Spada[2].* 1) Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) University of Washington Medical Center, Seattle, WA, USA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

Since the discovery of disease-associated CAG instability, *cis*-acting sequence elements, genomic context, and epigenetic modification have been thought to contribute to instability. The drastically different levels of repeat instability at different disease loci with repeats of identical sequence (spinobulbar muscular atrophy (SBMA) vs. SCA7) strongly supports the existence of *cis*-acting DNA elements that promote instability at certain loci. Similarly, the distinct patterns of repeat instability between tissues of the same patient argue for tissue-specific epigenetic or *trans*-factor regulation. However the mechanistic basis of this is yet to be described.

Binding sites for the CTCF chromatin insulator protein are adjacent to or flank the unstable CAG/CTG tracts at numerous disease loci. Using a mouse model of SCA7 CAG instability with (CAG)92, we tested the role of the 3'-CTCF binding site by assessing germline and somatic repeat instability in mice with either a wild-type or mutant CTCF binding site, respectively capable or incapable of binding CTCF protein. Transmitted and somatic instability was significantly enhanced in mice with mutant binding sites. CpG methylation, which ablates CTCF binding based on gel shift analysis, also enhanced CAG instability.

Our results thus implicate the CTCF binding site adjacent to the SCA7 CAG repeat as a *cis*-element regulating its instability, and indicate that CpG methylation is an epigenetic regulator of this element. These findings are the first data to implicate CTCF in genetic instability, and therefore have wide-reaching implications for instability at the many other disease loci where CTCF binding sites have been identified.

219

Genome-wide analysis of RUNX/AML target genes. *L. Cao, Y. Liu, J. Paschal, A.M. Bowcock.* Dept Genetics, Washington Univ, St Louis, MO.

RUNX1 is a member of the RUNX family of transcription factors and is involved in the development of hematopoietic cells. Recent genetic association studies have revealed a potential role for RUNX targets in the development of some inflammatory diseases including systemic lupus erythematosus, rheumatoid arthritis and psoriasis. In these instances, an associated SNP allele lies within a RUNX binding site, and leads to an increase in inflammation when compared to the effects of the other allele. RUNX1, 2 and 3 recognize the same target sequences and a RUNX3 knockout mouse has an inflammatory phenotype that includes asthma and inflammatory bowel disease. This provides further evidence that genes regulated by these transcription factors are important in the pathogenesis of inflammatory diseases. To identify RUNX targets that may play important roles in the prevention of autoimmune and inflammatory diseases we have performed chromatin immunoprecipitations (CHIP) from Jurkat T cells, using a RUNX1 antibody. RUNX1 pulldown DNA was either sequenced following conventional cloning and transformation, or after sequencing with 454 technology. With conventional cloning approaches we identified 61 potential RUNX1 targets that were pulled down more than twice. A search for RUNX1 transcription factor binding sites revealed that each of these 61 genes/regions contains at least 2 AML-1 binding sites. Four RUNX1 targets (CANX, ASCC1, SPINK5 and a novel gene) were further confirmed as being RUNX1 targets with electrophoretic mobility shift assays. SPINK5 (serine peptidase inhibitor, Kazal type 5) alterations are associated with the inflammatory skin disease Netherton syndrome and the common inflammatory skin disease, atopic dermatitis, and CANX (calnexin) is a major histocompatibility complex class I antigen-binding protein. Sequencing of RUNX pull down DNA fragments with 454 technology has permitted an analysis of over 24,000 potential RUNX targets and revealed a powerful method for the identification of additional targets that does not involve the bias created by conventional cloning and transormation methodologies.

220

A multi-lineage, whole-genome map of human DNaseI hypersensitive sites: identification of candidate functional elements underlying multiple common diseases. *P. Sabo, M. Kuehn, R. Thurman, J. Goldy, A. Haydock, M. Weaver, K. Lee, R. Sandstrom, S. Neph, W. Noble, M. Dorschner, J. Stamatoyannopoulos.* Dept. of Genome Sciences, University of Washington, Seattle, WA.

Recent whole-genome association studies have highlighted the importance of non-coding genetic variation in multiple human diseases. Identification of causal non-coding variants requires a detailed whole-genome map of diverse gene regulatory sequences. DNaseI hypersensitive sites (DHSs) in chromatin have long been known to mark the locations of human cis-regulatory elements including promoters, enhancers, repressors, insulators, and locus control regions. We created comprehensive, high-resolution whole-genome maps of DNaseI hypersensitive sites in fourteen human cell types using whole genome tiling DNA microarrays and high-throughput DNaseI tag sequencing by Solexa. We mapped >600,000 DNaseI hypersensitive sites, of which 45%; are cell type-specific. The vast majority of cell-type-specific DHSs are distant from transcriptional start sites; by contrast, >90%; of promoters are DNaseI hypersensitive in more than one cell type. This suggests that cell type-specific gene regulation is determined mainly by distal elements (enhancers, LCRs, etc.). Combining the DHS map with HapMap polymorphism data revealed distinct classes of DHSs that are under selection in modern human populations. Recent whole-genome association studies have identified numerous non-coding loci, some very far from genes, that contain risk alleles for common diseases including diabetes, inflammatory bowel disease, cardiovascular disease, and colon and prostate cancer. Strikingly, we find that the majority of risk alleles mapped far from genes are in fact proximal to dense clusters of DHSs, which presumably mark the functional elements harboring the causative alleles.

221

Identification and characterization of cell type-specific and ubiquitous chromatin regulatory elements. *G.E. Crawford[1], H. Xi[2], H.P. Shulha[2], J.M. Lin[2], T.R. Vales[1], Y. Fu[2], D.M. Bodine[3], R.D.G. McKay[4], J.G. Chenoweth[4], P.J. Tesar[4], T.S. Furey[1], B. Ren[5], Z. Weng[2].* 1) Institute for Genome Sciences & Policy, Duke University, Durham, NC; 2) Boston University, Boston, MA; 3) National Human Genome Research Institute, Bethesda, MD; 4) National Institute of Neurological Disorders and Stroke, Bethesda, MD; 5) Ludwig Institute for Cancer Research, University of California San Diego, La Jolla, CA.

The identification of regulatory elements from different cell types is necessary for understanding the mechanisms controlling cell type-specific and housekeeping gene expression. Mapping DNaseI hypersensitive (HS) sites is an accurate method for identifying the location of functional regulatory elements. We have used a high throughput method, called DNase-chip, to identify 3904 DNaseI HS sites from six cell types across 1% of the human genome. A significant number (22%) of DNaseI HS sites from each cell type are ubiquitously present among all cell types studied. Surprisingly, nearly all of these ubiquitous DNaseI HS sites correspond to either promoters or insulator elements: 86% of them are located near annotated transcription start sites (TSS) and 10% are bound by CTCF, a protein with known enhancer blocking insulator activity. We also identified a large number of DNaseI HS sites that are cell type-specific (only present in one cell type); many of these regions do not map to promoters, are enriched for enhancer elements and correlate with cell type-specific gene expression as well as cell type-specific histone modifications. Finally, we find that approximately 8% of the genome overlaps a DNaseI HS site in at least one the six cell lines studied, indicating that a large percentage of the genome is potentially functional. Collectively, these results show that ubiquitous chromatin structures are predominantly associated with promoters and insulators while enhancers tend to associate with cell type-specific chromatin structures.

222

Chromatin accessibility is associated with recombination hotspots of genomic rearrangements. *M.O. Dorschner[1], M.A. Weaver[2], A. Haydock[2], J. Goldy[2], K. Lee[2], S. Vong[2], F. Neri[2], A. Shafer[2], P. Sabo[2], J.A. Stamatoyannopoulos[1,2].* 1) Department of Medicine , University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA.

Despite our ability to identify and delineate genomic rearrangements, our understanding of the underlying molecular mechanisms that cause them lags behind. Many studies have implied that chromatin structure plays a role in mediating rearrangements, however few provide evidence to support their hypotheses. Our lab has generated detailed in vivo chromatin profiles of the human genome from many cell types using several high-throughput genome-scale technologies. These assays detect DNase I hypersensitive sites (DHSs) which coincide with active functional elements. Recently, we examined these chromatin accessibility data to determine if DHSs are located in close proximity to known recombination hotspots, in particular, those associated with genomic disorders and transclocations. Our analysis yielded several remarkable findings: 1) DHSs are collocated with recombination hotspots, even within segmental duplications known to mediate large rearrangements such a those causing Neurofibromatosis Type I, Charcot-Marie-Tooth, Smith-Magenis syndrome, Williams Beuren syndrome and many other genomic disorders; 2) DHSs are found at both meiotic and mitotic breakpoint clusters, including translocations involving IGHA1 and BCR; 3) all recombination hotspots were found within regions of open chromatin and 4) the terminal ends and subfragments of segmental duplications are often delineated by DHSs. These findings have major implications for the study of chromatin structure and its impact on recombination. A comprehensive map of DHSs will provide investigators with information to facilitate the localization of rearrangement breakpoints, particularly those found within segmental duplications. We hypothesize that variation in chromatin structure at recombination hotspots predisposes individuals to rearrangement. Such predispositions may be valuable when predicting genetic disease risk. Analysis of chromatin structure has the potential to further our understanding of the molecular pathogenesis of genomic rearrangements.

223

Combinatorial Potential of Human Enhancers. *L.A. Pennacchio[1,2], A. Visel[1], E.M. Rubin[1,2].* 1) Genomics Division, MS84-171, Lawrence Berkeley National Laboratory, Berkeley, CA; 2) DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA.

The expression of human genes are increasingly being found to be controlled by a modular architecture of multiple distant-acting enhancers that are individually sufficient to target gene expression to specific tissues. However, it is not known to what extent the local arrangement of enhancer modules with different specificities affects their individual impact on gene expression in time and space. To determine if enhancer modules can function in a combinatorial manner outside of their original genomic context, we concatenated developmental enhancers from different genes and defined how the artificial presence of heterologous enhancer modules affects their individual activities in transgenic mice. In all cases tested we observe compound patterns that are additive combinations of the individual enhancer activities and maintain their remarkable spatial and temporal precision, and we did not observe aberrant expression sites artificially created by potential positive interactions across enhancer modules. Conversely, even in cases where two elements drove expression in close anatomical proximity, such as within the developing limb bud, the compound patterns also showed no signs of cross-inhibition or interference between individual elements, indicating that mammalian enhancers do not commonly repress transcription in tissues where they are inactive. This additive modularity suggests that mammalian enhancers are discrete, functionally independent units with the potential to serve as fundamental building blocks of cis-regulatory architecture in evolution and synthetic biology.

224

A whole-genome association study of global gene expression. *L. Liang[3], A.L. Dixon[1,2], M.F. Moffatt[1], W. Chen[3], S. Heath[4], K.C.C. Wong[1], J. Taylor[2], I. Gut[4], M. Farrall[2], G.M. Lathrop[4], G.R. Abecasis[3], W.O.C. Cookson[1].* 1) National Heart and Lung Institute, Imperial College London, SW3 6LY, England; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, OX3 7BN, England; 3) Center for Statistical Genetics, Dept. of Biostatistics, SPH II, Ann Arbor, MI 48109-2029, USA; 4) Centre National de Genotypage, 91057 Evry Cedex, France.

Variation in gene transcription is important in mediating disease susceptibility, and global identification of genetic variants that regulate transcript abundance will be helpful in mapping human disease genes. We systematically mapped the effects of polymorphism on global gene expression by genome-wide association (GWA). We genotyped 408,298 SNPs to identify expression quantitative trait loci (eQTLs) from measurements of 54,675 transcripts representing 20599 known genes in EBV-transformed lymphoblastoid cell lines (EBVL) in 400 children from nuclear families recruited through a proband with asthma. We found that 15,084 transcripts (28%) representing 6660 genes had heritabilities (H2) > 0.3, exemplifying the wide extent of human genetic variability. We executed whole genome association scans for each of these heritable gene-expression traits and found individual peak association LOD scores between 3.68 and 59.1. We were able to identify significant SNP associations for 81% of traits with an overall H2 > 0.8 with our marker panel, suggesting good coverage of the eQTL genome. The most highly heritable traits were strikingly enriched with gene ontology descriptors for response to unfolded protein (chaperonins and heat shock proteins), regulation of progression through cell cycle, RNA processing, DNA repair, immune responses, and apoptosis. SNPs that regulate expression of these genes are candidates to modify degenerative diseases, malignancy, infection and inflammation. We provide several examples of how our genome-wide eQTL database can be used in the context of disease gene mapping, and we have developed web-based tools to aid others to apply our findings to loci associated with complex diseases.

225

Functional interactions of conserved non-coding (CNCs) sequences with other CNCs using circular chromosome conformation capture (4C). *D. Robyr, G. Duriaux-Sail, S. Polti, C. Wyss, S. Deutsch, S.E. Antonarakis.* Genetic Medicine and Development, University of Geneva Medical School, Geneva, Geneva, Switzerland.

The comparison of human chromosome 21 (Hsa21) sequences with the mouse syntenic regions led to the identification of roughly 3500 regions displaying an identity of >70% over a length of a least 100 nucleotides of ungaped alignment. About 65% (~ 2300) of these are conserved non-coding sequences (CNCs).Very little is known about the function of most CNCs. We speculated that a functional CNC may interact with its genomic target (i.e. an enhancer would bind to it's cognate gene promoter). Thus, the identification of any part of the genome that interacts directly with a CNC could provide clues on the function of the latter. We have generated libraries of CNC-interacting DpnII fragments by chromosome conformation capture (4C) whose identity is determined by subsequent sequencing. We have generated initial results concerning crosslinking of 18 Hsa21 CNCs with DNA fragments mapping hundreds of kilobases away from the "bait" on the same chromosome, or with fragments on other chromosomes. A total of 245 such potentially interacting DpnII DNA fragments have been identified so far. Interestingly, the median distance from the cloned DpnII fragments to the nearest conserved region is 767bp with a pvalue of 0.045 when compared to the distribution of the median of the distances of 1000 random samples of 245 fragments. These results provide initial evidence that the function of CNCs is mediated by their interaction with other conserved regions. We are now using high-throughput sequencing technology in order to increase the pace of characterization of the CNCs-interacting loci.

226

Ultraconserved Knockout Mice are Viable. *N. Ahituv[1,2,3], A. Visel[1], Y. Zhu[1], L.A. Pennacchio[1,4], E.M. Rubin[1,4].* 1) Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA; 2) Department of Biopharmaceutical Sciences, University of California, San Francisco, CA; 3) Institute for Human Genetics, University of California, San Francisco, CA; 4) US DOE Joint Genome Institute, Walnut Creek, CA.

Ultraconserved elements, sequences exhibiting 100% identity over 200bp or greater between human-mouse-rat, have been suggested to retain extreme evolutionary conservation due to their essential functional properties. To investigate the necessities of these elements *in vivo*, we removed four non-coding ultraconserved elements (ranging in length from 222 to 731 base pairs) from the mouse genome. To maximize the likelihood of observing a phenotype, we chose to delete elements adjacent to genes that exhibit marked phenotypes both when completely inactivated in the mouse as well as when their expression is altered due to genomic modifications. Remarkably, all four resulting lines of mice lacking these ultraconserved elements were viable and fertile, and failed to reveal any critical abnormalities when assayed for a variety of phenotypes including growth, longevity, pathology, and metabolism. In addition, more targeted screens based on the knowledge of the adjacent genes' biological characteristics did not show any notable abnormalities. These results, while clearly not inclusive of all the possible phenotypic impact of the deleted sequences, indicate that extreme sequence constraint does not necessarily reflect crucial functions required for viability.

227

Constitutional telomere shortening may be a predisposition to young onset microsatellite stable colorectal cancer. *L.A. Boardman[1], D.L. Riegert-Johnson[1], R.A. Johnson[2], S.L Slager[3], S.J Achenbach[3], S.N. Thibodeau[2], G.M. Petersen[4].* 1) Dept Internal Medicine, Mayo Clinic Colege of Medicine, Rochester, MN; 2) Dept of Laboratory Medicine and Experimental Pathology, Mayo Clinic College of Medicine, Rochester, MN; 3) Division of Biostatistics,Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; 4) Division of Epidemiology,Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN.

Background and Significance: Telomeres are repetitive base pairs sequences that cap linear chromosomes and protect them from unraveling. With progressive cell division, telomeres will shorten; leading to regulated cell senescence and apoptosis in healthy cells. A phenomenon associated with aging, telomere shortening has been associated with many diseases of aging, including cancers. Though telomere shortening has been documented in colorectal cancer (CRC) tumor cells, constitutional telomere shortening has not been rigorously studied in CRC patients. Methods: We evaluated telomere length by quantitative PCR in peripheral blood lymphocytes DNA from 114 CRC patients and compared to telomere length in 98 individuals serving as healthy controls on the basis of having no current or prior history of cancer. Statistical analysis: Results are presented using medians and interquartile ranges (IQR). The Wilcoxon Rank Sum Test was used to perform between group comparisons. All tests were two-sided and p-values <0.05 were considered statistically significant. Results: Peripheral lymphocyte telomere length was statistically significantly shorter in patients with CRC than in age matched controls ($p< 0.001$). Men with CRC had significantly shorter telomeres than women with CRC ($p=0.003$). There were no significant differences seen in telomere lengths between CRC patients or healthy controls on the basis of tobacco exposure. Conclusion: Constitutional telomere shortening is associated with an increased risk for MSS colorectal cancer in patients ≤ 50 years of age. Men with CRC had a higher degree of constitutional telomere shortening than did women with cancer.

228

TopoIIIα, a Member of the BRAFT Complex, functions in the Fanconi anemia pathway. *A. Hemphill, S. Philip, M. Al-Dhalimy, S. Olson, R. Moses.* Dept Molecular & Medical Gen, Oregon Health & Science Univ, Portland, OR.

Fanconi anemia (FA) is a recessive disorder characterized by an increased cellular sensitivity to interstrand crosslinks (ICLs), manifest at the cytogenetic level by the formation of chromosomal radials. FA is caused by a defect in any of at least 13 known genes. Following ICL damage, a core complex of at least 8 FA proteins (A, B, C, E, F, G, L, M) forms in the nucleus, resulting in the mono-ubiquitination of FANCD2. A number of these core complex proteins have also been shown to form a complex with Bloom protein (BLM), replication protein A (RPA), and TopoIIIα, known as the BRAFT complex. We have previously presented data showing that BLM is functionally epistatic to the FA core complex for the repair of ICLs. We have now used siRNA depletion of TopoIIIα to study its role after ICL damage. Depletion of TopoIIIα in a normal human fibroblast cell line results in decreased survival following exposure to ICLs, as well as increased formation of radials. To study the role of TopoIIIα in the FA pathway, we then depleted TopoIIIα in several FA cell lines. Unlike in normal fibroblasts, depletion of TopoIIIα did not result in an increased sensitivity to ICLs in the FA cell lines. Thus, as we have reported for BLM, TopoIIIα appears to be epistatic to the FA pathway for ICL repair. This indicates that the BRAFT complex is a functional complex for ICL repair.

229

The Fanconi Anemia pathway plays a critical role in recombinational telomere maintenance in ALT-immortalized human cells. *H. Root[1,2], M.S. Meyn[1,2].* 1) Program in Genetics & Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept of Molecular & Medical Genetics, Univ of Toronto, Toronto, ON, Canada.

Fanconi Anemia (FA) proteins are implicated in genetic recombination, a process involved in Alternative Lengthening of Telomeres (ALT) pathways. We find that FANCD2, A, and G form nuclear foci that localize to telomeric foci and PML bodies in ALT, but not in telomerase-positive or primary human cells. Co-IP experiments indicate ALT-specific in vivo interactions in late S/G2 cells between FANCD2, the Bloom syndrome helicase BLM, and the telomeric protein TRF2. FANCD2 localization to ALT telomeric foci is independent of ATM or ATR, but requires monoubiquitination by the FA core complex. Depletion of BLM significantly decreases FANCD2 association with ALT telomeric foci, but does not affect non-telomeric FANCD2 foci formation. This suggests that FANCD2 may have a function at ALT telomeres that is independent of its putative role in replication fork rescue.

ALT cells depleted of FANCD2 by siRNA exhibit increased telomere dysfunction-induced foci, telomere entanglements, and extrachromosomal telomeric DNA. FANCD2 depletion also has a severe ALT-specific effect on viability, nuclear morphology and chromosome stability. Nuclei with large holes, bridging, multiple lobes/micronuclei appear 3-5 days after FANCD2 depletion. These abnormalities only occur at low frequencies following FANCD2 depletion of telomerase positive cells and ALT cells forced to express telomerase, suggesting that these nuclear abnormalities result from ALT-specific telomere dysfunction. FANCD2-depleted ALT cells also exhibit supernumery centrosomes, re-replicated DNA, aneuploidy, cohesion and condensation problems. In contrast, these abnormalities are not found at high frequencies in FANCD2-depleted telomerase positive cells.We hypothesize that FANCD2 and the FA pathway play a critical role in limiting telomeric recombination and/or resolving telomeric recombinational events in ALT cells. In the absence of FANCD2, telomeres may be aberrantly entangled during mitosis, leading to mitotic failure and continued cell growth without proper segregation of DNA, resulting in multiple secondary abnormalities.

230

The telomeric protein TRF2 and Nijmegen Breakage syndrome protein NBS1 modulate the association of the ataxia-telangiectasia protein ATM with DNA damage. *P. Bradshaw[1], W. Wang[1], D.J. Stavropoulos[1,2], M.S. Meyn[1,2].* 1) Genetics & Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Molecular & Medical Genetics, Univ of Toronto, Toronto, ON, Canada.

Eukaryotes use complex networks to respond to as few as 2-4 induced double-strand breaks (DSBs) in genomic DNA. The ATM protein kinase plays a key role in the major human DSB response network by associating with damaged chromatin, then activating DNA repair, cell cycle checkpoints, and other damage responses. To better understand the ATM response, we used laser microbeam irradiation to induce DNA damage in human fibroblasts then followed the localization of endogenous ATM and GFP-tagged ATM to photo-induced DNA damage. We find that endogenous ATM rapidly forms foci that, unlike γH2AX, tightly colocalize with damaged DNA. GFP-ATM localizes to photo induced DNA damage within 3-5 seconds post irradiation and GFP-ATM accumulation plateaus within 2 minutes post-irradiation. In cells deficient for NBS1, a member of the MRN DSB sensing complex, GFP-ATM does not associate with damaged DNA in the first minute post-irradiation. In contrast, mutating the ATM 1981 phosphorylation site from serine to alanine delayed but did not block accumulation of GFP-ATM at damaged DNA. The telomeric ends of chromosomes normally do not trigger DNA damage responses, in part due to the telomeric protein TRF2. We find that TRF2 and TRF1 associate as rapidly as ATM with photo-induced DNA damage in non-telomeric DNA and that TRF2 over-expression impairs phosphorylation of ATM, H2AX and p53 following ionizing radiation. Additionally, over-expression of a DsRed-tagged TRF2, but not TRF1, attenuates GFP-ATM accumulation at DNA damage. Our data indicate that a functional MRN complex is required for ATM recruitment to damage sites, and optimal accumulation of ATM at damaged DNA may require phosphorylation at serine 1981. In contrast, interactions with TRF2 appear to dampen the ATM response to DSBs, supporting a model in which TRF2 interactions with ATM promote local repair of non-telomeric DNA damage while inhibiting ATM-dependent activation of global DNA damage responses such as cell cycle checkpoints and apoptosis.

231

The role of hMSH5 in DNA double-strand break repair. *J.D. Tompkins, N. Zhao, C. Her.* School of Molecular Bioscience and Center for Reproductive Biology, Washington State University, Pullman, WA. 99164-4660.

DNA double-strand breaks (DSBs) constitute the most common and dangerous form of DNA damage frequently occurring during normal DNA metabolism and exposure to chemotherapeutics. Although hMSH5 is generally known for its function in meiotic recombination, recent studies have demonstrated that the interplay between hMSH5 and various interacting partners are involved in mitotic DSB repair and DNA damage response. Specifically, we demonstrate that a DSB triggers the local recruitment of endogenous hMSH5 and hMSH4 proteins in somatic cells, and DSB-induced hMSH5 assembly at the break is dependent on functional hMRE11 and hRad51 proteins. Moreover, hMSH5 RNAi reduces the frequency of DNA recombination triggered by a defined DSB in human cells harboring a chromosomally integrated recombination reporter. The function of hMSH5 in DSB repair is potentially controlled by a dynamic interplay with c-Abl, in which the interaction between these two proteins coordinates the activation of c-Abl kinase and tyrosine phosphorylation of hMSH5 at tyrosine residue 742 (Y742). By disrupting the (Px)5 motif within the hMSH5 N-terminal c-Abl-interacting domain, the common polymorphic hMSH5 P29S variant alters the functional interaction with c-Abl in DNA damage response and repair. Coherent with the fact that compromised recombinational repair renders cells more sensitive to strand break-inducing agents; over-expression of hMSH5 P29S or hMSH5 Y742F mutant proteins sensitizes cells to ionizing radiation and cisplatin, respectively. Taken together, the current evidence strongly suggests an important role for hMSH5 in the process of recombinational repair and DNA damage response.

232

A randomised controlled trial of aspirin and resistant starch to prevent colorectal neoplasia in Lynch Syndrome: The CAPP2 Study. *J. Burn[1], D.T. Bishop[2], J-P. Mecklin[3], F. Macrae[4], G. Moeslein[5], S. Olschwang[6], M.L.S. Bisgaard[7], R. Ramesar[8], F. Elliott[2], G. Barker[1], J. Jass[9], H.T. Lynch[10], J. Mathers[1].* 1) Inst Human Genetics & Human Nutrition Research Centre, Newcastle University,UK; 2) Inst of Molecular Medicine, Leeds University, UK; 3) Jyvaskyla Central Hospital, Finland; 4) Royal Melbourne Hospital, Australia; 5) St. Josefs Hospital, Bochum-Linden, Germany; 6) Institut Paoli Calmettes, Marseille, France; 7) University of Copenhagen, Copenhagen, Denmark; 8) University of Cape Town, Observatory, South Africa; 9) Dept of Cellular Pathology, St. Mark's Hospital, London, UK; 10) Hereditary Cancer Inst. Creighton University, Omaha, USA.

There is strong epidemiological evidence in favour of aspirin and resistant starch being protective against colorectal cancer. We randomised 1009 eligible carriers of mismatch repair gene defects at risk of Lynch Syndrome (HNPCC), from 43 centres worldwide, in a factorial design to receive 600mg enteric coated aspirin or placebo and 30 grams of non-GM resistant starch (Novelose) or placebo for up to 4 years. 937 started treatment. 191 withdrew without an exit colonoscopy being recorded. The average duration of therapy among the 735 participants analysed was 29 months (range 7.4 to 74.4). A total of 141 participants developed colonic neoplasia including 22 carcinomas. This exceeded the number of events predicted on power anlaysis to be needed to detect an effect equivalent to epidemiological predictions. The numbers in each randomisation group were equal. The results of this genetically targeted trial, the largest to date and the first in Lynch syndrome, are currently under publication embargo. They will help determine chemoprevention in HNPCC families and patients with sporadic MSI high tumours. We have now proven that people who are at high risk of genetic disease are willing to participate in long term chemoprevention/dietary trials, are compliant and contribute a high level of statistical power. Geneticists should become more involved in development of disease prevention strategies. (New Engl. J. Med. submitted).

233

Molecular basis of the Li-Fraumeni syndrome (LFS): an update from the French LFS cohort. *G. Bougeard[1], S. Baert-Desurmont[1,2], C. Martin[2], S. Vasseur[2], L. Brugières[3], A. Chompret[4], D. Stoppa-Lyonnet[5], C. Bonaïti-Pellié[6], T. Frebourg[1,2], the French LFS Network.* 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Department of Genetics, Rouen University Hospital, Rouen, France; 3) Department of Pediatric Oncology, Institut Gustave Roussy, Villejuif, France; 4) Department of Medicine, Institut Gustave Roussy, Villejuif, France; 5) Department of Genetics, Institut Curie, Paris, France; 6) Inserm U535, Villejuif, France.

The Li-Fraumeni syndrome represents one of the most devastating genetic predispositions to cancers and is characterized by a wide spectrum of early-onset malignancies including sarcoma, brain tumours, adrenocortical tumours and premenopausal breast cancers.We have performed an extensive analysis of *TP53*, based on complete sequencing of the 11 exons and on QMPSF to detect genomic rearrangements, in 396 families suggestive of LFS, fulfilling the French LFS network criteria. We detected in 76 families (19%) germline alterations including 4 complete or partial genomic deletions of the *TP53* locus. These results constitute a definitive argument demonstrating that LFS results from a haploinsufficiency at the *TP53* locus. In this series, we confirm that the mean age of tumour onset in *MDMD2* SNP309 G allele carriers (19.2 years) is significantly different from that observed in patients homozygous for the T allele (29.3 years) and found that the mean ages of tumour onset in *MDMD2* G and *TP53*Arg alleles carriers, and in patients with the *MDMD2* T/T and *TP53* Pro/Pro genotype were clearly different (17.6 vs 39.2 years). The earlier development of tumours in *TP53* wt/mt mice compared to wt/- mice, recently reported, led us to compare the age of tumour onset between patients harbouring *TP53* missense mutations (56 patients) and those carrying other types of alterations (31 patients). As predicted by the murine models, we indeed observed a significant difference between both groups (21.1 years vs. 29.2 years). These results support the hypothesis that missense mutations not only inactivate the transcriptional activity of the wild-type protein but have also an additional oncogenic effect.

234

Identification of modifiers of BRCA1/2: results from combined analysis from the Consortium of Investigators of Modifiers of BRCA1/2. *A. Antoniou[1], J. Beesley[2], D.F. Easton[1], G. Chenevix-Trench[2], Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA).* 1) CRUK Genetic Epidemiology Unit, Cambridge, Cambridge, United Kingdom; 2) Queensland Institute of Medical Research, Brisbane, Australia.

Mutations in BRCA1 and BRCA2 confer high risks of breast and ovarian cancer. However, epidemiological evidence suggests that these risks are modified by other genetic or environmental factors. Studies of genetic modifiers of BRCA1/2 have been hampered by small sample size. To address this problem, CIMBA was established to conduct collaborative analyses of genetic polymorphisms as modifiers of risk in BRCA1/2 mutation carriers. To correct for the potential bias from non-random sampling of carriers with respect to their disease phenotype, we analysed the data by modelling the retrospective likelihood of the observed SNP genotypes and disease phenotypes conditional on the disease phenotypes. A SNP in the 5'UTR of RAD51, G135C, has been suggested as a possible modifier of breast cancer risk in BRCA1/2 carriers. We pooled genotype data on 8512 mutation carriers from 19 studies for this SNP. We found evidence for an increased breast cancer risk in CC homozygotes (HR=1.92, 95%CI: 1.25-2.94) but not in heterozygotes (HR=0.95, 95%CI: 0.83-1.07, 2df heterogeneity test: p=0.002). When BRCA1 and BRCA2 mutation carriers were analyzed separately, the increased risk was significant only among BRCA2 mutation carriers in whom we observed HRs of 1.17 (95%CI: 0.91-1.51) among heterozygotes and 3.18 (95%CI: 1.39-7.27) among rare homozygotes (2df, p=0.0007). SNPs in FGFR2, TNRC9 and MAP3K1 have been recently shown to be associated with breast cancer risk in the general population through a genome-wide association study of breast cancer. To evaluate whether these SNPs also modify the breast cancer risk in BRCA1/2 mutation carriers we genotyped approximately 11000 mutation carriers from 24 studies. The data are currently being analysed and the results will be discussed. The identification of genetic modifiers of risk may have important implications for the clinical management of BRCA1/2 mutation carriers.

235

Multiple ADH genes are associated with head and neck cancers in three large independent studies. *P. Brennan, M. Hashibe, V. Gaborieau, J. McKay On behalf of the Central Europe, ARCAGE and the Latin America head and neck cancer study.* Genetic Epidemiology Group, International Agency for Research on Cancer, Lyon, France.

The alcohol dehydrogenase (ADH) pathway comprises 7 distinct ADH genes and is a key candidate gene pathway for cancers of the head and neck. In order to elucidate the potential role of this pathway for head and neck cancers we have genotyped 15 candidate SNPs in the 7 ADH genes in a large case-control study comprising 811 head and neck cancer cases and 2598 controls from 5 countries of Central Europe (Russia, Poland, Romania, Czech Republic and Slovakia). When comparing the common homozygous genotype to possession of one or two variant alleles, two of the 15 SNPs provided a strongly significant protective effect against head and neck cancer; ADH2 R48H (rs1229984), OR=0.49, 95% CI (0.35-0.69), p<0.0001) and ADH7 A92G (rs1573496); OR =0.57, 95%CI (0.45-0.72), p<0.0001. We subsequently genotyped these two SNPs in a second study of head and neck cancer comprising 1323 cases and 1352 controls from 7 European countries. A similar effect was observed for ADH2 R48H (OR=0.59, 95% CI 0.44-0.78, p=0.0002), and a weaker effect was observed for ADH7 A92G (OR=0.79, 95% CI (0.63-0.97), p=0.03). Finally, in a third study of head and neck cancer from 3 Latin American countries (Brazil, Argentina and Cuba), including 1711 cases and 1285 controls and we identified similar effects for both ADH2 R48H (OR=0.61, 95%CI (0.46-0.82, p=0.0008) and ADH7 A92G (OR=0.70, 95% CI (0.55-0.90), p=0.0056). When results were pool across all 3 studies the effect of ADH2 was significant at p<10-11 and for ADH7 at p<10-7, after adjusting country, age, sex, tobacco and alcohol. These results would appear to confirm that variants in both ADH2 and ADH7 are associated with head and neck cancer. Linkage disequilibrium between these two variants was minimal and neither had an important effect on alcohol consumption. This would suggest that functional effects of these two variants, or other variants that are closely associated with them, independently alter the risk of head and neck cancer.

236

Confirmation study of prostate cancer risk variants at 8q24 in African Americans identifies a novel risk locus. *R. Kittles[1], C. Robbins[2], J. Benn Torres[1], S. Hooker[1], C. Bonilla[3], W. Hernandez[1], A. Candereva[2], C. Ahaghotu[4], J. Carpten[2].* 1) Dept Medicine, MC 6091, Univ Chicago, Chicago, IL; 2) Division of Integrated Cancer Genomics, Translational Genomics Research Institute, Phoenix, AZ; 3) Department of Clinical Pharmacology, University of Oxford, Oxford, UK; 4) Division of Urology, Howard University Hospital, Washington, DC.

Prostate cancer (Pca) is a common complex disease that disproportionately affects men of African descent. Recently, several different common variants on chromosome 8q24 have been shown to be associated with Pca in multiple studies and ethnic groups. The objective of this study was to confirm the association of 8q24 markers with Pca in African Americans. We genotyped 24 markers along 8q24 and 80 unlinked ancestry informative markers in a hospital-based case-control sample of 1,057 African American men (490 Pca cases and 567 healthy controls). Association analyses of 8q24 markers with prostate cancer risk were adjusted for both global and local 8q24 admixture stratification using estimates from ancestry informative markers. We report that rs7008482, which maps to the 8q24.13 region, is an additional independent prostate cancer risk variant (P= 5 x 10-4) and we also replicate the association of rs16901979 with prostate cancer (P=0.002). Other published risk variants in the region such as rs1447295 and rs6983267 did not replicate in our population. Both rs7008482 and rs16901979 independently predicted risk and remained significant (P<0.001) after controlling for each other. Our most significantly associated SNP, rs7008482 mapped to 8q24.13, approximately 2.2Mb proximal to the DG8S737/ rs1447295 region at 8q24.21. These findings are intriguing since SNP rs7008482 lies within an area on 126.2Mb surrounded by several interesting candidate genes which are amplified and overpressed in Pca. Further additional genotyping within this region and functional analyses are underway. Taken together, multiple studies, including ours strongly support the existence of several independent susceptibility loci within the 8q24 region of the genome.

237

Translational re-initiation of DNp63 protein causes Rapp-Hodgkin syndrome. *T. Rinne[1], K. Krahn[2], E. Lamme[3], J.C. Murray[4], B. van den Heuvel[5], J. Schalkwijk[3], H.G. Brunner[1], J. Zhou[1], H. van Bokhoven[1].* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Department of Genetics Research Laboratory, University of Iowa, Iowa City, USA; 3) Laboratory of Skin Biology and Experimental Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 4) Department of Paediatrics, University of Iowa College of Medicine, Iowa City, USA; 5) Laboratory of Paediatrics and Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

A group of human developmental disorders is characterized by combinations of ectodermal dysplasia, orofacial clefting and limb malformations. So far, seven different entities have been reported to be caused by dominant mutations in the transcription factor gene p63. 5% of p63-linked patients have Rapp-Hodgkin syndrome (RHS), which is characterized by generalized ectodermal dysplasia and orofacial clefting. We have recently identified a RHS family with an a-typical stop mutation (Q11X). Intriguingly, the N-terminal position of this nonsense mutation appears to be incompatible with the postulated dominant-negative/gain-of-function mechanism of other p63 mutations. Initial RNA analysis in patient cells revealed normal expression of both alleles. Protein analysis revealed an additional protein of reduced size. Through extensive proteomic analyses we could demonstrate that the smaller p63 protein was produced by translation re-initiation at the next methionine, causing N-terminal truncation of 25 amino acids for the DNp63 isoforms. This N-terminal truncation abrogates a non-canonical transactivation domain in the DN-specific isoforms, which was functionally shown by testing a natural target of p63, Keratin-14 promoter. These data establish that the Q11X mutation does not represent a null-allele, but gives rise to an abnormal DNp63 protein with dominant effects. Since other RHS mutations as well as mutations in the related Hay-Wells syndrome are invariably located in p63 the C-terminal a-tail, we conclude that the specific disruption of DNp63a is key to phenotypes of these syndromes, which comprise severe ectodermal dysplasia and abnormal orofacial development but not limb defects.

238

The Debré type of autosomal recessive cutis laxa is associated with brain dysgenesis or neurodegeneration and defective N-glycosylation. *L. Van Maldergem[1], M. Yuksel-Apak[2], H. Kayserili[2], E. Seemanova[3], S. Giurgea[4], J. Vigneron[5], M. Greally[6], E. Leao-Teles[7], L. Basel-Vanagaite[8], J. Jaeken[9], S. Mundlos[10], W.B. Dobyns[11].* 1) Ctr Génétique Humaine, Univ Liege, Liege, Belgium; 2) Div Med Genet, Instit Child Health, Univ Istanbul, Istanbul, Turkey; 3) Dpt Clin Genet, Motol Hospit, Charles Univ, Prague, Czech Republic; 4) Dpt Neurol, CHU Tivoli, La Louvière, Belgium; 5) Unité de génét méd, CHU Nancy, Nancy, France; 6) Coll Med & Med Sci, Arabian Gulf Univ, Manama, Bahrein; 7) Dpt Pediatr, Univ Porto, Porto, Portugal; 8) Dpt Med Genet, Schneider Children's Med Ctr, Petah Tikva, Israel; 9) Ctr Metabol Disord, Katholieke Univ Leuven, Leuven, Belgium; 10) Instit Med Genet, Charité, Campus Virchow, Berlin, Germany; 11) Dpt Hum Genet, Neurol & Pediatr, Univ Chicago, Chicago, Illinois.

Two main type of congenital cutis laxa are known. Both are very rare autosomal recessive conditions differing by presence or absence of pulmonary emphysema, clinical outcome and associated findings. We delineate the Debré type lacking pulmonary emphysema. It is associated with downward slant of palpebral fissures, megafontanelles, hip dislocation, inguinal herniae and developmental delay. Our follow-up data indicate that high myopia,generalized seizures associated with brain dysgenesis and defective glycosylation are also part of the clinical picture. Eleven patients from eight families are described. Clinical course was remarkable for progressive neurological impairment starting with psychomotor retardation, followed by onset of generalized seizures by the end of the first decade and a subsequent neurodegenerative course while skin overfolding becomes less pronounced. Frontoparietal pachygyria was observed on brain MRI in seven cases out of eight investigated and a Dandy-Walker malformation was observed in three cases. Although all unrelated patients had a type 2 isoelectrofocusing of serum sialotransferrins indicating defective N-glycosylation, linkage studies pointed to at least two different loci. We conclude that Debré type of cutis laxa is a N-glycosylation disorder with brain dysgenesis, a poor neurological outcome and is genetically heterogeneous.

239

Connective Tissue Conundrum: The EDS IV Clinical Spectrum. *B.D. Rink, C.L. Blout, M.E. Nunes.* Division of Genetics, Children's Hospital, Columbus, OH.

Ehlers-Danlos syndrome (EDS), vascular type (formerly EDS IV), an autosomal dominant condition attributed to abnormalities in type III collagen, presents with tissue fragility and characteristic physical features. Associated morbidity and mortality are due to vascular and/or visceral rupture. Penetrance is close to 100% for families identified with significant features. However, evaluation of families with *COL3A1* mutations and individuals with EDS IV features without biochemical abnormality argue for reduced penetrance. We present four such families illustrating the clinical difficulty in correlating genotype to phenotype. This provides further argument for broader clinical designation of type III collagenopathies, given the stigma associated with EDS IV. Through chart review and patient evaluation, several families were identified illustrating clinical and biochemical variability. Family 1, segregating a Gly814Arg *COL3A1* missense mutation, was ascertained by a mother suffering multiple arterial ruptures and cerebral aneurysm in her 30's. Both sons inherited the mutation, one with an obvious clinical diagnosis as a teenager and the other without any connective tissue or vascular features in his 20's. Family 2, segregating a R1024X *COL3A1* nonsense mutation, was ascertained via a cousin diagnosed with aortic aneurysm. Penetrance for vascular complication by age 50 in known mutation carriers is about 60% in this family. Family 3, evaluated for familial aortic root dilation, demonstrated classic stigmata of EDS IV. Family 4, segregating visceral rupture by the fifth decade, demonstrated identifiable but milder connective tissue features in two generations. Skin biopsy did not reveal a type III collagen abnormality in family 3 or 4. Ascertainment bias in laboratories evaluating type III collagen may overestimate the penetrance of severe features. Our families illustrate the diagnostic challenge clinicians face when evaluating patients with Ehlers-Danlos syndromes and suggest reduced penetrance and variable expressivity. A role for additional genes modifying the type III collagen phenotype is suggested. Further, *COL3A1* sequencing may provide additional diagnostic information for patients undetected by biochemical analysis.

240

Hereditary disorders of connective tissue may present with Chiari I malformation, occipitoatlantoaxial hypermobility, and functional cranial settling. *C. Francomano[1], T. Milhorat[2], P. Bolognese[2], M. Nishikawa[2], N. McDonnell[3].* 1) Greater Baltimore Med Ctr, Harvey Institute Human Genetics, Baltimore, MD; 2) The Chiari Institute, North Shore-Long Island Jewish Health System, Manhasset, NY; 3) National Institute on Aging, NIH, Baltimore, MD.

We report an association of hereditary disorders of connective tissue (HDCT) and Chiari malformation 1 (CM1), presenting with lower brain stem symptoms attributable to occipito-atlantoaxial hypermobility and functional cranial settling. The prevalence of hereditary disorders of connective tissue (HDCT) was determined in a prospectively collected cohort of 2,813 patients with CM1. All patients underwent a detailed medical and neuroradiological workup that included an assessment of articular mobility. Using reconstructed 3D-CT and plain x-ray images, osseus structures comprising the craniocervical junction were investigated morphometrically in 114 patients with HDCT/CM1 and compared to those in patients with CM1 alone (n = 55) and normal controls (n = 55). The diagnosis of Ehlers-Danlos syndrome (EDS) or other HDCT was made in 357 of 2,813 of patients with CM1 (12.7%). The clinical features of HDCT/CM1 were distinguished from those of CM1 alone by clinical stigmata of HDCT, a greater female preponderance (7:1 vs. 3:1), and a greater incidence of lower brain stem symptoms (0.43 vs. 0.05), retroodontoid pannus formation (0.71 vs. 0.16), and hypoplasia of the oropharynx (0.45 vs. 0.02). In patients with HDCT/CM1, upon sitting or standing there was reduction of the basal-dens interval (3.6 mm), enlargement of the basal-atlas interval (3.0 mm), and reduction of the clivus-axis angle(10.8º), clivus-atlas angle (5.8º), and atlas-axial angle (5.3º). These changes were reducible by cervical traction or returning to the supine position. In normal controls and patients with CM1 alone, these measurements did not change with position.

Conclusions: The identification of HDCT in 12.7% of patients with CM1 establishes an association between these previously unrelated disorders. Patients with HDCT and symptoms suggestive of CM1 should be evaluated with brain MRIs in the supine and upright positions.

241

Generalized Arterial Calcification of Infancy (GACI): Clinical Course and Prevalence of *ENPP1* mutations. *F. Rutsch[1], P. Böyer[1], Y. Nitschke[1], N. Ruf[2], G. Weissen-Plenz[3], P. Nürnberg[4], R. Terkeltaub[5].* 1) University Children's Hospital, Münster, Germany; 2) Max-Delbrück Center, Berlin, Germany; 3) Medicine, University Hospital, Münster, Germany; 4) Cologne Center for Genomics, Cologne, Germany; 5) Medicine, VAMC, UCSD, La Jolla, USA.

GACI is often associated with autosomal recessively inherited defects in Ecto-nucleotide pyrophosphatase 1 (ENPP1). The resulting deficiency in extracellular inorganic pyrophosphate leads to calcification of arteries and intima proliferation, and, occasionally, periarticular calcifications. Clinical data available from 52 clinically diagnosed GACI patients were analyzed retrospectively. Mutation analysis of *ENPP1* was performed by direct sequencing in all patients. 27 of the 52 patients were delivered prematurely. 12 of these presented signs of fetal distress. 35 patients demonstrated signs of heart failure soon after birth, 21 patients developed arterial hypertension. 26 patients presented with pulmonary symptoms, 20 needed ventilatory support. Arterial calcifications were demonstrated predominantly in the aorta and coronary arteries by imaging studies. Additionally, autopsy confirmed calcification of pulmonary and renal arteries in 16 deceased patients. 10 patients showed periarticular calcifications. 21 patients were surviving at the time of data collection, 31 had died, 15 by heart failure. 9 of 14 patients treated with bisphosphonates survived, and calcifcations resolved in 8 of them. Of the 38 patients not treated with bisphosphonates, 12 patients were surviving. In 4 of these, spontaneous resolution of calcifications was demonstrated. Homozygous or compound heterozygous mutations in *ENPP1* were found in 40 of the 52 patients. 26 of these patients died in infancy. Also, 5 of the 12 patients without *ENPP1* mutations died in infancy. *ENPP1* mutations account for 77% of clinically diagnosed GACI cases. 40% of all patients studied survived the critical period of infancy. There is no significant difference in disease manifestations and clinical course in patients with and without *ENPP1* mutations. Bisphosphonate treatment in GACI seems to be reasonable, although a standardized treatment regimen does not exist.

242

Evidence for T(Brachyury) as a Candidate Gene for Vertebral Malformations. *P. Giampietro[1], C. Raggio[2], J. Staubli[1], E. McPherson[1], L. Ivacic[1], K. Rasmussen[1], F.S. Jacobsen[1], F. Faciszewski[1], R.M. Pauli[3], J. Burmester[1], I. Glurich[1], O. Boachie-Adjei[2], R. Blank[3].* 1) Marshfield Clinic, Marshfield, WI; 2) Hospital for Special Surgery, New York, NY; 3) University of Wisconsin, Madison, WI.

No major genes for sporadically occurring congenital vertebral malformations (CVM) in humans have been identified. In contrast, multiple mouse mutations feature abnormal vertebral phenotypes. We have identified body patterning genes in mice as candidates for causing human CVM. T is a critical gene to establish mesodermal identity. In mice, T mutations act as recessive lethals and have a dominant abnormal tail phenotype. In humans, some, but not all, investigators report that the C allele of a T/C polymorphism in intron 7 has been reported to be preferentially transmitted to offspring with spina bifida. We therefore hypothesized that mutations in T (Brachyury) contribute to the pathogenesis of other human vertebral malformations. To test this idea, we sequenced the complete coding region and 500 bp of the T gene in 50 thoroughly characterized patients with CVM. Three patients were heterozygous for an A338V missense mutation in exon 7 that did not occur in an ethnically diverse, 443 person reference population. Alanine is conserved at this residue in mouse, rat, rabbit, dog, and Xenopus tropicalis, but not in armadillo, elephant, opossum, chicken, or tetraodon. Valine did not occur at this residue in any of the species in which alanine was not conserved. The individuals harboring the A338V mutation had diverse spinal phenotypes, including Klippel-Feil syndrome, sacral agenesis, and spondylothoracic dysplasia. A fourth patient, with T12 and L1 hemivertebrae, harbored an exon 7 splice junction C to T variant. This previously unreported variant was tested in 347 control subjects, and 11 heterozygotes and 2 T/T individuals were found. We believe that the A338V mutation is pathogenic and that the splice junction variant may increase CVM risk. Presumably, epistatic interactions between the T protein and other developmental genes and the environment modulate the phenotypic consequences of T mutations.

243

Molecular mechanisms underlying congenital scoliosis. *K. Staehling-Hampton[1], A.S. Cornier[2], K.M. Delventhal[1], J.F. Caubet[3], J.B. Emans[3], H. Welsh[1], P. Turnpenny[4], O. Pourquie[5].* 1) Molecular Biology, Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Genetics, San Juan Bautista School of Medicine, Puerto Rico; 3) Department of Orthopaedic Surgery, Children's Hospital Boston, Boston, MA; 4) Clinical Genetics Department,Royal Devon & Exeter Hospital, Exeter, UK; 5) Stowers Institute for Medical Research/HHMI, Kansas City, MO.

Congenital vertebral malformations are rare, occurring in 1 to 2 out of every 10,000 births. Conditions that fall into this category include: generalized vertebral malsegmentation (e.g., spondylothoracic dysostosis), regionalized conditions (e.g., Klippel-Feil syndrome) or conditions involving only one or two vertebrae (e.g., Alagille syndrome). Although most cases of congenital scoliosis were previously thought to be sporadic, recent evidence points to a considerable genetic component. To date, three genes have been associated with vertebral malformations in humans. All of these genes are associated with NOTCH signaling, which is involved in the segmentation of the spine. Using a candidate gene approach, we selected genes associated with NOTCH signaling and vertebral anomalies in mouse mutants and humans. We then sequenced these genes in a cohort of 30 patients with vertebral malformations from Boston Children's Hospital. We identified a novel homozygous mutation in a 12-year-old female of Puerto Rican descent with Jarcho Levin syndrome, a severe form of spondylothoracic dysostosis (STD). This patient harbors an E103stop mutation in the gene coding for the transcription factor MESP2. Sequencing of Mesp2 in a broader sampling of Puerto Rican patients indicates that this mutation is a common mutation in STD patients in the Puerto Rican population. In conclusion, we have identified a founder effect mutation accounting for the classical Puerto-Rican form of Jarcho-Levin syndrome.

244

Molecular analysis of the SHOX gene in 409 children with short stature. *C. Huber[1], M. Rosilio[2], A. Munnich[1], V. Cormier-Daire[1], The French SHOX Genesis Module.* 1) Department of Medical Genetics and INSERM U781, Necker Hospital, Paris, France; 2) Lilly France, Suresnes, France.

We present the results of the molecular analysis of the SHOX gene and the PAR1 region in a series of short stature children with normal endocrine screening. This study was part of GeNeSIS, the international observational study conducted by Eli Lilly and Company. The aim of the study was to determine 1) the prevalence of SHOX anomalies in short stature children who were followed by pediatric endocrinologists, 2) the frequency of clinical evidence of Madelung Deformity (MD) in children with SHOX anomalies and 3) the value of a family history of short stature for deciding on whether SHOX testing should be performed. The only inclusion parameter was height SDS below -2. 409 children were included by 38 participating centres (from January 2003 to February 2007). The series comprises 248 girls and 161 boys with age ranging from 2 to 17 years, 172 were at a prepubertal stage. The SHOX molecular screening included extensive microsatellite analysis of the PAR1 region and direct sequencing of the SHOX gene. We observed a total of 103 SHOX anomalies including 49 deletions of variable sizes encompassing SHOX, 37 deletions located downstream of the SHOX gene and 17 point mutations. Among the 103 anomalies, 50 were identified in children with clinical evidence of MD, with a large preponderance of girls. In the 53 others, the proportion of boys and girls was similar. The absence of MD in this group may reflect the difficulty to clinically diagnose dyschondrosteosis (DCS) in young children before puberty and in males. 78 SHOX anomalies were inherited and short stature was observed in 69 parents highlighting the importance of evaluating the family. We conclude that SHOX deficiency is a frequent cause of short stature. Evaluation of the family history, taking especially signs of DCS such as MD into account, may guide the physician to identify subjects who should undergo SHOX testing. Finally, the observation of a large proportion of deletions located downstream of the SHOX gene emphasizes the necessity of an extensive microsatellite analysis of the PAR1 region.

245

Carrier Frequency of Recurring Mutation Causing Severe/Lethal Recessive Type VIII Osteogenesis Imperfecta in African-Americans. *W.A. Cabral[1], A.M. Barnes[1], F.D. Porter[2], J.C. Marini[1].* 1) Bone and Extracellular Matrix Branch, NICHD/NIH, Bethesda, MD; 2) Heritable Disorders Branch, NICHD/NIH, Bethesda, MD.

The majority of cases of Osteogenesis imperfecta (OI) are caused by dominant mutations in either of the two genes encoding type I collagen, *COL1A1* and *COL1A2*, with an incidence of 1/20,000 births. Two recessive forms of OI have recently been shown to be caused by defects in the genes encoding cartilage-associated protein (*CRTAP*) or prolyl 3-hydroxylase 1 (*LEPRE1*). Although recessive OI accounts for approximately 5% of OI cases overall, we have identified a recurring mutation in the *LEPRE1* gene, IVS5+1G>T (Cabral and Chang et al, Nat Genet (2007) 39:359-365). The common mutation occurs in a compound heterozygous or homozygous state in 6 of 8 probands (9 of 16 alleles) with severe/lethal recessive type VIII OI (OMIM #610915). All six probands with the IVS5+1G>T mutation were born to carrier parents of West-African (Nigerian or Ghanaian) or African-American descent, suggesting the existence of a stable mutant allele in this population. In order to determine the carrier frequency of the IVS5+1G>T mutation in African-Americans, we screened genomic DNA extracted from 1429 random African-American newborn metabolic screening cards from Pennsylvania. Five carriers were identified, predicting a carrier frequency of 1 in 286 African-Americans newborns (0.35%) in Pennsylvania. Our results predict a 1 in 330,000 rate of occurrence of lethal type VIII OI in African-Americans due to homozygosity for the LEPRE1 IVS5+1G>T mutation. The proportion of African-Americans currently in Pennsylvania who trace their ancestry to contemporary Nigeria or Ghana is unknown. This mutation may have a higher frequency in states (i.e. Maryland or Virginia) whose pre-Civil War slave populations include a higher proportion of individuals originating in this area of West Africa. In addition, screening of this population has identified a previously reported SNP, g.IVS5+115A>G occurring with an allele frequency of 2.1%. This polymorphism is not linked to the mutant allele, but may be useful for haplotype analysis of the African-American population.

246

Genotype and phenotype correlation of *CRTAP* or *P3H1* mutations with recessive osteogenesis imperfecta. *D. Baldridge[1], R. Morello[1], J. Lennington[1], T.K. Bertin[1], M. Weis[2], D.R. Eyre[2], A. Green[3], J. Walsh[3], D. Lambert[3], D. Krakow[4], D.L. Rimoin[4], D.H. Cohn[4], U. Schwarze[5], P.H. Byers[5], B. Lee[1,6].* 1) Dept Mol & Human Gen, Baylor Col Med, Houston, TX; 2) Orthopaedics and Sports Med, U of Washington, Seattle, WA; 3) Natl Centre for Med Gen, Our Lady's Hosp, Dublin, Ireland; 4) Med Gen Inst, Cedars-Sinai Med Center, Los Angeles, CA; 5) Dept Pathology, U of Washington, Seattle, WA; 6) Howard Hughes Med Inst, Houston, TX.

Autosomal dominant osteogenesis imperfecta (OI) or brittle bone disease is a heritable disorder caused by mutations in the two genes encoding type I collagen (*COL1A2* or *COL1A2*). Recently, dysregulation of hydroxylation of a single proline residue in the α-helical domain of fibrillar collagens has been implicated in the pathogenesis of recessive forms of OI. Cartilage-associated protein (CRTAP) interacts with prolyl-3-hydroxylase-1 (P3H1), and Crtap null mice lack fibrillar collagen prolyl 3-hydroxylation and display an OI-like phenotype. In our study of 72 OI subjects we report on a spectrum of recessively-inherited phenotypes, including OI types II and III, resulting from mutations in either *CRTAP* (3 patients) or *P3H1* (15 patients). The latter group includes a recurring mutation in patients from the Irish Traveller population, a community with a high degree of consanguinity and an increased incidence of OI. We report on nonsense, frameshift, and splice site alterations that lead to loss of mRNA and loss of function. In addition, the first case of homozygosity for a missense mutation in *CRTAP* was identified and associated with a milder phenotype. At the protein level, patient fibroblasts showed decreased collagen prolyl 3-hydroxylation. Patients with *CRTAP* or *P3H1* loss of function mutations were indistinguishable clinically, as both groups presented with multiple fractures at birth, decreased bone modeling (especially of the femur), extreme low bone mineral density and poor prognosis. Treatment with bisphosphonates appeared to be beneficial in at least one child with a *P3H1* null mutation. These results expand the genotype-phenotype correlations for the recently described recessive OI and support a DNA based approach to the diagnosis of OI.

247

High-resolution genetic characterization of 51 unique human populations from the Human Genome Diversity Project. *D. Absher[1], J. Li[1], H. Tang[2], S. Ramachandran[3], A. Southwick[1], G. Barsh[2], M.W. Feldman[3], L. Cavalli-Sforza[2], R.M. Myers[1,2].* 1) Stanford Human Genome Center, Palo Alto, CA; 2) Dept Genetics, Stanford University, Palo Alto, CA; 3) Dept Biological Sciences, Stanford University, Palo Alto, CA.

The Human Genome Diversity Project (HGDP) began fifteen years ago as an attempt to characterize genetic diversity in a worldwide collection of humans, and to study the history and geography of mutation, drift and selection in human populations. The HGDP samples include more than 1,050 individuals from 51 unique populations distributed globally and have been studied previously by using microsatellite markers and a few thousand SNPs. To characterize further this valuable resource, we performed the first high-resolution genetic study of the HGDP collection, where we genotyped 660,000 SNPs in 1,043 of the individuals with the Illumina Infinium platform. The extensive genomic coverage of this panel revealed fine structures both between and within populations at an unprecedented resolution. Principal component analyses clearly delineated sub-populations that were indistinguishable in previous studies, for example, between the southern and northern Han Chinese. Estimates of genetic distance from these data allowed us to refine genetic relationship trees down to the individual level. Furthermore, clustering analysis at the individual level recapitulates the continental clusters previously constructed with microsatellite markers, and generates intriguing hypotheses regarding migratory and admixture history of human populations. Finally, we analyzed the genotyping intensity data for evidence of copy number variation (CNV) and identified many examples of population-specific CNVs. We believe these data and analyses will serve as a valuable resource for both population studies of human diversity as well as disease association studies in diverse ethnic backgrounds.

248

Linkage disequilibrium and haplotype variation in Sub-Saharan Africa. *M. Jakobsson[1], F.A. Reed[2], T.J. Pemberton[3], G. Coop[4], D.F. Conrad[4], J.D. Wall[5], J.K. Pritchard[4], S.A. Tishkoff[2], N.A. Rosenberg[1].* 1) Dept. Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Biology, University of Maryland, College Park, MD; 3) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 4) Department of Human Genetics, University of Chicago, Chicago, IL; 5) Department of Epidemiology and Biostatistics, University of California, San Francisco, CA.

In most populations, the HapMap provides an excellent resource for the design of association mapping studies aimed at finding disease-susceptibility genes. However, the major exception to this general rule has been Sub-Saharan Africa. While Africa is the region in which association mapping is most challenging, current knowledge of African linkage disequilibrium (LD) is based largely on a limited sample of populations. We investigate LD at 2,810 SNPs in 8 Sub-Saharan African populations - Beja, Borana, Fulani, Hadza, Iraqw, Mada, Sandawe, and Sengwer. These data are analyzed jointly with SNP data on a worldwide panel of 53 populations, including 7 additional Sub-Saharan populations.

Within Sub-Saharan Africa, isolated hunter-gatherer populations stand out in having the largest number of private haplotypes. These populations also have the largest number of "missing" haplotypes - haplotypes that are found in all except a single population. For a few East African populations - but not for other Sub-Saharan populations - we find extensive haplotype sharing with populations of the Middle East. We also find that every Sub-Saharan population has considerably less LD than every non-African population, confirming that the low level of African LD is a continent-wide pattern. The portability of tag SNPs based on the HapMap Yoruba sample is smaller in all Sub-Saharan populations compared to the portability of the HapMap CEU and CHB/JPT samples in non-African populations. These results have implications both for the history of human migrations out of Africa and for improving the prospects for association studies in populations of African descent.

249

Ancestral reconstruction of segmental duplications reveals punctuated cores of human genome evolution. *Z. Jiang[1], H. Tang[2], M. Ventura[3], M.F. Cardone[3], R. Hubley[4], A. Smit[4], X. She[1], P.A. Pevzner[5], E.E. Eichler[1,6].* 1) Department of Genome Sciences, University of Washington, Seattle, WA 98195; 2) School of Informatics and Center for Genomics and Bioinformatics, Indiana University,Bloomington, IN 47408; 3) Department of Genetics and Microbiology, University of Bari, 70126 Bari, Italy; 4) Institute for Systems Biology, Seattle WA, 98103; 5) Department of Computer Science and Engineering, UCSD, La Jolla, CA 92093; 6) Howard Hughes Medical Institute.

Human segmental duplications are hotspots for non-allelic homologous recombination leading to genomic disorders, copy-number polymorphisms and gene/transcript innovations. The complex structure and history of these regions has precluded a global evolutionary analysis. Combining a modified A-Bruijn graph algorithm with comparative genome sequence data, we identify the origin of 4,692 ancestral duplication loci/ and use these to cluster 437 complex duplication blocks into 24 distinct groups. The sequence divergence data between donor-acceptors pairs and a comparison with the chimpanzee and macaque genome support a "punctuated model" of evolution whereby at least 18 regions were formed by bursts of multiple independent duplication events. Our analysis reveals that human segmental duplications are frequently organized around "core duplicons" which are enriched for transcripts and associated with genes undergoing positive selection. We hypothesize that the rapid expansion and fixation of some intrachromosomal segmental duplications during great-ape evolution has been due to the selective advantage conferred by these genes/transcripts embedded within these core duplications. Using these data, we have developed a new program package called DupMasker which can be used to analyze the mosaic structure of duplication blocks in newly sequenced primate genomes.

250

Impact of diet on the evolution of human amylase gene copy number. *G.H. Perry[1,2], N.J. Dominy[3], K.G. Claw[2], A.S. Lee[1], H. Fiegler[4], R. Redon[4], J. Werner[2], F.A. Villanea[3], J.L. Mountain[5], R. Misra[2], N.P. Carter[4], A.C. Stone[2], C. Lee[1,6].* 1) Brigham & Women's Hospital, Boston, MA; 2) Arizona State University, Tempe, AZ; 3) University of California, Santa Cruz, CA; 4) The Wellcome Trust Sanger Institute, Hinxton, UK; 5) Stanford University, Stanford, CA; 6) Harvard Medical School, Boston, MA.

Recent studies have shown that copy number variation (CNV) among humans is surprisingly common, and there has been intense speculation that CNVs may have played important roles in human evolution. However, few studies have yet directly tested evolutionary hypotheses for CNVs. We performed a detailed evolutionary study of a multi-allelic CNV, the salivary amylase gene (AMY1), which encodes for an enzyme responsible for starch hydrolysis. Specifically, we questioned whether different selective pressures have acted on AMY1 CNV for populations with traditionally high-starch diets (e.g., agricultural societies and hunter-gatherer groups in arid environments) versus populations with traditional diets containing substantially reduced amounts of starch (e.g., rainforest and circum-arctic hunter-gatherers and some pastoralist groups). AMY1 gene copy number was found to correlate positively with salivary amylase protein levels, and individuals from populations with high-starch diets have higher AMY1 copy numbers than those with traditionally low-starch diets. Comparisons with other CNV loci, using data from a whole genome platform, suggested that the observed level of differentiation of AMY1 copy number is highly significant. Our data are consistent with a model of positive or directional selection on AMY1 copy number in high-starch populations, but neutral evolution (i.e., genetic drift) on AMY1 copy number in low-starch populations. This is one of the first examples of positive selection on a human CNV and our study shows that natural selection, in response to behavioral variation, can readily shape the structure of our genomes. Other CNV loci may also be subject to similarly strong pressures of natural selection, related to behavioral or environmental changes.

251

***SGK* expression is increased by an ancestral allele showing a latitudinal cline in human populations.** *F. Luca[1], M. Zou[2], S. Kashyap[2], S. Conzen[2], A. Di Rienzo[1].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Medicine, University of Chicago, Chicago, IL.

Serum and Glucocorticoid regulated-Kinase (*SGK*) encodes a glucocorticoid-induced anti-apoptotic protein required for cell survival in breast epithelium. In "triple negative" breast cancer, *SGK*overexpression may contribute to tumor growth. *SGK* is also an important mineralocorticoid receptor (MR) target in the kidney, leading to increased salt and water reabsorption. Glucocorticoid receptor (GR) and MR can use the same hormone responsive elements (HREs), thus genetic variation in HREs is expected to affect both GR- and MR-mediated transcriptional efficiency. We tested the hypothesis that variations in the regulatory sequences of *SGK* exist and were selected in human populations based on their ancestral requirements for salt/water retention. Such variants could account for some of the differences observed between people of African vs European ancestry in the prevalence of hypertension and triple negative breast cancer.

We identified 3 conserved sequence elements upstream of *SGK* that contain: 1) 6 SNPs with large allele frequency differences between Africans and Europeans and 2) predicted HREs. These elements were a) resequenced in 14 Europeans and 14 African samples and b) tested for enhancer activity by reporter gene assays. In order to test for a correlation between allele frequency and climate, the 6 SNPs were genotyped in 52 human populations worldwide.

By combining population genetics and a molecular approach, we identified a genetic variant upstream of *SGK* in which the ancestral allele increases *SGK* expression in response to glucocorticoid. The frequency of this allele is highest in Sub-Saharan African populations and is strongly correlated with latitude and temperature variables in worldwide samples. Because of the critical role of *SGK* in sodium homeostasis, this variant is likely to have evolved under a spatially-varying selective pressure related to climate.

252

A scan for genetic determinants of human hair morphology: EDAR is associated with Asian hair thickness. *A. Fujimoto[1], R. Kimura[1], J. Ohashi[1], U. Samakkarn[2], W. Settheetham-Ishida[3], T. Ishida[4], Y. Morishita[5], T. Furusawa[6], M. Nakazawa[7], R. Ohtsuka[8], R. Yuliwulandari[1], L. Batubara[9], M.S. Mustofa[10], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo; 2) Rawai Health Centre; 3) Department of Physiology, Faculty of Medicine, Khon Kaen University; 4) Department of Biological Sciences, Graduate School of Science, The University of Tokyo; 5) Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo; 6) Division for International Relations, The University of Tokyo; 7) Socio-Environmental Health Sciences, Graduate School of Medicine, Gunma University; 8) National Institute for Environmental Studies; 9) Pharmacology Department, Yarsi University; 10) Biology Department, Yarsi University.

Hair morphology is one of the most differentiated traits among human populations. To identify hair morphology-determining genes, the levels of local genetic differentiation in 170 genes that are related to hair morphogenesis were evaluated by using data from the International HapMap project. Among highly differentiated genes, EDAR harboring an Asian-specific non-synonymous SNP (1540T/C, 370Val/Ala) was identified as a strong candidate. Association studies between genotypes and hair morphology suggested that the EDAR-1540T/C polymorphism is associated with hair thickness. The mean area of hair section in each genotype and in each population indicated that 1540T/C explains a major part of the difference in hair thickness between populations. The geographic distribution of 1540T/C and the long-range haplotype test suggest that 1540C arose after the divergence of Asians from Europeans and has been subject to positive selection in East Asian populations.

253

Genetic determinants of hair, eye and skin pigmentation in Europeans. *P. Sulem[1], D. Gudbjartsson[1], S. Stacey[1], A. Helgason[1], T. Rafnar[1], K.P. Magnusson[1], F. Jonasson[2], B. Sigurgeirsson[3], K. Thorisdottir[3], R. Ragnarsson[3], K.R. Benediktsson[3], K.K. Aben[4], L.A. Kiemeney[5], J.H. Olafsson[3], J. Gulcher[1], A. Kong[1], U. Thorsteinsdottir[1], K. Stefansson[1].* 1) deCODE Genetics, Reykjavik, Iceland; 2) Department of Ophthalmology, Landspitali-University Hospital, Reykjavik, Iceland; 3) Department of Dermatology and Department of Anatomopathology, Landspitali-University Hospital, Reykjavik, Iceland; 4) Comprehensive Cancer Center East and Department of Epidemiology and Biostatistics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 5) Department of Epidemiology and Biostatistics and Department of Urology, Radboud University Nijmegen Medical Center, The Netherlands.

The color of hair, skin and eyes are highly heritable and visible phenotypic traits in humans. However, relatively few common sequence variants that account for variation of normal human pigmentation have been identified to date. Here we present results from a genome-wide association scan for variants associated to hair, eye and skin pigmentation among 2,986 Icelanders. The most significantly associated SNPs located in six regions were tested and their association replicated in a second sample of 2,718 Icelandic individuals and a sample of 1,214 Dutch individuals. Genome-wide significance was detected for single nucleotide polymorphisms (SNPs) in the six different regions. Association signals to four of these regions are reported here for the first time, linking a variant on 14q to eye and hair color, a variant on 12q to hair color, two coding variants in a gene on 11q to eye color and freckles and finally a variant on 6p to freckles. We observe the reported effect of MC1R variants on hair and skin types and of variants near OCA2 on eye and hair color. We note that the two new variants associating to freckling did not associate to red hair, unlike the MC1R variants. The two new eye color genes predispose to blue versus green eyes but do not affect brown eye color, unlike the OCA2 variants.

254

Patterns of microsatellite variation within the KITLG and TYRP1 genes: Implications for the evolutionary history of skin pigmentation in human populations. *S. Beleza[1], C. Martinho[1], I. Alves[1], E. Parra[2], M. Shriver[3], J. Rocha[1].* 1) IPATIMUP, Porto, Portugal; 2) Department of Anthropology, University of Toronto, Canada; 3) Department of Anthropology, Penn State University, PA, USA.

A major motivation for searching for genes affected by recent adaptation is to determine how different environmental selective pressures have shaped contemporary phenotypic variation. Skin pigmentation has long been considered a trait largely affected by selection, but only recently have several analyses shown signatures of positive selection in pigmentation candidate genes. The Tyrosinase Related Protein-1 (TYRP1) and the Kit Ligand (KITLG) genes are particularly notable in showing large between-population differentiation when compared to other loci and a European-specific decrease in genetic diversity due to the presence of high frequency extended haplotypes in SNP database analyses. To further elucidate the evolutionary history of these two genes, we have characterized the patterns of microsatellite haplotype variation within lineages defined by the tag SNPs rs2733831 (FST=0.49) and rs642742 (FST= 0.70) encompassing 267 kb and 1 Mb around TYRP1 and KITLG, respectively, in 240 chromosomes from European and African ancestry. We have observed two distinct genealogical patterns associated with each gene. In the TYRP1 gene, the rs2733831 genealogy shows a paraphyletic pattern where the derived G lineage encompasses a limited subset of observed haplotype diversity, in spite of its high 0.53 frequency in Europeans, consistent with a very recent incomplete selective sweep. The KITLG gene rs642742 SNP is associated with a reciprocal monophyletic pattern where both the ancestral A and the derived G lineages have identical levels of intra-allelic diversity, suggesting that the rise in frequency of the G allele to 0.80 in Europeans may represent a nearly complete more ancient sweep associated with high levels of diversity recovery. Our data show that microsatellites may be useful markers for elucidating recent evolutionary events and suggest that the multiple selective episodes that might have shaped pigmentary traits occurred over different time scales and/or under diverse selective coefficients.

255

Inference of the peopling of the world under sequential bottlenecks with admixture. *G. Hellenthal, D. Falush.* Department of Statistics, University of Oxford, Oxford, UK.

Extracting information about migrations from autosomal data represents a formidable statistical challenge. We here present a statistical approach, based on the copying model introduced by Li and Stephens (2003), that uses the detailed information on ancestry provided by the structure of variation in haplotypes to infer patterns of colonization. In inferring a human history, our approach has two principal advantages over most previous models. Firstly, it makes no geographical assumptions but instead infers a pattern of colonization using genetic data alone. Secondly, our model allows each population to have multiple sources, allowing us to detect both geographically near and distant sources of admixture and hence to provide a richer approximation to the complex historical processes of human migration. We demonstrate the accuracy of our approach using data simulated under a coalescent with recombination model with various migration scenarios.

We apply our model to the SNP data for the 52 populations of the Human Genome Diversity Project described in Conrad et al. (2006). Our results are broadly consistent with existing serial dilution out-of-Africa models but add several interesting details. For example: (1) while European populations have received multiple independent contributions from both the Near East and Central Asia, Far Eastern populations derive most of their ancestry from two central Asian populations; (2) there is evidence for gene flow between populations on opposite sides of the Arctic Circle; (3) the Melanesians have an important source of ancestry from African hunter-gatherer populations independent of the main out-of-Africa bottleneck; (4) North and South Americans have important ancestral contributions from distinct Asian sources, implying multiple waves of migration into the Americas. A detailed depiction of the peopling of the world is available in animated form.

.

256

Waves of expansion? Interpreting principal components analyses of human genetic variation. *J. Novembre[1], M. Stephens[1,2].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Statistics, University of Chicago, Chicago, IL.

Obtaining an efficient summary of population genetic structure is crucial for many applications, including controlling for false positives in genome-wide association studies. One commonly applied technique for summarizing population genetic variation is principal components analysis (PCA). In this talk I will present results that describe how PCA behaves when applied to spatially structured populations. In these settings the principal components have a striking regularity that has previously gone unrecognized among geneticists. The results provide insight into how in practice PCA results should be interpreted and how they will perform when used to correct for population structure in GWA. As an example of the implication of the results, we suggest a new interpretation of Cavalli-Sforza et al's classic principal components maps of genetic variation in humans.

257

Overlap between genome-wide linkage and association scan signals: insights from type 2 diabetes. *E. Zeggini[1], N.J. Timpson[1], T.M. Frayling[2], M.N. Weedon[2], K.S. Elliott[1], C.M. Lindgren[1], H. Lango[2], J.R.B. Perry[2], N.W. Rayner[1], R.M. Freathy[2], A.T. Hattersley[2], M.I. McCarthy[1], UK Type 2 Diabetes Genetics Consortium, Wellcome Trust Case Control Consortium.* 1) University of Oxford, UK; 2) Peninsula Medical School, Exeter, UK.

Linkage analysis has, until recently, been the only feasible approach to obtain a genome-wide overview of disease susceptibility. Genome-wide association scans (GWAS), made possible by advances in the field, now allow comprehensive examination of common variation at a higher resolution. Over 30 linkage scans have been carried out for type 2 diabetes (T2D), highlighting a handful of regions with evidence for linkage (chr 1q, 2q, 3q, 8p, 10q, 12q, 20q). Causative variants remain to be identified for these. As part of the Wellcome Trust Case Control Consortium, we recently completed a GWAS for T2D, which, together with 4 further published scans, has increased the number of proven genes to 9 (*PPARG*, *KCNJ11*, *TCF7L2*, *HHEX/IDE*, *SLC30A8*, *CDKAL1*, *CDKN2A*, *IGF2BP2*, *FTO*). We examined the overlap between the location of previously reported T2D linkage peaks and GWAS signals. We compared the observed and expected total number of independent associations (with p<0.001 in the WTCCC scan) within regions demarked by 1-LOD-drop intervals under T2D linkage peaks and found no convincing evidence of regional over-representation (p>0.16). The number of expected hits was derived from the total number of association signals and the number of independent loci (conservatively set at r^2<0.8) in each linkage region. The only region for which we observed some evidence (albeit not significant at p=0.16) for an excess of independent associations was 10q (in which *HHEX* and *TCF7L2* reside). Importantly, however, none of the GWAS signals could generate a detectable linkage signal, as shown by estimating lambda(s) values attributable to each one (ranging from 1.002 [*SLC30A8*] to 1.025 [*TCF7L2*]). It is possible that rarer, more penetrant variants on 10q are responsible for the observed linkage, but establishing this will require extensive resequencing efforts. In conclusion, we observed little/no overlap between linkage and association scan signals for T2D.

258

Meta-analysis of 4552 type 2 diabetes (T2D) cases and 5576 controls on ~1.9 million genotyped and imputed SNPs spanning the human genome. *L.J. Scott[1], B.F Voight[2,3], J.L. Marchini[4], R. Saxena[2,3], C.J. Ding[1], N.P. Burtt[2], G. Abecasis[1], E. Zeggini[5] for the FUSION, DGI, and WTCCC/UKT2D studies.* 1) Dept Biostatistics and Center for Statistical Genetics, Univ Michigan, Ann Arbor, MI; 2) The Broad Institute of Harvard and MIT,Cambridge MA; 3) Massachusetts General Hospital, Boston MA; 4) Dept Statistics, Oxford Univ, Oxford, UK; 5) Oxford Centre for Diabetes and Wellcome Trust Centre for Human Genetics, Oxford Univ, Oxford, UK.

We have performed a meta-analysis of 1467 T2D cases and 1464 controls from the Diabetes Genetics Initiative (DGI), 1924 T2D cases and 2938 controls from the Wellcome Trust Case Control Consortium (WTCCC) and 1161 T2D cases and 1174 controls from the Finland United States Investigation of NIDDM genetics (FUSION) studies. The DGI and WTCCC samples were genotyped on the Affymetrix GeneChip500K Array Set and the FUSION samples on the Illumina HumanHap300 BeadChip. We imputed genotypes for ~2.0 million additional SNPs from the phased CEU HapMap data using the Mach 1.0 and IMPUTE programs. For all high quality genotyped and imputed SNPs with allele frequency > 1 % in each sample, we combined the T2D odds ratios using a fixed-effects meta-analysis. Of the ~1.9 million SNPs analyzed, 107 had p-values < 1×10^{-6} (1.9 expected) and 263 had p-values < 1×10^{-5} (19 expected). An initial association analysis by the DGI, WTCCC, and FUSION recently identified and confirmed multiple loci associated with T2D. After excluding SNPs from these regions we continued to observe an excess of significant results; 23 SNPs from five regions had p-values < 1×10^{-6} (1.9 SNPs expected) and 101 SNPs from 12 regions had p-values < 1×10^{-5} (19 SNPs expected). Of the 12 regions, four were identified based solely on imputed SNPs and six were identified based on genotyped SNPs from at least two of the studies. As an initial follow-up of the strongest novel T2D associations, we are genotyping SNPs on stage2/ replication samples of 10,037 T2D cases and 12,389 controls. Our results suggest that additional genes for T2D will likely be identified from this more comprehensive approach.

259

Genome-wide association scans in cohorts from Sardinia and Finland identify a locus for fasting glucose levels. *W.M. Chen[1], A.U. Jackson[1], A. Scuteri[2,6], M.R. Erdos[3], M. Uda[4], W.L. Duren[1], S. Sanna[1,4], H.M. Stringham[1], A. Mulas[4], H. Shen[5], L.J. Scott[1], S. Najjar[6], A.R. Shuldiner[5], J. Tuomilehto[7], E. Lakatta[6], R.N. Bergman[8], D. Schlessinger[6], M. Boehnke[1], G.R. Abecasis[1], R.M. Watanabe[8].* 1) Biostats, U of Michigan, Ann Arbor, MI; 2) Operativa Geriatria, INRCA, Rome, Italy; 3) National Human Genome Research Institute, Bethesda, MD; 4) Istituto di neurogenetica e neurofarmacologia, CNR, Cagliari, Italy; 5) U of Maryland, Baltimore, MD; 6) National Institute on Aging, Baltimore, MD; 7) Public Health Institute of Finland, Finland; 8) School of Medicine of USC, LA, CA.

Fasting glucose levels are a function of glucose production and utilization. Glucose is tightly regulated within a narrow range and dysfunction of this regulation can lead to type 2 diabetes. We carried out two independent genome-wide association (GWA) scans for fasting glucose levels: a scan of 4,305 Sardinians in large pedigrees from the ProgeNIA study genotyped using the Affymetrix 500K chip set and a scan of 1,256 mostly unrelated nondiabetic Finnish individuals from the FUSION study genotyped on the Illumina HumanHap300 chip. In both GWA scans, an additive genetic model was used to test for association between fasting glucose levels and SNPs, adjusting for familiality and covariates including sex, age, and age^2. To minimize the impact of outliers and skewed distribution on the association testing, quantile normalization was applied to each trait prior to GWA scans. Analysis of our two GWA scans identified a SNP that is strongly associated in both the ProgeNIA ($p = 4.0 \times 10^{-7}$) and FUSION ($p = 1.9 \times 10^{-3}$) studies, and achieves clear genome-wide significance in our two-study meta-analysis ($p = 2.8 \times 10^{-9}$). This SNP is located within 10kb of a gene that encodes an enzyme involved in the release of glucose into bloodstream. To confirm and follow-up the signal, we currently are genotyping additional SNPs in the region and following up in additional samples. Preliminary replication analysis on 973 Finish individuals and 451 Amish individuals are promising, showing a significant result in the same direction ($p = 6.5 \times 10^{-5}$ for replication data only and 1.1×10^{-12} for combined data).

260

Type 2 diabetes whole genome association study in four populations: the DiaGen Consortium. *J.T. Salonen[1], P. Uimari[1], J.-M. Aalto[1], M. Pirskanen[1], B. Todorova[1], T.-P. Tuomainen[2], J. Luedemann[3], M. Nauck[3], W. Kerner[4], R.H. Stephens[5], J.M. Gibson[5], B. Ollier[5], N. Pendleton[5], W. Mahoney[6], D. Meyre[7], J. Delplanque[7], P. Froguel[7], O. Luzzatto[8], B. Yakir[8], A. Darvasi[8].* 1) Oy Jurilab Ltd, Kuopio, Finland; 2) University of Kuopio, Kuopio, Finland; 3) Ernst Moritz Arndt University of Greifswald, Greifswald, Germany; 4) Center of Cardiology and Diabetes, Karlsburg, Germany; 5) University of Manchester, Salford and Manchester, UK; 6) Nanogen Inc, San Diego, CA; 7) Institut Pasteur de Lille, Lille, France; 8) The Hebrew University of Jerusalem, Jerusalem, Israel.

Type 2 diabetes (T2D) is a common, polygenic chronic heritable disease. DiaGen is a whole-genome association (WGA) study in 500 familial cases and 497 age- and gender-matched controls from two founder (East Finns, Ashkenazi Jews) and two heterogeneous (Germans, English) populations. The Illumina HumanHap300 tagging array was used with mean call rate of 99.5%. Statistical inferences for single SNPs were based on stratified analysis across populations using the Cochrane-Mantel-Haenszel statistic. Correction for multiple testing was based on 10,000 permutations. Only one SNP in the TCF7L2 gene reached the corrected statistical significance. Intragenic SNPs close to genome-wide significance were in AHI1 and LOC441171. The SNPs rs1535435 and rs9494266 are located within LD block of 178 kb with AHI1 gene and LOC441171 on 6q23.3. The association of these SNPs with T2D was retested and confirmed in 2573 cases and 2776 controls from France (odds ratio 2.52 for homozygote, 95% confidence interval 1.43 to 4.47, p=0.001 for rs1535435 and 2.25, 1.33 to 3.79, p=0.002 for rs9494266), confirming AHI1 as a novel T2D susceptibility gene. The replication of the TCF7L2 finding provides strong evidence for the robustness of the WGA study approach at least for the identification of genes with allelic odds ration greater than 1.7. The identification of genes affecting common diseases such as T2D may provide novel insights as to the biology of the diseases and hence serve as the basis for efficient treatments and better diagnostic tools such as disease predisposition, molecular subtyping and pharmacognenetic tests.

261

A two-stage genome-wide association study for type 2 diabetes. *R. Sladek[1,2], L. Shen[2], D. Meyre[3], G. Rocheleau[1], C. Dina[3], J. Rung[2], L. Shen[1], A. Mazur[1], C. Polychronakos[1,4], D.J. Balding[5], P. Froguel[1,6].* 1) Department of Human Genetics, McGill University, Montreal, PQ, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 3) 8090-Institute of Biology, Pasteur Institute, Lille, France; 4) Departmnent of Pediatrics, McGill University, Montreal, Canada; 5) Department of Epidemiology and Public Health, Imperial College, London, United Kingdom; 6) Section of Genomic Medicine, Imperial College, London, United Kingdom.

The recent availability of high-density genotyping arrays, which combine the power of association studies with the systematic nature of a genome-wide search, led us to undertake a two-stage, genome-wide association study to identify T2DM susceptibility loci. In the first stage of this study, we obtained genotypes for 392,935 single-nucleotide polymorphisms (SNPs) in 694 T2DM and 669 control subjects. Markers with the most significant differences in genotype frequencies between cases and controls were fast-tracked for testing in a second cohort consisting of 5,511 cases and controls. Our strategy identified 4 novel loci containing variants that confer T2DM risk, in addition to confirming the known association with the TCF7L2 gene. To complete the second stage, we have designed a custom iSelect panel to genotype the 5% most significant associations in the first stage. Based on a joint analysis of the two stages, this strategy will provide 82% power to detect an association conferring a heterozygous relative risk of GRR=1.3 for a minor allele frequency of MAF=0.20 (see table). Relevant methods, including strategies for correcting population stratification using ancestry informative markers and principal component analysis, will be presented.

MAF	GRR 1.2	GRR 1.3	GRR 1.4	GRR 1.5
0.05	0	5	29	65
0.10	3	36	81	95
0.20	21	82	97	100
0.30	40	92	99	100
0.40	51	95	100	100

.

262

Glucagon is a Thrifty Gene in Mexican Americans. *C.S. Carlson[1], M.O. Goodarzi[2], A. Reiner[3], X. Guo[2], L.J. Raffel[2], A. Xiang[4], T.A. Buchanan[4], W.A. Hsueh[5], D. Siskovick[3], J.I. Rotter[2], M.J. Rieder[3], D.A. Nickerson[3].* 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) University of Washington, Seattle, WA; 4) USC Keck School of Medicine, Los Angeles, CA; 5) David Geffen School of Medicine at UCLA, Los Angeles, CA.

The glucagon gene (GCG) encodes several hormones that play important roles in glucose homeostasis: GCG, GLP1 and GLP2. On the basis of genomic re-sequencing data, patterns of sequence diversity at GCG in European Americans (EA) are consistent with a recent positive selective sweep, while patterns in Mexican Americans (MA) suggest positive selection for a second, highly divergent haplotype in the Americas. This MA haplotype is tagged by the G allele of an A/G SNP (rs6732914). We used a GEE approach to assess the association between the G allele and fasting glucose in the Mexican American Coronary Artery Disease study, a population of adult offspring of CAD patients, and observed a mean increase in fasting blood glucose of 2.4 mg/dl in G allele carriers as compared to AA homozygotes (p= 0.045). This association was robust to adjustment for population stratification using 145 microsatellite genotypes. We confirmed this association in the Mexican American Hypertension and Insulin Resistance study, in which the families of hypertensive probands had glucose phenotypes available, where QTDT analysis demonstrated a significant increase in fasting glucose for G allele carriers (p=0.016).

In vitro tests of the A/G polymorphism in a GFP expression system show significantly reduced mRNA expression from the G allele. The SNP alters the poly-A cleavage site, so the decreased expression is likely attributable to altered mRNA turnover. The G allele is common in Mexican Americans and African Americans (populations at high risk of diabetes) but rare in European Americans and Asian Americans, providing support to the 44 year-old hypothesis that glucagon is a thrifty gene. This polymorphism may have important consequences for the utility of glucagon-derived therapeutics.

263

A functional common polymorphism in the Vitamin D-Responsive Element (VDRE) of the GH1 promoter contributes to Isolated Growth Hormone Deficiency (IGHD) susceptibility. *P. Momigliano-Richiardi, M. Godi, S. Mellone, L. Tiradani, Y. Carlomagno, A. Petri, G. Corneli, D. Vivenza, S. Bellone, C. Santoro, G. Bona, M. Giordano.* Department of Medical Sciences, University of Eastern Piedmont, Novara, Italy.

High penetrance mutations in the growth hormone gene (GH1) have been found in the severe growth hormone (GH) deficiency and in the familial forms of IGHD. However most of the IGHD subjects present with a low but detectable serum GH, no family history and no deleterious mutations. The involvement of (GH1) polymorphisms in sporadic isolated growth hormone deficiency (IGHD) was investigated by a case-control study. Seven SNPs in the GH1 promoter (at positions -308,-278,-75,-57,-31,-6,-1), one intronic (IVS4+90) and two SNPs in the LCR region, 14.5 Kb upstream, were analysed in 118 IGHD patients and in two control groups, namely normal stature (N=200) and short stature individuals with normal GH secretion (N=113). The variation -57T within a VDRE showed a positive significant association when comparing patients both with normal (p=0.006) or with short stature (p= 0.0011) controls. The genotype -57TT showed an OR of 2.93 (1.44-5.99) and 2.99 (1.42-6.31) respectively. The functional relevance of the -57T variation was demonstrated by a reporter gene (luciferase) assay, performed in the presence of vitamin D. The addition of vitamin D induced a repressive effect on in vitro GH1 gene transcription. This inhibition was significantly (p=0.012) stronger for the promoter haplotype carrying the associated variation -57T (hp#1) with respect to hp#2, bearing -57G. When replacing the T with a G at -57 on hp#1 the transcriptional activity became comparable to that of the same haplotype in the absence of vitamin D, suggesting that the T at position -57 is necessary to determine the greater vitamin D induced inhibitory effect of hp#1. The functional role of the -57 SNP was confirmed by EMSA experiments showing a different band shift pattern of the T and G sequences. In conclusion, the common -57T/G polymorphism contributes to IGHD susceptibility indicating that in the majority of the patients IGHD is a multifactorial disease.

264

A genome-wide association study in 5,402 individuals identifies several susceptibility variants for body mass index. *R.J.F. Loos[1], S. Li[1], J.H. Zhao[1], E. Wheeler[2], S. Debbenham[3], D. Strachan[4], D. Hadley[4], K. Papadakis[4], W. McArdle[5], P. Deloukas[2], M. Inouye[2], R. McGinnis[2], M. Sandhu[3], I. Barroso[2], N.J. Wareham[1].* 1) MRC Epidemiology Unit, Cambridge, UK; 2) The Wellcome Trust Sanger Institute, Hinxton, UK; 3) Institute of Public Health, University of Cambridge, UK; 4) St George's, University of London, UK; 5) University of Bristol, UK.

It is well recognized that genes contribute to the development of obesity. However, the identification of genetic variants for BMI and obesity has been largely unsuccessful.

We applied genomewide association (GWA) to identify variants that contribute to BMI in 5402 men and women. Using inverse variance weighted meta-analysis, we combined 3 GWA studies (Affymetrix 500K GeneChip). The studies included two population-based cohorts; [1] EPIC-Obesity (n=2,418) and [2] the British Birth Cohort 1958 (WTCCC)(n=1,480). The 3rd study comprised the control samples of the Diabetes Genetics Initiative (n=1,504). After QC (SNP call rate ≥0.90, HWE $p>10^{-6}$ and MAF≥5%), 352,700 SNPs were considered for analyses. All analyses were based on an additive model. BMI was log-transformed and standardised by sex and age.

We identified 51 SNPs that showed consistent and significant association with BMI ($p<10^{-4}$). A total of 21 SNPs clustered in 9 loci, which included novel loci and FTO ($p=1.4x10^{-5}$). Notably, the strongest association ($p=3.4x10^{-6}$) was observed for a cluster of 9 intronic SNPs (MAF=15%) at chr 17p with a 0.13 z-score (or 0.56 kg/m^2) increase per minor allele. A FOX gene variant (MAF=20%) showed a protective association ($p=1.9x10^{-5}$, β=-0.08 z-score/allele) and similar associations were observed for 3 intronic SNPs (MAF=31%) at chr 12p ($p=1.5x10^{-5}$, β=-0.09 z-score/allele). Several other SNPs showed highly significant associations and will be discussed.

Our study has identified previously unknown gene variants that contribute to variation in BMI in Caucasians. Further replication is sought in a 4th population-based cohort (n=6,205) that is currently being analysed.

265

Analysis of 16784 individuals shows that BMI-altering *FTO* genotypes are associated with obesity-related quantitative traits in the general population. *R.M. Freathy[1], N.J. Timpson[2,3], D.A. Lawlor[3], P. Elliott[4], A. Pouta[5], A. Ruokonen[5], S. Ebrahim[6], B. Shields[1], Y. Ben-Shlomo[3], L. Ferrucci[7], G. Paolisso[8], M.J. Neville[2], F. Karpe[2], C.N.A. Palmer[9], A.D. Morris[9], M.R. Jarvelin[4,5], G. Davey Smith[3], M.I. McCarthy[2], A.T. Hattersley[1], T.M. Frayling[1].* 1) Exeter,UK; 2) Oxford,UK; 3) Bristol,UK; 4) Imperial College,UK; 5) Oulu,Finland; 6) London Sch Hygiene & Trop Med,UK; 7) NIA, NIH,USA; 8) Napoli,Italy; 9) Dundee,UK.

We recently showed that common variation in the *FTO* gene alters body mass index (BMI;$P=3x10^{-35}$) and type 2 diabetes risk (T2D;odds ratio 1.27; $P=5x10^{-8}$). Raised BMI is associated with alterations in obesity-related traits, but their relationship with *FTO* genotype is not known. We aimed to test the association between *FTO* genotype and obesity-related traits in the general population. We hypothesised that the changes in quantitative traits associated with the *FTO* risk allele would reflect the *FTO*-BMI association and the correlations between BMI and the traits. We studied the association between *FTO* genotype and obesity-related traits by analysing 16784 white Europeans. Each copy of the rs9939609 A allele was associated with a 0.09SD (95%CI 0.07-0.11) higher BMI ($P=4x10^{-16}$), as well as with higher fasting glucose (0.03SD [0.002-0.05]; P=0.04), insulin (0.04SD [0.01-0.06]; P=0.005), triglycerides (0.03SD [0.004-0.05]; P=0.02), systolic (0.02SD [-0.004-0.04]; P=0.1) and diastolic (0.02SD [-0.001-0.05]; P=0.06) blood pressure and lower fasting HDL-cholesterol (0.03SD [0.01-0.06]; P=0.01). Effect sizes were all consistent with those expected for the per-allele change in BMI. Our results suggest that common *FTO* variation is associated with altered obesity-reated traits to an extent that is entirely consistent with its effect on BMI, strengthening evidence that raised BMI is causal to these adverse outcomes. The associations observed are modest, despite the variant's large impact on BMI and T2D risk and the use of >16000 subjects. This highlights the importance of large sample sizes for identifying effects of established diabetes risk alleles in the general population.

266

The yin and yang of T2D and cancer risk: evidence of pleiotropy from genome-wide association studies. *K.S. Elliott[1], E. Zeggini[1], N.W. Rayner[1], M.N. Weedon[2], C.M. Lindgren[1], N.J. Timpson[3], T.M. Frayling[2], C.J. Groves[1], R.M. Freathy[2], J.R.B. Perry[2], H. Lango[2], B. Shields[2], A.T. Hattersley[2], M.I. McCarthy[1].* 1) Wellcome Trust Ctr Human Genetics, Oxford, UK; 2) Peninsula Med Sch, Exeter, UK; 3) Uni of Bristol, UK.

Many of the T2D-susceptibility genes identified to date (*PPARG*, *TCF7L2*, *CDKN2A*, *HHEX* and *IGF2BP2*) are also implicated in neoplasia. Furthermore, several of the genes causal for monogenic forms of diabetes also have oncogenic potential. One possibility (consistent with the opposing effects of *CDKN2A* over- and underexpression) is that at some such variants, one "pro-proliferation" allele predisposes to cancer, whilst the other is associated with poor cellular regeneration which reduces the complement of pancreatic beta-cells with age (predisposing to diabetes). To test this hypothesis, we used data from the Wellcome Trust Case Control Consortium genome-wide association (GWA) scan (1924 cases, 2938 controls, 393,453 SNPs) to determine whether common variants impacting on cancer risk are also associated with T2D. We found strong evidence that this was true for the prostate-cancer-susceptibility SNP, rs4242382, on 8q24. The G-allele (low risk for prostate Ca) is associated with T2D in the GWA scan (OR 1.19 [1.07-1.29], p=0.002), a finding replicated in 3,757 further cases and 5,346 controls (p=0.036) (combined data: OR=1.17 (1.08-1.42), p=0.0003). We extended these findings to other recently-published cancer associations (6 loci for breast and 4 for prostate [the latter all on 8q24] and found evidence for T2D-associations affecting the low-risk breast cancer allele of rs2107425 near *H19*, *IGF2* and *INS* (p=0.0009). More extensive examination of genome-wide data for T2D and cancer susceptibility is in progress. Since we would only expect a subset of cancer susceptibility genes to be expressed in the pancreatic islet (and therefore would not expect reciprocal risk to be a feature of all such genes), these findings are consistent with the idea that variants which influence proliferative and regenerative processes may have pleiotropic effects on cancer and diabetes.

267

Long-term oral cysteamine therapy attenuates the morbidity and mortality of nephropathic cystinosis in adults. *W. Gahl[1], J.Z. Balog[1], K. O'Brien[1,2], G. Golas[1,2], R. Kleta[1,2], I. Bernardini[1].* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Office of Rare Diseases, NIH, Bethesda, MD.

Nephropathic cystinosis, a lysosomal storage disorder due to defective transport of cystine out of lysosomes, results from mutations in CTNS. Almost half the patients in North America and Europe are homozygous for a 57-kb deletion in CTNS. Without treatment, children with cystinosis suffer from renal Fanconi syndrome and its complications, growth retardation, photophobia, and end-stage renal failure requiring kidney transplantation. Treatment with oral cysteamine (Cystagon), which can reduce cellular cystine levels by 95%, dramatically slows glomerular deterioration and normalizes growth; Cystagon is approved by the FDA for use in pre-transplant cystinosis patients. Based upon our examinations of 100 adult cystinosis patients between 1985 and 2006, we report striking rates of mortality (33%; mean age 29 years) and morbidity (24-75% for each complication), specifically related to hypothyroidism, hypergonadotropic hypogonadism (in men), pulmonary insufficiency, swallowing abnormalities, myopathy, retinopathy, vascular calcifications, and diabetes. Homozygosity for the 57-kb CTNS deletion did not correlate with these individual complications, but did correlate with mortality and with the overall severity of the morbidity. In adults, long-term (>8years) oral cysteamine therapy was associated with significantly greater height and weight, older age at renal transplant, lower serum cholesterol levels, and lower rates of morbidity and mortality. In fact, as duration of cysteamine therapy increased, the frequencies of myopathy, diabetes mellitus, pulmonary dysfunction, swallowing abnormalities, vascular calcification, retinopathy and death decreased. We conclude that all cystinosis patient should receive oral cysteamine therapy, and the registration for Cystagon should be amended to include post-transplant cystinosis as an indication. In addition, we should redouble our efforts to diagnose and treat cystinosis early, including attempts at newborn screening.

268

Phase 3 extension 96-week study data for Naglazyme (galsulfase) enzyme replacement therapy in MPS VI patients. *P. Harmatz[1], R. Giugliani[2], I. Schwartz[2], N. Guffon[3], C.Sa. Miranda[4], E. Teles[4], J.E. Wraith[5], M. Beck[6], M. Scarpa[7], Z.F. Yu[8], J. Rhorer[8], S. Swiedler[9], S. Turbeville[9], H. Nicely[9], J. White[9], C. Decker[9].* 1) Childrens Hospital, Oakland, USA; 2) Med Genet Serv HCPA, Brazil; 3) Hosp Edouardo Herriot Pavillon, France; 4) Hosp de Sao Joao and IBMC, Portugal; 5) RMCH, Manchester, UK; 6) Children's Hosp, U of Mainz, Germany; 7) Pediatrics, U of Padova, Italy; 8) Statistics Collaborative, Inc, USA; 9) BioMarin Pharmaceutical Inc, USA.

Background: MPS VI is a rare, fatal lysosomal storage disease. ERT with rhASB (galsulfase) has shown positive results in clinical studies. This study reports the findings of the phase 3 open-label extension study. Methods: Efficacy and safety are reported through 96 weeks for the phase 3, open-label extension study. Endpoints included 12-minute-walk test (12MWT), 3-minute-stair-climb (3MSC), level of urinary glycosaminoglycans (GAGs) and pulmonary function. Results: Patients receiving rhASB (n=19) improved by a mean of 183 meters from baseline to week 96 in the 12MWT ($p < 0.001$). The placebo group (n=18), which was switched to active drug at week 24, improved by a mean of 117 meters from week 24 to week 96 ($p < 0 .001$). Similar improvements in the rate of stairs climbed (3MSC) were also observed ($p < 0.001$). Both groups demonstrated a sustained reduction in urinary GAGs. Forced vital capacity improved in the rhASB-treated group by 0.11 L/min from baseline to week 96 ($p = 0.039$), and in the placebo group by 0.07 L/min from week 24 to week 96 ($p < 0.001$). All but one patient developed anti-rhASB antibodies, with 90% developing a persistent antibody response, 26% developing an enzyme-neutralizing antibody response, 39% developing a receptor-binding neutralizing antibody response, and 55% developing a persistent IgE response. These antibodies were not associated with IARs or lack of clinical benefit. Pharmacokinetic parameters did vary between week 1 and week 24, but difference was not associated with IARs, antibodies, or a PK/PD correlation. Conclusions: These data support continued improvement in endurance, pulmonary function, and urinary GAGs with an acceptable safety profile.

269

Sapropterin dihydrochloride (sapropterin) increases phenylalanine (Phe) tolerance in children with phenylketonuria (PKU) maintained on a Phe-restricted diet. *D. Gruskin[1], A. Dorenbaum[2], J. Bebchuk[3], N. Longo[4].* 1) Emory Univ Schl Med,Decatur,GA; 2) BioMarin Pharmaceutical Inc.,Novato,CA; 3) Statistics Collaborative Inc,Washington,DC; 4) U Utah,Salt Lake City,UT.

Intro:Current PKU management focuses on blood Phe control using a Phe-restricted diet, but non-compliance with the diet may increase as children approach adolescence. This double-blind, placebo-controlled, Phase 3 study investigated the efficacy of sapropterin on Phe tolerance in children with PKU on diet therapy who respond to sapropterin. Methods:In Part 1, 90 subjects (4-12 yrs) with a diagnosis of PKU with hyperphenylalaninemia (≥1 blood Phe measurement ≥360μmol/L) and controlled (blood Phe ≤480μmol/L) on a Phe-restricted diet for ≥6 months received sapropterin 20mg/kg/day, for 8 days. Responders (≥30% reduction in blood Phe and blood Phe 300μmol/L[5mg/dL] on Day 8, arbitrarily defined) entered Part 2 and were randomized 3:1 to sapropterin or placebo for 10 weeks. Phe supplement was prescribed at Wk 3 and adjusted bi-weekly according to blood Phe levels. Primary endpoint was daily Phe supplement tolerated during 10 weeks while maintaining adequate blood Phe control (≤360μmol/L[6mg/dL]). Results:Of 89/90 patients in Part 1, 50 were responders eligible for Part 2, 46 were randomized (sapropterin=33;placebo=12) and 1 did not receive drug. At Wk 3 prior to Phe supplementation, mean (SD) decrease in blood Phe compared with Wk 0 was 148.5(134.2)μmol/L with sapropterin ($p<0.001$) and 96.6(243.6)μmol/L with placebo ($p= 0.2$). By Wk 10, mean (SD) daily Phe supplement tolerated was significantly increased from Wk 0 (0 mg/kg/day) with sapropterin (20.9[15.4]mg/kg/day; $p<0.001$) and with placebo (2.9[4.0]mg/kg/day;$p=0.027$). Mean (SD) daily Phe intake (dietary+supplement) increased (Wk 0-Wk 10) from 16.8(7.6) to 43.8(24.6)mg/kg/day with sapropterin ($p<0.001$), and from 16.3(8.4) to 23.5(12.6)mg/kg/day with placebo ($p=0.079$). In the sapropterin group, mean (SD) blood Phe at Wk 10 was 340.0(234.5)μmol/L. Sapropterin had an acceptable safety profile (Grange et al). Concl:Sapropterin significantly increases Phe tolerance while maintaining adequate blood Phe control in children with PKU on a Phe-restricted diet.

270

Antisense-mediated exon 51 skipping restores local dystrophin expression in muscle of Duchenne muscular dystrophy patients. *A. Aartsma-Rus[1], J.J.G.M. Verschuuren[2], A.A.M. Janson[3], G. Platenburg[3], G-J.B. van Ommen[1], J.C.T. van Deutekom[1,3].* 1) Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands; 2) Department of Neurology, Leiden University Medical Center, Leiden, the Netherlands; 3) Prosensa B.V. Leiden, the Netherlands.

Antisense-mediated reading frame restoration is currently one of the most promising therapeutic approaches for Duchenne muscular dystrophy (DMD). In this approach, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing. They have been successful in repairing the disrupted open reading frame accompanied by the generation of internally deleted, partially functional Becker-like dystrophins. Proof of concept has been achieved in cultured muscle cells from patients, as well as in the *mdx* mouse model. As an essential step towards broad clinical studies and future applications, we here evaluated the effect of a single, intramuscular dose of DMD AON PRO051. Four DMD patients with different mutations were included on basis of eligible mutation, adequate condition of the target muscle, and positive *in vitro* PRO051 skip-response. A dose of 0.8 mg PRO051, without any excipient, was injected locally into tibialis anterior muscle and a biopsy was taken after 4 weeks. Exon 51 skipping on RNA level and restoration of dystrophin expression was confirmed for each patient, as demonstrated by RT-PCR, immunohistochemical and western blot analyses. Dystrophin levels were ~10% of wild type levels, except for one patient who suffered from a severe loss of muscle fibers and profound signs of dystrophy in his tibialis muscle . The AON was well tolerated and did not provoke serious adverse events in any of the patients. Our results provide a strong basis for subsequent studies on systemic treatment of DMD patients.

271

Translational read-through of a nonsense mutation in ATP7A is associated with treatment responsiveness in Menkes disease. *A. Donsante[1], J. Tang[1], A. Yergey[2], P. Backlund[2], S.G. Kaler[1].* 1) Unit on Pediatric Genetics, NICHD, NIH, Bethesda, MD; 2) Lab. of Cellular and Molecular Biophysics, NICHD, NIH, Bethesda, MD.

Nonsense mutations usually lead to the termination of protein translation. However, functional stop codon read-through has been described in bacterial, viral, and yeast genes, mediated by ribosomal jumping or tRNA mispairing. We report read-through translation of a nonsense mutation in the human copper transport gene, ATP7A, associated with an excellent clinical response to early treatment in Menkes disease, an X-linked recessive disorder of copper metabolism. We previously proposed that internal initiation or translation re-initiation could mediate a favorable treatment response in the context of a premature stop signal in the 5' region of ATP7A (*Nature Genet* 13:21-22, 1996). *In vitro* evidence for re-initiation at this locus was reported recently (*Am J Hum Genet* 79:214-229, 2006). In a Menkes disease patient, we identified a novel C to T transition, changing codon 201 from CGA to UGA. In the context of his excellent neurologic outcome in response to therapy, we suspected that translation reinitiation downstream of R201X produced some truncated but partially functional copper transporter. However, Western analyses using antibodies against N- and C-terminal segments of ATP7A detected small amounts of the full-length (178 kDa) protein in patient fibroblasts, consistent with translational read-through of R201X. Sequencing of cDNA excluded RNA editing as an explanation. Immunohistochemistry and confocal microscopy detected trace amounts of a perinuclear, anti-ATP7A reacting material, consistent with normal trans-*Golgi* localization of ATP7A. In a yeast complementation assay, the R201X allele yielded ≈10% residual functional copper transport. We expressed peptides with the wild type or mutant sequence in 293 cells. We detected full length products from both by Western blot, with lower amounts in the mutant. Amino acid sequencing by MALDI mass spectroscopy confirmed 201R in the wild type; analysis of the mutant peptide is in progress. These findings are consistent with translational read-through and represent the first evidence of this phenomenon in mammals with a phenotypic effect.

272

Gene expression profiling of rheumatoid arthritis patients treated with anti-tumour necrosis factor. *E.J.M. Toonen[1], P. Barrera[2], H. Scheffer[1], T.R.D.J. Radstake[2], P.L.C.M. van Riel[2], B. Franke[1], M.J.H. Coenen[1].* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands; 2) Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands.

Rheumatoid arthritis (RA) is a severe inflammatory disease and genetic factors are known to play an important role in disease susceptibility, prognosis as well as therapy response. Treatment strategies blocking TNFα have proven very successful showing beneficial effects in at least 60% of the patients with RA. The reason why a subset of patients does not respond is unknown. In this study we test the hypothesis whether gene expression profiles can be used to predict anti-TNF response as well as the effect of therapy on these profiles. Expression profiles of white blood cells of 50 patients before therapy (baseline) and 14 weeks after therapy start were analyzed using the Affymetrix GeneChip® Human Exon 1.0 ST Array. The search was focused on the differences between treatment responders and non-responders at baseline and on differences in effect (before and after therapy) between these groups. Treatment responses were assessed using European League Against Rheumatism (EULAR) response criteria. Genes that showed at least a two-fold lower expression in non-responders compared to responders at baseline in the pilot to this project included the inflammatory genes IL1B, TLR10 and CD274. These genes might be candidates predicting anti-TNF treatment outcome before therapy start. Genes that were two-fold or more downregulated in responders (but not non-responders) after three months of therapy included IL1R1, IL1R2 and CXCL11. This analysis might give new leads concerning the mechanism underlying the mode of action of anti-TNF. HLA-DQA2 showed a three-fold upregulation in non-responders compared to responders at baseline, which might indicate that the HLA system is involved in determining the responsivity to anti-TNF treatment. Confirmation of the results to verify the validity of the identified markers predicting anti-TNF outcome in the larger sample is needed to elucidate the role of the identified genes in anti-TNF response.

273

Clinical practice protocols for 3-methylcrotonyl CoA carboxylase (3-MCC) deficiency. *G.L. Arnold[1], D.D. Koeberl[2], B.A Barshop[3], B.K. Burton[4], S. Cederbaum[5], A. Feigenbaum[6], C.O. Harding[7], D. Kronn[8], D. Matern[9], J.B. Gibson[10], C.L. Garganta[11], N. Braverman[12], N. Longo[13], S.G. Kahler[14], the 3-MCC working group.* 1) U Rochester, Rochester NY; 2) Duke U Med Ctr, Durham NC; 3) UCSD, San Diego CA; 4) Children's Mem Hosp, Chicago IL; 5) UCLA, Los Angeles CA; 6) Hosp for Sick Children, Toronto, Ontario; 7) Oregon Health & Science U, Portland OR; 8) NY Med College, Valhalla NY; 9) Mayo Med Ctr, Rochester MN; 10) U Texas HSC, San Antonio TX; 11) Tufts-NEMC, Boston MA; 12) Johns Hopkins Med Ctr, Baltimore MD; 13) U Utah Med Ctr, Salt Lake City UT; 14) UAMS, Little Rock AR.

3-MCC deficiency is among the most common inborn errors of metabolism identified on expanded newborn screening (1:35,000 births). However, evidence based guidelines for diagnosis and management of this disorder are lacking. Using the traditional Delphi method, a panel of 15 experts in inborn errors of metabolism was convened to develop consensus based clinical practice guidelines for the diagnosis and management of 3-MCC screen positive infants and their mothers.

Panelists reviewed the initial evaluation of the screen positive infant mother dyad, diagnostic guidelines, and management of diagnosed patients. The panel agreed on biotinidase, acylcarnitine profile, organic acid and plasma carnitine analyses for screen positive infants, organic acid and plasma acylcarnitine profile for the mother and an expanded panel of tests for ill appearing infants. Follow-up testing is commonly inconclusive; the clinician may consider retesting in one month (for liver maturity), or after weaning (in a breast fed infant when mother has elevated 3-MCC metabolites). Final diagnosis can be made on metabolite levels alone in some cases, but lymphocyte assay is recommended in most cases (fibroblast assay if lymphocyte assay is not diagnostic). Treatment recommendations include prevention of catabolic stress by avoidance of fasting, and carnitine supplementation especially in deficient or symptomatic individuals. Formal data are unavailable regarding the role of leucine restricted diets or supplemental use of biotin or glycine. [Recommendations Grade D (case report/series or expert opinion based)].

274

Bronchoscope-guided, targeted lobar aersolization of HDAd into nonhuman primate lungs results in uniform, high level pulmonary transduction, long-term transgene expression and negligible toxicity. *A. Beaudet[1], P. Hiatt[2], N. Brunetti-Pierri[1], R. McConnell[2], D. Palmer[1], R. Zuo[1], F. Vetrini[1], M. Finegold[3], P. Ng[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pulmonary Pediatrics, Baylor College of Medicine, Houston, TX; 3) Department of Pathology, Baylor College of Medicine, Houston, TX.

Uniform delivery of gene therapy vectors to all lung lobes is important for cystic fibrosis (CF) gene therapy. However, this important objective has not been achieved in large animals. To address this obstacle, we have developed an approach to deliver vector into all lung lobes. In this strategy, an intracorporeal nebulizing catheter is inserted into a bronchoscope to permit visual targeted aerosolization of vector specifically into each lung lobe. Using this approach, $1x10^{12}$ vp of HDAd-K18LacZ mixed with 0.1% LPC (to transiently open tight junctions) was sequentially aerosolized into each of the major lung lobes of a baboon. A very slight (< 2%), transient and fluctuating decrease in oxygen saturation that did not warrant supplemental oxygen was noted. The entire procedure was otherwise well tolerated and there were no changes in chest X-rays. X-gal staining of the lungs revealed extensive transduction of the epithelium in the large and small airways in all targeted lobes. Substantial expression from the K18 promoter was almost exclusively restricted to the airway epithelial cells and submucosal glands, the target cells for CF gene therapy. We also investigated the duration of pulmonary transgene expression. In these studies, we aerosolized a HDAd expressing the baboon α-fetoprotein (bAFP) from the K18 promoter into the lungs of baboons. By measuring serum AFP levels, we found that pulmonary transgene expression from transduced airway epithelial cells can be detected for at least 177 days post-vector. These results demonstrate for the first time that exceedingly high levels of transduction of the airway epithelial cells and submucosal glands throughout all lung lobes in a large animal can be achieved with negligible toxicity resulting in long-term trangene expression. This should pave the way towards successful clinical CF gene therapy.

275

A farnesyltransferase inhibitor prevents cardiovascular disease in a progeria mouse model. *B.C. Capell[1], M. Olive[1], M.R. Erdos[1], K. Cao[1], D.A. Faddah[1], K.N. Conneely[2], H. San[1], X. Qu[1], H. Avallone[3], F. Kolodgie[3], R. Virmani[3], E.G. Nabel[1, 4], F.S. Collins[1].* 1) NHGRI, NIH, Bethesda, MD; 2) University of Michigan School of Public Health, Ann Arbor, MI; 3) CVPath, Gaithersburg, MD; 4) NHLBI, NIH, Bethesda, MD.

Hutchinson-Gilford progeria syndrome (HGPS) is the most dramatic form of human premature aging. Death occurs at a mean age of 13, usually from heart attack or stroke. HGPS is almost always caused by a *de novo* point mutation in the *LMNA* gene that results in production of a mutant lamin A protein, termed "progerin", that is permanently modified by a lipid farnesyl group. It is hypothesized that progerin remains associated with the nuclear membrane due to its farnesyl-anchor, thus acting as a dominant negative, disrupting the lamina, leading to the blebbed nuclei that are the cellular hallmark of the disease. Treatment with farnesyltransferase inhibitors (FTIs) has been shown to prevent and even reverse this nuclear abnormality in cultured HGPS fibroblasts. In a study extending over a year, we show that the dose-dependent administration of the FTI, tipifarnib (R115777, Zarnestra®) to a transgenic mouse model of HGPS can ameliorate a cardiovascular phenotype (loss of vascular smooth muscle cells (VSMC) in the media of the large arteries) that is strikingly similar to the cardiovascular disease seen in HGPS. Twenty-eight mice were randomly assigned to receive oral administration of 450 mg/kg/d, 150 mg/kg/d, or vehicle-only beginning at one month of age. Following sacrifice at 9-12 months of age, five blinded observers scored pathology levels examining both VSMC loss and proteoglycan accumulation. Using levels of the biomarker non-farnesylated HDJ-2 as a measure of *in vivo* FTI activity, we found a highly significant association between FTI activity and the prevention of the cardiovascular phenotype. Experiments currently underway will determine whether this FTI can also reverse this cardiovascular disease in HGPS mice that are allowed to reach 6 months or 9 months of age before treatment is started. Our results provide encouraging evidence in support of a clinical trial of FTIs for this rare and devastating disease.

276

Small molecule correction of an inherited learning defect in *Neto1* mutant mice. *D. Ng[1], M. Kanisek[3], G.M. Pitcher[2], R.K. Szilard[3], A. Sertie[1], S.J. Clapcote[3], J.C. Roder[3], M.W. Salter[2], R.R. McInnes[1].* 1) Developmental Biology; 2) Brain & Behaviour, Hosp for Sick Children, Toronto, Canada; 3) Lunenfeld Res Inst, Toronto, Canada.

The NMDA receptor (NMDAR) is a principle ionotropic excitatory glutamate receptor in the brain, where it is crucial for synaptic plasticity, learning and memory. We previously demonstrated that Neto1, a largely neurospecific transmembrane protein with two extracellular CUB domains, associates with NMDARs. Here we report that *Neto1*$^{-/-}$ mice had normal levels of the NR2A and NR2B subunits of the NMDAR in the hippocampus, but reduced post-synaptic localization of NR2A-containing NMDARs and diminished amplitude of basal NMDAR-mediated excitatory postsynaptic currents. Long-term potentiation (LTP) at hippocampal CA3-CA1 synapses, a form of NMDAR-dependant synaptic plasticity, was depressed in acute hippocampal slices from *Neto1*$^{-/-}$ mice by approximately 50% ($n = 11$, $p < 0.001$), indicating that Neto1 is critical to synaptic plasticity. Consistent with the depressed LTP, *Neto1*$^{-/-}$ mice have impaired spatial learning: in the initial learning phase of the Morris water maze, *Neto1*$^{-/-}$ mice performed normally, but in subsequent learning trials, performance was severely impaired ($n = 10$; effect of genotype: $F_{1,16} = 5.50$, $p < 0.05$). We next hypothesized that administration of CX546, an ampakine that indirectly enhances NMDAR signaling by modulating AMPA receptors, might correct the NMDAR-dependant abnormalities. Remarkably, both the LTP and spatial learning deficits in *Neto1*$^{-/-}$ mice were completely restored to wild-type levels by CX546 ($n = 10$; effect of genotype: $F_{1,10} = 13.30$, $p < 0.01$), at doses that in wild-type mice had no effect on LTP and only a modest effect on learning. We conclude that 1) Neto1 is required to establish or maintain the normal abundance of NR2A-containing NMDARs at the postsynaptic density; 2) Neto1 has a role in NMDAR-dependant synaptic plasticity and spatial learning; 3) an inherited defect of synaptic plasticity and spatial cognition can be pharmacologically rescued, a finding with important therapeutic implications for human neurological disease.

277

Deficiency of PORCN, a regulator of Wnt signaling, causes focal dermal hypoplasia. *K.-H. Grzeschik[1], D. Bornholdt[1], F. Oeffner[1], A. Koenig[2], M. Boente[3], H. Enders[4], B. Fritz[1], M. Hertl[2], U. Grasshoff[4], K. Hoefling[3], V. Oji[5], M. Paradisi[7], C. Schuchardt[8], Z. Szalai[9], G. Tadini[10], H. Traupe[6], R. Happle[2].* 1) Human Genetics, University of Marburg, Germany; 2) Dermatology, University of Marburg, Germany; 3) Dermatology, Hospital San Miguel de Tucumán, Argentina; 4) Human Genetics, University of Tuebingen, Germany; 5) Medical Microbiology, University of Bonn, Germany; 6) Dermatology, University of Muenster, Germany; 7) Pedatric Dermatology, IDI, Roma, Italy; 8) Klinik Pieper. St. Blasien-Menzenschwand, Germany; 9) Pediatric Dermatology, Children's Hospital, Budapest, Hungary; 10) Dermatological Science, University of Milan, Italy.

Focal dermal hypoplasia (FDH, Goltz syndrome, MIM 305600) is an X-linked dominant, male-lethal, mostly sporadic multisystem birth defect affecting a multitude of tissues of ectodermal and mesodermal origin.

Using a stepwise, generally applicable approach employing i) genetic mapping of FDH in rare familial cases, ii) comparative genome hybridization on custom made high resolution arrays (HR-CGH) to search sporadic cases for small deletions in candidate chromosome areas associated with this Mendelian trait, iii) point mutation analysis in genes highlighted by overlapping deletions, we identify *PORCN*, located in Xp11.23, as the gene mutated in FDH. Focusing the CGH analysis by independent methods, such as genetic mapping, on restricted candidate areas eliminates ambiguities which might arise from the wealth of copy number variants in the human genome unrelated to the phenotype under study. Contiguous gene deletions or stop mutations affecting PORCN result in loss of function of this putative O-acyltransferase, crucial for cellular export of Wnt signaling proteins. The defect is detectable at the cellular level. Hence, FDH is a human developmental disorder caused by deficient Wnt signal production. Extreme skewing of X-inactivation or postzygotic mosaicism reduce the deleterious consequences of mutations in female patients. Due to the severity of the PORCN deficiency in cells with active mutant X-chromosome, effects of missing neighbouring genes in contiguous deletions are covered by epistasis.

278

Spectrum of *PORCN* mutations in Focal Dermal Hypoplasia. *V.R. Sutton[1], X. Wang[2], J.O. Peraza-Llanes[3], Z. Yu[2], R. Rosetta[4], Y.C. Kou[2], T.N. Eble[1], A. Patel[1], C. Thaller[5], P. Fang[1], P.H. Fernandes[1], I.B. Van den Veyver[1,2].* 1) Molecular & Human Genetics; 2) Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX; 3) Pediatrics, IMSS, Merida, Mexico; 4) Pediatrics; 5) Biochemistry & Molecular Biology, Baylor College of Medicine, Houston, TX.

Focal dermal hypoplasia (FDH), also known as Goltz syndrome (OMIM 305600), is a genetic disorder that affects multiple organ systems early in development. Features of FDH include skin abnormalities, (hypoplasia, atrophy, linear pigmentation and herniation of fat through dermal defects); papillomas of the mucous membranes; patterning defects of the hands and feet, including syndactyly, polydactyly, camptodactyly and oligodactyly; osteopathia striata; colobomas and other ocular abnormalities; and hypodontia/oligodontia. FDH displays X-linked dominant inheritance; 95% of cases are sporadic and only 10% of cases are males. Using array-based comparative genomic hybridization, we identified a 219-kb heterozygous deletion in Xp11.23 in two girls with FDH. The deleted region contained seven known genes, *SLC38A5, FTSJ1*, *EBP*, *PORCN*, *OATL1*, *RBM3* and *WDR13*. Sequencing of genes in the deleted region revealed heterozygous mutations in *PORCN* in eleven additional females and mosaic mutations in all four males analyzed. Seven of thirteen mutations result in stop codons, all of which suggests that FDH is due to loss of function of PORCN. The two females with deletions were found to have 100% skewing of X-inactivation (XCI) while 3 of 7 with point mutations had skewed XCI. We have also screened individuals with Aicardi syndrome and have found no mutations in *PORCN*. This proves that FDH, Aicardi syndrome and Microphthalmia with linear skin defects are not allelic. *PORCN* encodes the human homolog of Drosophila porcupine. Drosophila and murine Porcupine are transmembrane endoplasmic reticulum proteins required for post-translational modification and secretion of Wnt proteins. In situ hybridization of E12.5 mouse embryos was performed and revealed expression in axial and appendicular cartilage, retina, tooth buds, and dorsal skin, which suggests that the pleiotropic features of FDH can be explained by defective Wnt signaling.

279

Mutation of FAM20C leads to lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. *M.A. Simpson[1], R. Hsu[1], L.S. Keir[1], J. Hao[2], G. Sivapalan[1], L.M. Ernst[3], E.H. Zackai[3], L.I. Al Gazali[4], G. Hulskamp[5], H.M. Kingston[6], T.E. Prescott[7], A. Ion[1], M.A. Patton[1], V. Murday[8], A. George[2], A.H. Crosby[1].* 1) St George's University of London, London, UK; 2) University of Illinois at Chicago, Chicago, USA; 3) The Children's Hospital of Philadelphia, Philadelphia, USA; 4) UAE University, UAE; 5) Universität Münster, Münster, Germany; 6) St Mary's Hospital, Manchester, UK; 7) Rikshospitalet University Hospital, Oslo, Norway; 8) Royal Hospital for Sick Children, Yorkhill, Glasgow, UK.

The generation and homeostasis of bone tissue throughout development and maturity is controlled by the carefully balanced processes of bone formation and resorption. Disruption of this balance can give rise to a broad range of skeletal pathologies. Lethal osteosclerotic bone dysplasia (Raine syndrome, OMIM:259775) is an autosomal recessive disorder characterized by generalized osteosclerosis with periosteal bone formation and a distinctive facial phenotype. Affected individuals survive only days or weeks. We have identified and defined a chromosome 7 uniparental isodisomy and a 7p telomeric microdeletion in an affected case. The extent of the deleted region at the 7p telomere was established by genotyping microsatellite markers across the telomeric region. The region is delimited by D7S2563 and contains 5 transcriptional units. Sequence analysis of FAM20C in 7 additional affected cases revealed 5 homozygous mutations and 2 compound heterozygotes. The mutations identified include 5 non-synonymous base changes all affecting evolutionarily conserved residues and 4 splice site changes which are predicted to have a detrimental effect upon splicing. FAM20C is a member of the FAM20 family of secreted proteins, and has demonstrated calcium binding properties, we also show by in situ hybridisation its expression profile in mineralising tissues during development. This study defines the causative role of FAM20C in this lethal osteosclerotic disorder and its crucial role in normal bone development.

280

Molecular genetics of Meckel syndrome. *T. Attié-Bitach[1,2], L. Baala[1], S. Saunier[3], S. Audollent[2], M. Delous[3], R. Khaddour[1], C. Ozilou[2], J. Martinovic[2], A. Munnich[1,2], F. MacDonald[4], M-C. Gubler[3], S. Schneider-Maunoury[5], F. Encha-Razavi[1,2], C. Johnson[6], M. Vekemans[1,2].* 1) INSERM U781, Hopital Necker-Enfants Malades, Paris, France; 2) Département de Génétique, Hopital Necker-Enfants Malades, APHP, Paris, France; 3) INSERM U574, Hôpital Necker-Enfants Malades, Paris, France; 4) West Midlands Regional Genetics, Birmingham Women's Hospital Birmingham, U.K; 5) CNRS UMR7622, Laboratoire de Biologie du Développement, Paris, France; 6) Section of Ophthalmology and Neuroscience, St. James's University Hospital, U.K.

Meckel syndrome (MKS) is a lethal autosomal recessive syndrome characterized by cystic kidneys, brain malformations, polydactyly, and liver bile duct proliferation. Recently, two genes have been identified: MKS1/FLJ20345 on 17q and MKS3/TMEM67 on 8q. Both encode ciliary proteins. Our molecular studies of MKS1 and MKS3 in a large fetal cohort of 120 MKS and 45 MKS-like indicate that MKS1 and MKS3 genes are each responsible for about 10 % of MKS cases. A strong phenotype-genotype correlation is observed regarding the type of CNS malformation, the frequency of polydactyly and bone dysplasia. In addition, MKS3 mutations were identified in fetuses with vermis agenesis and patients with Joubert syndrome (JS) defining MKS3 as the sixth JS locus (JBTS6). A genome wide linkage study performed in consanguineous MKS families excluding MKS1-3 showed homozygozity on chromosome 12q21 in 4 families. CEP290 homozygous truncating mutations were identified in 3/4 families, and compound heterozygote mutations were found in MKS cases and in 4 families presenting a "cerebro-reno-digital" syndrome, with a phenotype intermediate between MKS and JS, demonstrating a continuum spectrum from JS to MKS. Finally, using SNP mapping in MKS and JS, we mapped a new locus on chromosome 16q12 and identified truncating mutations in the novel gene RPGRIP1L in MKS fetuses with severe craniofacial defects. Inactivation of its mouse orthologue recapitulates the cerebral, renal, liver, craniofacial and limb defects of MKS. Mutations of this ciliary encoding protein in both JS or MKS syndromes identify novel JS (JBTS7) and MKS (MKS5) loci.

281

Mutations in the gene encoding the basal body protein RPGRIP1L, a novel nephrocystin-4 interactor, cause Joubert syndrome. *D. Doherty[1], H. Arts[2], S.E.C. van Beersum[2], M.A. Parisi[1], S.J.F. Letteboer[2], N.T. Gorden[2], T.A. Peters[3], T. Märker[4], K. Voesenek[2], A. Kartono[2], H. Ozyurek[5], F.M. Farin[6], H.Y. Kroes[7], U. Wolfrum[4], H.G. Brunner[2], F.P.M. Cremers[2], I.A. Glass[1], N.V.A.M. Knoers[2], R. Roepman[2].* 1) Dept Pediatrics, Univ Washington, Seattle, WA; 2) Department of Human Genetics, Radboud University Nijmegen Medical Centre and Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands; 3) Department of Otorhinolaryngology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) Institut für Zoologie, Johannes Gutenberg University, Mainz, Germany; 5) Department of Pediatrics, Ondokuz Mayis University, Samsun, Turkey; 6) Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA; 7) Division of Biomedical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands.

Protein-protein interaction analyses have uncovered a ciliary and basal body protein network that, when disrupted, can result in a variety of disorders including nephronophthisis (NPHP), Leber congenital amaurosis, Senior-Løken syndrome (SLSN) and Joubert syndrome (JBTS). However, details of the molecular mechanisms underlying these disorders remain poorly understood. We have identified the RPGRIP1-like protein (RPGRIP1L), a homolog of RPGRIP1 (RPGR interacting protein 1), a ciliary protein previously shown to be defective in a subset of Leber congenital amaurosis. RPGRIP1L is ubiquitously expressed, and its protein product localizes to basal bodies. RPGRIP1L interacts with nephrocystin-4, and disease-causing mutations in each of these genes disrupts the RPGRIP1L-nephrocystin-4 interaction. We analyzed RPGRIP1L as a candidate gene for JBTS and found nonsense, missense and splice site mutations in families with the characteristic mid-hindbrain malformation (the molar tooth sign). This work identifies RPGRIP1L as a gene responsible for JBTS and establishes a central role for cilia and basal bodies in the pathophysiology of this disorder.

282

Domain-specific mutations in FBN1 cause a congenital form of scleroderma: Stiff Skin Syndrome. *B. Loeys[1], D. Riegert-Johnson[2], P. Whiteman[3], V. McDonnell[4], P.J. Coucke[1], A. De Paepe[1], D. Judge[6], P. Handford[3], L. Sakai[5], H.C. Dietz[6].* 1) Ctr for Medical Genetics, Ghent University, Belgium; 2) Mayo Clinic, Rochester; 3) St Catherine's College, Oxford; 4) Regional Genetics Center, Belfast, Ireland; 5) Shriner's Hospital, Portland; 6) Johns Hopkins Univ, Baltimore.

Stiff skin syndrome (SSS) is characterized by generalized indurated skin and limited joint mobility. In contrast to classic scleroderma, SSS is congenital and has no visceral involvement. Because the "tight skin"(Tsk) mouse is heterozygous for an in-frame Fbn1 duplication a role for FBN1 mutations in SSS was hypothesized. In humans, FBN1 deficiency causes Marfan syndrome and related disorders. We studied one sporadic patient and three autosomal dominant families with SSS. All lacked the skeletal, ocular and cardiovascular findings of MFS. Each family demonstrated a missense mutation in FBN1, either substituting or creating a cysteine residue in the TB4 motif of fibrillin-1. These cysteines are essential for proper TB folding and unique to TB4 is the presence of an RGD sequence, mediating matrix cell interactions via integrin binding. In contrast to MFS, pulse-chase analysis of SSS dermal fibroblasts showed preserved fibrillin-1 secretion and matrix deposition. Structural analysis of mutant recombinant peptides encompassing TB4 showed little effect on domain folding and the RGD-motif remained exposed. Immunohistochemistry of SSS skin revealed abnormal keratinocyte morphology with intense accumulation of fibrillin-1 and elastin at the dermal-epidermal junction and gross architectural matrix disturbance throughout the dermis. EM showed increased collagen expression and densely packed collagen bundles with little intervening proteoglycans. Immunogold EM of showed dense, thick and branched microfibrils that lacked the normal periodic staining of microfibrillar lattices. These data suggest that in SSS, a loss of longitudinal growth and increased lateral growth of microfibrillar aggregates occurs; both events are plausibly informed by integrin-mediated matrix-cell attachments. The relevance of this pathogenetic mechanism to classic scleroderma warrants further investigation.

283

Complex genetic approaches to monogenic disease: Cystinosis as an example. *E.K. Moses, J.E. Curran, M.P. Johnson, J. Charlesworth, T.D. Dyer, S.A. Cole, H.H. Goring, J. Blangero.* Dept Genetics, SFBR, San Antonio, TX.

Cystinosis is a rare, autosomal recessive disorder characterized by defective transport and accumulation of cystine. Mutations in the *CTNS* gene account for all known causes of cystinosis. However, little is known about the function of CTNS. In this study, we describe a novel approach for elucidating the biological functions of a monogenic disease locus via the application of complex genetic analysis to normal variation in gene expression. To examine the potential functions of the *CTNS* gene, we utilized a unique dataset of whole-genome lymphocyte transcriptional profiles from 1,240 individuals in large extended Mexican American families. Quantitative expression levels of the *CTNS* transcript were significantly heritable ($h^2 = 0.33$, $p = 1.3 \times 10^{-16}$). Resequencing of the *CTNS* gene in 182 normal individuals identified over 180 variants. Association analysis in the sequenced subset revealed strong evidence for *cis*-regulation (with p-values as low as 1.0×10^{-10}). When we genotyped these variants in all 1,240 individuals, evidence for *cis*-regulation dramatically increased (with p-values as low as 2.4×10^{-39}). Using the most highly associated SNPs, we then performed association analysis on the transcriptional profiles to identify genes that are causally downstream of *CTNS*. Preliminary analyses revealed multiple potential downstream genes related to the mediation of polyglutamine tracts and the unfolded protein response that are influenced by *CTNS* sequence variation. Using a genome-wide scan to search for potential upstream *trans*-acting regulatory genes influencing *CTNS*, we identified the *VPS13A* gene (known to be involved in protein sorting) on chromosome 9 as a plausible positional and functional candidate for a *trans*-acting regulator of *CTNS* expression. This was further supported by the identification of genetic variation in *VPS13A* showing strong evidence for association with *VPS13A* expression (i.e., *cis*-effect; $p = 1.9 \times 10^{-7}$) and with *CTNS* expression (i.e., *trans*-effect; $p = 0.029$). Overall, our results suggest that the *CTNS* gene may be important for general cellular maintenance via a role in the elimination of potentially toxic cellular waste.

284

Synergistic heterozygosity for functional TGFβ1 SNPs and BMPR2 mutations modulate age of diagnosis and penetrance of Familial Pulmonary Arterial Hypertension (FPAH). *J.A. Phillips III[1], J.S. Poling[1], C.A. Phillips[1], K.C. Stanton[1], E.D. Austin[2], J.D. Cogan[1], L.A. Wheeler[2], J.E. Loyd[2].* 1) Division of Medical Genetics; 2) Division of Pulmonary Medicine, Vanderbilt University School of Medicine, Nashville, TN.

Intro: FPAH is a progressive, autosomal dominant disease with pulmonary artery occlusion, heart failure and early death. FPAH is caused by mutations in the BMPR2 gene, which encodes a receptor in the TGFβ Superfamily. Two TGFβ1 SNPs (-509 C/T and codon 10 T/C) both increase levels of TGFβ1. The TGFβ and BMP pathways acting through SMADs 2/3 and 1/5/8, respectively can have opposing effects on apoptosis, differentiation and proliferation. Hypothesis: Synergistic Heterozygosity for functional TGFβ1 SNPs increases TGF/BMP signaling imbalance in BMPR2 mutation heterozygotes to modulate the age at diagnosis and penetrance of FPAH. Methods: TGFβ1 SNPs were genotyped by sequencing genomic DNAs of BMPR2 mutation heterozygotes and correlating TGFβ1 SNP haplotypes and FPAH phenotype. Results: BMPR2 mutation heterozygotes having least (CC) or more active (CT or TT) -509 TGFβ1 SNP genotypes had mean ages at diagnosis (AAD) of FPAH of 40.2 and 33.5 yrs, respectively (p=0.046 Mann Whitney). Those with least (TT) or more active (CT or CC) codon 10 TGFβ1 SNP genotypes had mean AAD of 42.3 and 34.1 yrs, respectively (p=0.022 Mann Whitney). Kaplan Meier analysis of BMPR2 heterozygotes having no active versus 1-4 active TGFβ1 SNP alleles had mean AAD of 44.4 and 33.4 yrs, respectively (p=0.004 log rank). Heterozygotes for all BMPR2 mutations that did not elicit nonsense mediated decay who had 0, 1 or 2 active -509 or 0-1, 2 or 3-4 active -509/codon 10 alleles had 27, 70 and 80% or 28, 70 and 75% penetrance of FPAH (p=0.002 and 0.003 ANOVA), respectively. Conclusions: 1) TGFβ1 SNP genotypes associate with AAD and penetrance of FPAH in BMPR2 mutation heterozygotes, 2) more active TGFβ1 SNP alleles may increase the TGFβ/BMP signaling imbalance to lower the AAD and increase the penetrance of FPAH, and 3) modulation of rare BMPR2 mutations by common TGFβ SNPs is an example of Synergistic Heterozygosity in reciprocal, interactive pathways.

285

***PDE8B*, encoding a high affinity cAMP phosphodiesterase, is mutant in Micronodular Adrenocortical Hyperplasia.** *A. Horvath, C. Giatzakis, E. Levine, P. Osorio, A. Robinson-White, K. Tzang, S. Boikos, M. Nesterova, C.A. Stratakis.* NIH, NICHD, SEGEN, CBethesda, MD.

Adrenocortical micronodular hyperplasia (MAH) represents a distinct type of cortisol-producing neoplasm. Genetic aberrations in cAMP signaling pathway have been found to play role in many types of adrenal tumors (ADTs); we recently identified mutations of a phoshodiesterase (PDE) gene (*PDE11A*) on 2q31-33 in 7 out of 17 MAH patients. Genome-wide allelotyping in that study (Nat Genet 2006,38:794-800) indicated that another locus on 5q14, harboring another PDE, *PDE8B*, was likely to contain a disease-related gene. In this present study, 20 patients with MAH were tested for sequence alterations of the *PDE8B* gene. We identified a single base substitution that resulted in a proline-to-histidine change in an evolutionarily-conserved residue of the protein (c.914A>T/p.H305P) in a female patient with Cushing syndrome due to MAH. The substitution was not present among 1030 unrelated control individuals. To estimate the effect on the protein function, we performed in vitro studies on HEK293 cells. We introduced the c.914A>T substitution in pCMV6-XL6 constructs containing the *PDE8B* open reading frame and measured significantly higher cellular cAMP after transfection with mutant *PDE8B* construct (15.9±2.6 pmol/ml/ug of protein for the mutant vs 5.03±0.06 for the wild type, P=0.002). *PDE8B* was found to be expressed highly in the adrenal cortex in both human and mouse tissues. A novel *PDE8B* isoform that includes one additional exon of 53 bp at the 5' end and skips the currently recognized first exon *PDE8B* was identified. This isoform showed high expression in the adrenal gland and other endocrine tissues. Mouse tissues show early expression of the *PDE8B* gene in the developing adrenal and other endocrine tissues. In conclusion, we describe here that, in addition to *PDE11A*, another PDE - *PDE8B* - is mutated in a patient with MAH. The identification of mutations of one more PDE in patients with MAH underscores the role of cAMP signaling in ADT formation and points to the possible involvement of other molecules of this pathway in ADT genetics.

286

Importance of functional studies for diagnosing effects of rare disease-causing missense mutations. *K.V. Krasnov[1], M. Tzetis[2], J. Cheng[1], G.G. Germino[1], W.B. Guggino[1], G.R. Cutting[1].* 1) Johns Hopkins Univ, Baltimore, MD; 2) Athens Univ, Athens, Greece.

Over 1,300 putative disease-causing mutations are reported in the Cystic Fibrosis (CF) Mutation Database and almost half (~630) are rare mutations predicted to substitute a single amino acid. We have investigated functional consequences of rare disease-associated mutations that alter amino acids in CFTR cytosolic loop 4 that are completely conserved across 36 diverse species. Three substitutions of the conserved R1070 residue are associated with different disease consequences: patients with R1070P and R1070Q have severe pancreatic insufficient (PI)-CF, while those with R1070W have mild pancreatic sufficient (PS)-CF. Intriguingly, CFTR bearing each of these mutations maintains chloride channel function in non-polarized cells. To determine whether R1070 mutations cause disease by affecting CFTR localization in native epithelia, we used the FLP-In system to create stable polarized MDCK cell lines that express wildtype or mutant CFTR from the same genomic integration site. Confocal microscopy revealed that R1070P was cytoplasmic, R1070Q was apical, and R1070W was apical and cytoplasmic. Quantitative biotinylation studies revealed that R1070P was not membrane inserted, R1070Q was inserted into the apical membrane at wildtype-like levels, and R1070W had apical membrane protein at levels considerably lower than wildtype. Localization of R0170P and R1070W were distinctly different from wildtype CFTR, which is consistent with their proposed deleterious role in CF patients. However, the profile of R1070Q was inconsistent with a PI-CF phenotype. Re-analysis of 12 patients bearing R1070Q revealed that each carried an in cis nonsense mutation (S466X). Discovery of the in cis S466X mutation reconciles the apparent discrepancy between functional studies of R1070Q and the phenotype of patients bearing this mutation. Our results demonstrate that substitutions of evolutionarily conserved amino acids are not necessarily deleterious and emphasize that functional studies in relevant model systems are valuable for the interpretation of the disease-causing potential of rare missense mutations.

287/W

Genetic alterations in bilateral breast cancer. *A. Shadeo, J. Chae, J. Kennett, W.L. Lam.* Department of Cancer Genetics, British Columbia Cancer Research Centre, Vancouver, BC, Canada.

Introduction: 0.7% of women diagnosed with breast cancer will develop a second primary cancer and, this scenario is called Bilateral Breast Cancer (BiBC). It has been reported that BiBC accounts for 2-11% of all breast cancer cases. BiBC has the greatest concordance with familial history and early onset of disease occurrence. Mutations in known genes such as BRCA1, BRCA2, TP53, PTEN and CHEK2 account for one third of the hereditary breast cancer cases thus leaving the majority of the genetic culprits unidentified. Genetic instability is a characteristic of malignant cells. Paired organs, such as the breast, offer a unique opportunity to study the genetic causal events in breast cancer such that the cells that become malignant in both primary occurrences are identical in terms of original genetic make-up and exposure to environmental factors. Hypothesis: Comparison of somatic genetic alterations in matched bilateral breast tumours and matched normal tissue will allow us to distinguish the discrete causal events from the random genetic changes that are associated with genetic instability in tumours. Relevant alterations would appear in both sets of tumours and would suggest a role in disease development. Results: We have used a whole genome tiling resolution array CGH platform (SMRT aCGH), which allows for breakpoint detection at approximately 50 kb resolution, to identify discrete regions of genetic alterations in ten pairs of frozen BiBC cases from the Manitoba Breast Tissue Bank and fifteen single occurrence breast cancers. 659 gene loci were found gained more frequently in intersecting BiBC pairs in comparison to single occurrence breast cancer whereas 233 were more commonly lost. Conclusion: In this study we have successfully assessed comprehensive genomic copy number profiles of 35 primary breast cancer cases (20 BiBC and 15 single occurrence). We have identified 892 genes which are frequently altered in both primary BiBC cases.

288/W

Characterization of chromosome aberrations in meningiomas combining standard cytogenetic and array CGH technique. *J. Han[1], K.E. Kim[1], R.Y. Goh[1], K.S. Woo[1], J.E. Sim[2], K.U. Kim[2], L.G. Shaffer[3].* 1) Department of Laboratory Medicine, Dong-A University College of Medicine, Busan, Korea; 2) Department of Neurosurgery, Dong-A University College of Medicine, Busan, Korea; 3) Health Research and Education Center, Signature Genomic Laboratories, LLC, Washington State University, Spokane, WA, USA.

Meningioma is the most frequent tumor of neuroectodermal origin in humans. It is usually benign. However, certain histological variants show more aggressive biological behavior and are clinically associated with a high risk of local recurrence and a less favorable prognosis. Cytogenetic and molecular biological studies are important to identify characteristic genetic aberrations associated with meningioma tumorigenesis and progression. We studied 40 meningioma tumor samples. On the cytogenetic level, we observed abnormal karyotypes in 30 (75.0%) patients, while two cases did not produce enough metaphases and 8 of 40 (20.0%) patients had normal karyotypes. The most common numerical alterations were loss of chromosome 22 (about 73.3% of abnormal cytogenetic cases). In 10 cases, monosomy 22 was found as a single primary abnormality and in the rest of 12 patients, it was combined with another numerical or structural chromosome abnormalities. Hyperidploidy and hypodiploidy were observed in three and one case, respectively. The remaining four abnormal cases included two balanced and two unbalanced rearrangements.A complex karyotype with more than three chromosome abnormalities was found in nine patients, eight of which contained monosomy 22. In cases with complex structural rearrangements, array CGH technique has permitted the best identification of abnormalities. At present, it is well established that meningiomas are genetically heterogeneous tumors. Our results also indicate that meningiomas show variable patterns of chromosomal instability. Conventional cytogenetic techniques may hamper the identification of all tumor cell clones present in samples due to the relatively low percentage of neoplastic metaphases. In turn, array CGH analyses can be systematically employed to reveal a combination of aberrations not seen by using one method alone.

289/W

FREQUENCY OF p190BCR-ABL AND p210BCR-ABL FUSION TRANSCRIPTS IN COLOMBIAN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AND PHILADELPHIA POSITIVE -ACUTE LYMPHOID LEUKEMIA(ALL-Ph+). *C.A. Aya[1], C.E Muskus[2], F. Cuéllar-Ambrossi[3], J.D. Torres[4], F. Quintero-Rivera[5], G. Vásquez-Palacio[1].* 1) Unidad de Genética Médica, Facultad Medicina Universidad de Antioquia, Medellin, Antioquia, Colombia; 2) Programa de Estudio y Control de Enfermedades Tropicales, Universidad de Antioquia, Medellín Colombia; 3) Unidad de Transplantes de Sangre y Médula, HUSVP, Universidad de Antioquia, Medellín Colombia; 4) Laboratorio de Hematología Adultos, HUSVP, Universidad de Antioquia, Medellín Colombia; 5) Department of Pathology & Laboratory Medicine, School of Medicine at UCLA,Los Angeles, CA, USA.

The t(9;22)(q34;q11) generates different BCR-ABL fusion transcripts. Three BCR-ABL fusion proteins have been described: p190BCR-ABL(e1a2), p210BCR-ABL (b2a2 or b3a2) and p230BCR-ABL (e19a2) These fusion events are related to ALL, CML and CNL, respectively. The aim of this study was to determine the frequency of p190BCR-ABL and p210BCR-ABL fusion transcripts in patients with CML and ALL-Ph+, from a Colombian population. Total RNA for the cDNA synthesis was isolated from peripheral blood samples of 38 patients. 63.16% (24/38) of the patients had CML (15-78 years) and were either recently diagnosed or already receiving Imatinib treatment. The remaining 36.84%(14/38) were diagnosed with B-ALL (n= 3 (<15 years); n=11 (>15 years)). The p210BCR-ABL transcript was seen in 95.8% (23/24) of the CML and in 27.3% (3/11) of the ALL (all >15 years) cases. We also found p190BCR-ABL fusion in 33.3%(1/3) <15 years, and in 9.1% (2/14) >15 years. Co-expression of p210BCR-ABL and p190BCR-ABL was detected in a patient with CML resistant to Imatinib . Our study demonstrated that the frequency of p210BCR-ABLtranscript in the CML population studied is similar to previous reports. In our ALL patient population the p210BCR-ABL transcript was found to be the most common, this transcript is usually less common (5-40%) in comparison to the p190BCR-ABL. Our results show a high frequency of BCR-ABL positive ALL patients (<15 years) compared to what it has been previously reported. This may due to a small sample population or represent a new subgroup of BCR-ABL positive childhood-ALL.

290/W

Molecular analysis of additional partner chromosome-*BCR* junctions of complex *BCR-ABL1* rearrangements. *S.M. Benjes[1], C.M. Morris[1, 2].* 1) Cancer Genetics Research Group, University of Otago at Christchurch, Christchurch, New Zealand; 2) Cytogenetics Unit, Canterbury Health Laboratories, Christchurch, New Zealand.

It is well established that in chronic myeloid leukaemia (CML) recombination occurring between the *BCR* gene on chromosome 22 and the *ABL1* gene on chromosome 9 creates a *BCR-ABL1* fusion protein responsible for the clinical phenotype. However, the molecular mechanism underlying *BCR-ABL1* recombination remains poorly understood. In about 90% of CML patients recombination results in a cytogenetically visible, simple reciprocal exchange involving the long arms of chromosome 9 and 22. In the remaining cases, recombination between *BCR* and *ABL1* can be more complex involving additional chromosomal sites that may be visible cytogenetically or cryptically concealed within a normal appearing karyotype. These complex translocations are of interest because they give the opportunity to study recombination sequences not only within the *BCR* and *ABL1* genes, but also on the other chromosome sites involved. Previously, we have isolated *BCR* gene fragments linked to other participating chromosomes from four patients with complex rearrangements and identified Alu repeat sequences as a common feature at the additional chromosome recombination sites. A combination of inverse-PCR and DNA sequence analysis has now been applied to isolate and characterise sequence features at *BCR* recombination sites in a new series of 20 CML patients having complex *BCR-ABL1* rearrangements. By this approach, *BCR* fragments linked to additional participating chromosomes have been isolated from seven further patients. Alu, L1 and other repetitive sequences were found at or within 441 bp of the additional partner chromosome breaks. Analysis of ~10 kb of DNA sequence extracted from databases and surrounding the recombination sites identified GC composition ranging from 38% to 62% and repetitive DNA content from 13% to 69%. Distribution of these elements and recombinogenic motifs identified at the additional partner recombination sites will be presented in context of current understanding of DNA alignment, disruption and end-joining processes.

291/W

Diagnosis of t(9;14)(p13;q32) in post-transplant lymphoproliferative disorder. *S.L. Betz[1], M.A. Vigil[1], K.W. Rao[1,2].* 1) Department of Pathology, McLendon Clinical Laboratories, University of North Carolina Hospitals, Chapel Hill, NC; 2) Departments of Pediatrics and Genetics, University of North Carolina School of Medicine, Chapel Hill, NC.

Post-transplant lymphoproliferative disorder (PTLD) is a diverse group of lymphoid proliferations that arises in immunosuppressed recipients of solid organ or bone marrow allografts. Clonal cytogenetic abnormalities are frequent in monomorphic PTLD, however, there are few reports of 14q32 rearrangements. Here we report a 3 year old patient with a history of renal transplant, a high EBV titer, lymphadenopathy, and a high clinical suspicion for PTLD. A bone marrow biopsy and fine needle liver aspiration were performed. Liver aspirate demonstrated strong diffuse positive staining for CD20, BCL-2, and EBV (ISH) EBER stains within large malignant cells, and was classified as monomorphic PTLD, diffuse large B-cell lymphoma subtype. G-banded cytogenetic analysis demonstrated the presence of del(9)(p13), add(11)(q23), and add(14)(q32) in 5 of 20 metaphase cells. In order to further identify the origin of material on the abnormal chromosomes, analysis with Vysis' MLL, p16, and CCND1/IGH FISH probes was performed. This analysis, using interphase and metaphase FISH, demonstrated the presence of a t(9;14) and a der(11) with loss of the MLL locus and 14q and 9p material attached to the distal long arm. Final cytogenetics were reported as 46,XY,t(9;14)(p13;q32),der(11)t(11;14)(q21;q12)t(9;14)(p13;q32)[5]/46,XY[14]. The t(9;14)(p13;q32) is a rare recurrent chromosomal aberration, detected in B cell lymphoproliferative disorders, and results in translocation of PAX5 to chromosome 14. While limited information exists about the t(9;14) and PTLD, the 2 reports in the literature suggest the presence of this rearrangement may be associated with a poor outcome. Our patient, diagnosed with PTLD 5 months after transplant, developed severe respiratory distress with pulmonary hemorrhage and expired 5 weeks after the initial PTLD diagnosis. This report provides further evidence that the t(9;14) is associated with PTLD, and that this rearrangement may be associated with a particularly aggressive variant of PTLD.

292/W

Cytogenetic features associated with FLT3 abnormalities in acute myeloid leukemia (AML). *A. Block[1], S.N.J. Sait[1], S. Kakati[1], P. Starostik[2], M. Barcos[3], G. Deeb[3], M. Wetzler[4], E.S. Wang[4].* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Molecular Diagnostics Lab, Roswell Park Cancer Inst, Buffalo, NY; 3) Department of Pathology, Roswell Park Cancer Inst, Buffalo, NY; 4) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY.

Accumulation of acquired genetic alterations and epigenetic changes in hematopoietic progenitor cells have been implicated in the development of acute myeloid leukemia (AML). Molecular analyses have associated the fms-related tyrosine kinase 3 (FLT3) gene with multistep pathogenesis in AML. In order to evaluate the cytogenetic contribution to the genomic instability in FLT3-positive AML, we identified 29 AML patients (pts) treated at our institution with either FLT3 internal tandem duplication (FLT-ITD; 16 female, 8 male) or missense mutations of the aspartic acid 835 of the kinase domain (D835; 1 female, 4 male). Median age was 58 years (range 22-89). FLT3 mutations were found at diagnosis in 10 pts and at relapse in 19 pts. Median overall survival from FLT3+ AML diagnosis was 6.0 months. FAB morphological classification included M1, M2, M4, M5a and M5b. Karyotypic abnormalities (abn) at diagnosis were described in 7/24 pts with FLT3-ITD and 3/5 pts with D835. Cytogenetically abnormal cases displayed extensive ITD expansion as compared to cytogenetically normal cases. 7/19 karyotypically normal AML at diagnosis later developed clonal abn at relapse. Using CALGB hierarchical criteria, no pts were observed with "favorable" cytogenetic abn. Recurrent intermediate and unfavorable risk abn included +8, +11, t(6;11), t(9;11), 11q23 abn and del(7q). Complex karyotypes (≥3 abn) were observed in 6 pts. Most structural abn did not involve recurring breakpoints. Further identification of prognostic factors in this group of pts may optimize treatment and more precisely predict patient outcome.

293/W

Analysis of promoter methylation for 15 genes in different types of leukemias. *K. Bodoor, A. Alkhateeb, Y. Haddad.* Biology Department, Jordan Univeristy of Science and Technology, Irbid 22110, Jordan.

Leukemia is classified according to the differentiation stage of blood precursor cells into acute lymphoid (ALL), acute myloid (AML), chronic lymphoid (CLL) and chronic myeloid (CML). Epigenetic modifications including DNA hypermethylation of tumor suppressor genes and global genomic hypomethylation is thought to play a major role in carcinogenesis. A growing number of genes are being identified to be inactivated through aberrant methylation in different types of cancers including leukemia. Identification of these genes and studying their methylation profile will shed light on the biology of leukemia and might offer novel therapeutic opportunities. In this study we investigate the methylation status of 15 cancer-related genes in patients of different leukemia types. After obtaining confirmed consent, 71 leukemia patients were recruited. Patients were classified as 15 ALL, 12 AML, 23 CLL, 12 CML and 9 with unclassified type. Genomic DNA is extracted from blood samples and target genes are analyzed using methylation-specific polymerase chain reaction (MS-PCR) technique. MS-PCR entails initial modification of genomic DNA by treatment with sodium bisulfite which chemically converts cytosine residues into uracil and leaves methylated cytosines unchanged and thus provides a means of differentiation between methylated and unmethylated DNA. Results are visualized by gel electrophoresis using ethidium bromide or by realtime PCR using SYBR green. The genes investigated are ATF2, DAPK, ECad, GSTP1, hMLH1, MGMT, p14, p15, p16, RAF1, RASSF1A, RARβ2, THBS1, TIMP3, TMS1. These genes play a role in different cellular processes like apoptosis, cell cycle regulation, and DNA repair and have been shown to be differentially methylated in leukemias and/or other malignancies. Preliminary data for the genes p14, p15, p16, and RASSF1A showed aberrant methylation of these genes with different types of leukemia. A comprehensive methylation pattern for the 15 genes in these 71 patients would be correlated with variables such us leukemia types, sex, and drug treatment in order to develop methylation criteria for differentiating and monitoring leukemia types.

294/W

Clonal chromosome evolution in a patient with germ cell cancer and treatment-related MDS. *D. Boles[1], D. King[1], J. Parker[1], B. Carstarphen[1], C. Stollmack[1], S. Chai[2], S. McClure[3].* 1) Cytogenetics Laboratory, Presbyterian Reference Laboratory, Charlotte, NC; 2) Carolinas Cancer Care, Charlotte, NC; 3) Presbyterian Pathology Group, Presbyterian Hospital, Charlotte, NC.

Therapy-related AML/myelodysplastic syndrome is a frequent finding in patients with germ cell tumors after chemotherapy that includes etoposide or ifosfamide. Our patient is a 24 year old male with a history of germ cell tumor and rhabdomyosarcoma who developed therapy related MDS/AML after separate treatments with BEP (bleomycin, etopside, cisplatin) and TIP (paclitaxel, ifosfamide, cisplatin). Standard G-band analysis of unstimulated 24 hour bone marrow cultures revealed two abnormal subclones: 47,XY,+iso(12)(p10), a common finding in germ cell cancers, and 48,XY,+8,+iso(12)(p10). The iso(12)(p10) was confirmed by FISH. The final karyotype was: 47,XY,+iso(12)(p10)[3]/48,idem,+8[15].ish i(12)(p10)(TEL-x4,AML1x2)[8/8].nuc ish(TELx4,AML1x2)[195/344],(TEL,AML1)x2[107/344]. Trisomy 8 is a frequent abnormality in patients with therapy-related MDS; however, the co-occurence of iso(12)(p10) and trisomy 8 in the same cells is unusual. The probable evolution of this cancer is that cells with iso(12)(p10) as the sole abnormality, and presumably derived from a primordial germ cell, then acquired an extra copy of chromosome 8 (an AML/MDS marker). The hematopoietic process that would result in this karyotype is unclear but cumulative genetic defects in a stem cell with myeloid potential are suggested.

295/W

Application of high resolution genomic analysis to define clonal relationships between synchronous lung tumors. *J.M. Campbell, T.P. Buys, J. Chae, C. MacAulay, S. Lam, W.L. Lam.* BC Cancer Agency, Vancouver, BC, CANADA.

Background: Lung cancer is the leading cause of cancer death worldwide. Because pulmonary metastases and multiple independent primary tumors are staged and managed differently in the clinic, determining the clonal relationship between tumors found in the same patient is essential for proper patient management. While most molecular analyses of suspected cases of multiple primary lung tumors (MPLTs) have relied on the status of specific genetic markers, critical genetic events (e.g. loss of chromosome arm 3p) could have occurred as independent events and do not truly reflect clonal expansion. Objectives: To assess clonal origins of multiple lung tumors from the same patient using the boundaries of multiple segmental genetic alterations for use as signature markers for clonality. To define key genetic alterations in lung tumorigenesis and disease progression by their independent emergence in each of the lung tumors from a given case. Study Design: Patients presenting with multiple lung tumors were identified and DNA was extracted from microdissected tumor cells. These DNA samples were then profiled by tiling path array CGH. SeeGH visualization software was then used to define segmental DNA alterations and chromosomal breakpoints. Results: Alignment of genomic profiles identified multiple shared and unique segmental alterations in tumors from the same patient. Shared chromosomal breakpoints in the tumors suggested a clonal relationship. Genomic changes unique to each tumor suggested subsequent tumor evolution (i.e. independent alterations). Although alteration boundaries for some regions did differ between tumors from the same patient, some of these unique alterations encompassed similar tracts of the genome, suggesting independent alteration of the same genes. Genomic determinations of clonality were contrasted with clinical determination of multiple primaries or intra-pulmonary metastases. Conclusion: We demonstrate the application of genomic profiles to delineate clonality of lung tumors from the same patient through the identification of shared breakpoints. This is an effective means of establishing the clonal relationship between such tumors.

296/W

The development of a sarcoma FISH profile to detect recurrent translocations in soft tissue sarcomas. *A.W. Carlson, R.A. Knudson, B.M. Shearer, R.P. Ketterling.* Division of Laboratory Genetics, Mayo Clinic, Rochester, MN.

Soft tissue sarcomas (STS) belong to a histologically and genetically heterogeneous group of cancers, accounting for approximately 1% and 7% of all adult and childhood malignancies, respectively. Establishing a precise diagnosis can be challenging due to similar clinical presentations, morphologic appearance and overlapping immunohistochemical staining patterns. The discovery of specific translocations associated with various sarcoma subtypes has led to the development of genetic assays to detect these recurrent rearrangements. Interphase FISH, adapted to formalin-fixed paraffin-embedded tissue, is a rapid and highly sensitive technology that allows for the detection of several specific gene rearrangements associated with various sarcoma subtypes. We have validated the application of several commercially available FISH probes to detect rearrangements involving various sarcoma-related genes, including FKHR (13q14) for alveolar rhabdomyosarcoma; CHOP (12q13) for myxoid liposarcoma; SYT (18q11.2) for synovial sarcoma; EWSR1 (22q12) for Ewings/PNET, clear cell sarcoma and desmoplastic small round cell tumor; and ALK (2p23) for inflammatory myofibroblastic tumor. We have evaluated at least 20 tumors of each sarcoma subtype and proven the effectiveness of FISH testing for detection of these recurrent gene rearrangements. In addition, via the inclusion of 25 normal paraffin-embedded tissues in the evaluation of each probe set, we have established normal cut-offs for each sarcoma probe. The recent clinical implementation of this "sarcoma FISH profile" has allowed the application of these FISH assays into various clinical algorithms for both diagnostic and follow-up testing in specific sarcoma subtypes. The detection of tumor specific translocations represents an extremely useful diagnostic tool as an adjunct to classical surgical pathology.

297/W

Identification of commonly aberrant genomic regions using high resolution oligo array CGH of FFPE breast cancer samples. *C. Carmack[1], R. Davis[2], A. De Witte[1], B. Poirier[2], E. Lin[1], A. Borowski[2], J. Ghosh[1], J. Gao[1], S. Giles[1], E. LeProust[1], D. Amorese[1], D. Roberts[1], S. Shams[3], J. Gregg[2].* 1) Agilent Technologies, Santa Clara, CA; 2) University of California Davis Medical Center, Sacramento, CA; 3) BioDiscovery, Inc. El Segundo, CA.

Background: A number of published studies have suggested that DNA copy number can be an important therapy determinant for cancer. Perhaps the best example of the importance of copy number to cancer therapy is the effectiveness of Trastuzumab (antibody to Her2/neu) in the treatment of breast tumors with amplifications in the EGFR family member ERBB2. While it is anticipated that copy number determination may also aid in the discovery of potential therapeutic targets, it is still unclear where the genomic regions are and what further prognostic or diagnostic information they may provide for breast cancer.

Material and Methods: We have designed two Comparative Genomic Hybridization (CGH) arrays. The first is a whole genome array consisting of 244,000 in situ synthesized 60-mer oligonucleotides spanning the entire human genome with an average genomic distance between probes of ~12 Kbp. A second array, containing 44,000 features was specifically designed to focus on chromosome 17 with an average probe spacing of 1kb across the chromosome and ~400bp through the region encompassing ERBB2 (17q21.2-q21.3), ~30k probes and ~8k probes respectively. Using these arrays, we performed an analysis of copy number changes in breast carcinomas and in breast carcinoma cell lines. We defined the smallest commonly amplified regions in cases with amplification at ERBB2. We also define and classify other common aberrations including amplification at 1p and deletion at 16q.

Results and Discussion: Using both whole genome and focused array data, we define several common aberrations in breast cancer samples. Further, patient chromosomal/gene copy number determinations are analyzed with respect to their ERBB2 and Estrogen Receptor protein levels, clinical classification, and outcome.

298/W

COMPLEX CHROMOSOMAL ABNORMALITIES IN AN ADOLESCENT WITH ALVEOLAR RHABDOMYOSARCOMA. *N. Chen[1], Q. Tao[1], H. Liu[1], W. Nugent[1], H.O. Shah[1,2], J. Lin[1,2].* 1) Department of Pathology, Nassau University Medical Center, East Meadow, NY; 2) Health Science Center, State University of New York, Stony Brook, NY.

Rhabdomyosarcoma (RMS), the most common pediatric soft tissue sarcoma, likely results from dysregulation of the precursor cells during skeletal myogenesis. RMS can be classified into three subtypes histologically: the most common embryonal rhabdomyosarcoma (ERMS), the less common but more aggressive alveolar rhabdomyosarcoma (ARMS), and the rare adult variant pleomorphic rhabdomyosarcoma (PRMS). Cytogenetically, ARMS is characterized by a t(2;13)(q35;q14) (PAX3/FKHR fusion protein) or a t(1;13)(p36;q14) (PAX7/FKHR fusion protein), whereas ERMS by gaining chromosomes 2, 8, 11, 12 and 13. In addition, loss of heterozygosity (LOH) at 6p, 11p, 16q and 18p has been frequently observed in both types. We present here an 18 year-old male with negative family history who presented initially with generalized lymphadenopathy, bilateral pleural effusion and hydronephrosis. FNAs on neck and left inguinal masses showed highly atypical cells, suspicious for lymphoma and recommend for tissue diagnosis. A 3.5 cm neck mass was removed and histologically showed small blue cell tumor which is positively stained for Desmin, Vimentin, Actin, MyoD1 and Myogenin. Final diagnosis is ARMS, stage IV. Patient underwent chemotherapy and radiation therapy for 6 months and resulted in a complete remission. One year later patient developed 2 subcutaneous nodules in the right thigh and back, which were proved to be recurrence of ARMS. Cytogenetic study showed a hypertetraploid composite karyotype with multiple structural and numerical aberrations: 99~100,XXYY,+X,+Y,+1,add(1)(p13)x2,add(2)(q35)x2 or der(2)t(2;13)(q35;q14),-4,+5,+6,-7,-8,+11,+11,+12,-13,-16,-16,-17,-18,+20,+20,+5~6mar[cp2]/46,XY[4]. Chromosomal abnormalities in this case include an add or der(2) probably resulting from a translocation between chromosomes 2q35 and 13q14, which is observed in approximately 80% ARMS. However, the traslocation complexed with many other chromosomal changes is rare in ARMS. Further studies may help to unveil more details of pathogenesis in RMS and provide useful information for predicting tumor biological behavior.

299/W

A case of myeloma with *C-MYC* double minutes. *J.M. Cowan.* Cytogenetics Laboratory, Tufts-New England Medical Ctr, Boston, MA.

Double minutes (dmin) are rare observations in a variety of malignant cells. They have been shown to be acentric, circular fragments of DNA that pass to daughter cells by random assortment. Dmin are more frequent in fresh cultures than in cultured cells and may transform into homogeneously staining regions after prolonged culture. The amplicon often involves *C-MYC* region, though *C-MYC* may not be the target gene. C-MYC rearrangements have been reported in 15% of primary multiple myeloma. We present a rare case of myeloma with *C-MYC* amplification in the form of dmin.

HF is a 56 yo. female, who presented with a history of progressive multiple myeloma-plasmacytoma, diagnosed 12/2005. As part of a workup for stem cell transplantation, her bone marrow aspirate was sent to the lab.

03/2006: 47,XX,t(11;14)(q13;q32),der(17)t(1;17)(q21;p13),+18[3]/47,idem,-X,add(5)(q33),+7,+8,add(13)(q34),+der(14)t(11;14)[1]/39-43,X,-X,del(5)(q13q33),del(6)(q15), t(6;15)(p23;q22),der(12)t(12;13)(q24.3;q12), del(13)(q21q33),-20[3] /46,XX[15]. FISH with a *C-MYC* probe revealed amplification in 79/415 cells. Review of metaphases revealed rare double minutes.

08/2006: following autologous stem cell transplant May 2006: 46,XX[20].nuc ish(-MYCx2)[300]

02/2007: following stem cell transplant from brother October 2006: 46,XX,del(5)(q31q33),t(11;14)(q13;q32),add(13)(q34),add(17)(p13),20~40dmin [12]/46,idem-,del(6)(q15q21)[2]/46,XY[6].nuc ish(CCND1x3),(IGHx3)(CCND1 con IGHx2)[212/336]/(MYC-x2)(5'MYC sep 3'MYCx2)(5'MYCx20~40)[105/300]

04/2007 46,XY.nuc ish(CCND1,IgH)x2[300]/(MYCx2)[300]

It has been demonstrated previously that cells become more chemosensitive after the loss of dmin. In this case the remaining cells lack dmin but are also male (donor) cells, suggesting that the observed response to therapy is the result of increased drug sensitivity. Since the number of dmin per cell increased with time, it is suggested that the response is linked to the presence of dmin in the cells.

300/W

Tiling resolution array CGH, expression, and methylation analyses of dup(1q) in Burkitt lymphomas and pediatric high hyperdiploid acute lymphoblastic leukemias reveal clustered near-centromeric breakpoints and overexpression of genes in 1q22-32.3. *J. Davidsson[1], A. Andersson[1], K. Paulsson[1], M. Heidenblad[1], M. Isaksson[1], A. Borg[2], J. Heldrup[3], M. Behrendtz[4], I. Panagopoulos[1], T. Fioretos[1], B. Johansson[1].* 1) Department of Clinical Genetic, Lund University Hospital, Lund, Scania, Sweden; 2) Department of Oncology, Lund University Hospital, and Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, Lund, Sweden; 3) Department of Pediatrics, Lund University Hospital, Lund, Sweden; 4) Department of Pediatrics, Linköping University Hospital, Linköping, Sweden.

Although gain of 1q occurs in 25% of Burkitt lymphomas (BLs) and 10% of pediatric high hyperdiploid ALLs, little is known about its molecular genetic characteristics and functional outcome. Ten dup(1q)-positive BLs/ALLs were investigated by tiling resolution array CGH analysis, which revealed that proximal breakpoints in all cases were near-centromeric, in eight of them clustering within a 1.4 Mb segment in 1q12-21.1. The 1q distal breakpoints were heterogeneous, being more distal in the ALLs than in the BLs. The minimally gained segments in the ALLs and BLs were 57.4 Mb [dup(1)(q22q32.3)] and 35 Mb [dup(1)(q12q25.2)], respectively. Satellite II DNA on 1q was not hypomethylated, as ascertained by Southern blot analyses of 15 BLs/ALLs with and without gain of 1q, indicating that aberrant methylation was not involved in the dup(1q) origin, as previously suggested for other neoplasms with 1q rearrangements. Global gene expression analyses revealed that five genes in the minimally gained region - B4GALT3, DAP3, RGS16, TMEM183A, and UCK2 - were significantly overexpressed in dup(1q)-positive ALLs compared to ALLs without dup(1q). The DAP3 and UCK2 genes were among the most overexpressed genes in the BL case with gain of 1q investigated. The DAP3 protein has been reported to be highly expressed in invasive glioblastoma multiforme cells, whereas expression of the UCK2 protein has been correlated with sensitivity to anticancer drugs. However, involvement of these genes in dup(1q)-positive ALLs and BLs has previously not been reported.

301/W

Molecular relationship between HER2 and BRCA1 in invasive breast carcinoma. *B. Deyarmin[1], R.E. Ellsworth[1], C.D. Shriver[2].* 1) Clinical Breast Care Project, Windber Reseaerch Institute, Windber, PA; 2) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC.

Background: Deletion of 17q is one of the most common alterations found in sporadic breast cancers and often results in inactivation of the BRCA1 tumor suppressor gene, rendering cells susceptible to additional DNA damage. In contrast, the HER2 gene, located within 5 Mb of the BRCA1 gene, is found to be amplified in ~20% of breast tumors. Although efforts have sought to identify the smallest common region amplified in patients with HER2 amplification, the status of BRCA1 in these patients, whether co-amplified or deleted, is unknown, thus FISH analysis of BRCA1 was performed in breast tumors with documented HER2 amplification and/or allelic imbalance at 17q12-q21. Methods: HER2 status was determined for 77 invasive breast tumors using the PathVysion HER2 kit. For BRCA1 analysis, DNA probes were generated by nick translation from BAC clone RP11-831F13 in conjunction with a CEP 17 probe. Chromosomal content was defined using copy number values of the CEP17 probe: monosomy = <1.75, disomy = 1.76-2.25, and polysomy = >2.25. Chromosomal gains and losses for HER2 and BRCA1 were defined as copy number ratios of >3.0 and <1.75, respectively. Results: The majority (73%) of breast tumors were aneusomic (42% polysomy, 31% monosomy) for CEP17. Frequency of HER2 gains and losses was 73% and 4%, respectively. For BRCA1, 56% of specimens had a loss of BRCA1, but no tumors showed a gain of BRCA1. HER2 and BRCA1 status were discordant in 35% of specimens and were divided equally between polysomy, disomy, and monosomy. Conclusions: The high frequency of BRCA1 deletion tumors selected for HER2 amplification suggests that amplification and deletion occur independently within a 5 Mb region. 20% of samples had copy number <1.75 at CEP17 and BRCA1 with normal or amplified chromosomal content at HER2 suggesting an early large deletion of 17q followed by targeted amplification of the HER2 region. This data, in conjunction with the high levels of repetitive elements within the BRCA1 gene region, suggest that genomic instability of BRCA1 may influence HER2 amplification.

302/W

Chromosomal and molecular signatures oligodendrogliome. *M. Gadji[1], D. Fortin[2], A.-M. Tsanaclis[3], R. Drouin[1].* 1) Genetics - Dept of Pediatrics, Faculty of Medicine and Health Sciences, Sherbrooke, Quebec, Canada; 2) Service of neurosurgery, Department of Surgery, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada; 3) Department of Pathology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada.

The pathogenesis of oligodendrogliomas is largely unknown. Combined loss of chromosome arms 1p and 19q has proven to be a powerful predictor of chemotherapeutic response and survival in oligodendrogliomas. The mechanism of this dual chromosomal arm loss is unexplained. Recently, two studies using cytogenetic analysis of cultured low grade oligodendroglial tumours, showed a chromosomal translocation of 1p and 19q [t(1,19)(q10;p10)], arguing to the combined loss on 1p/19q was mediated by this translocation. However, this translocation was indicated in 12% of tumours without 1p/19q deletion. We prospectively studied the oligodendroglial tumours diagnosed at the CHUS using cytogenetic and molecular genetic techniques. To date, eighteen cases composed of 7 anaplastic olidendrogliomas, 5 anaplastic oligoastrocytomas, 5 oligodendrogliomas and 1 primary neuroectodermal tumor were studied using biopsy samples after surgical resection. Thirteen cases were successfully cultured and GTG banding was performed. The karyotypes of 5 cases displayed 3 normal karyotypes, one hypotriploidy karyotype and one with a translocation between chromosome 1q and chromosome 7p [t(1;7)(q10;p10)]. To study the 1p/19q deletion status of our patients, we used fluorescent in situ hybridization technique (FISH) using specific commercial probes for chromosomes 1 and 19. To define the length of the deletions and genes related, we will use LOH (Loss Of Heterozygosity) screening with microsatellite markers specific to chromosome 1 and chromosome 19. The combination of karyotyping and molecular investigations will allow us to define a new mechanism of codeletion of chromosomal arms 1p and 19q.

303/W

The Role of Germ Cells in Cancer. *R. Goradia, S. Merchant.* Cytogenetics Center, Rolling Hills, CA.

Germ cells may play a crucial role in the origin of cancer. Current literature suggests a correlation of various forms of cancer to chromosomal abnormalities. Although not all chromosomal defects result in cancer, every form of cancer appears to be associated with one or more chromosomal abnormalities. These abnormalities originate from genomic imbalances during recombination. Germ cells and nongerm cells divide mitotically. Both of these cell types maintain the entire genome content and replicate from their original cell. However, only germ cells divide meiotically. During a meiotic division of a germ cell its homologous chromosomes exchange/recombine their genomic material with one another altering the original genome. This recombination produces diversity. However, when an abnormal recombination occurs, either spontaneously or due to radiation or chemicals exposure etc., a genomic imbalance may occur resulting in structural rearrangements of one or more chromosomes. These abnormalities may result in cancer. Any breakage of DNA that may occur during mitosis is due to external factors but breakage of DNA occurring during meiosis is due to internal factors. Genomically imbalanced germ cells may migrate to adjoining organs or infiltrate the lymphatic system and blood vessels, and manifest themselves at various levels of severity. Infertility is the highest level of severity followed by embryonic lethality, developmental abnormality and then cancer. Therefore expression of cancer may be delayed for many years. Chances of accelerated expression of cancer would be higher in cases of preexisting chromosomal abnormalities such as Down syndrome. Finally, cancer develops in multi stages. Its origin may be a germ cell whose genome has been altered, and has migrated to a different location with delayed expression.

304/W

Development of a clinical array CGH test for identification of genomic imbalances in hematological malignancies. *S. Gunn[1], M.E. Gorre[1], B. Tirtorahardjo[1], X.T. Reveles[2], R.S. Robetorye[2], P. Cottter[1], M.S. Mohammed[1].* 1) Combimatrix Molecular Diagnostics, Irvine, CA; 2) UT Health Science Center, San Antonio, TX.

Chromosomal imbalances are a hallmark of many hematological malignancies and specific recurrent changes in genomic copy number have been shown to correlate with disease severity. As these correlations become established, their translation into clinical tests will enable prognosis and risk-adapted treatment decisions at diagnosis. Commercial FISH panels have recently become available for the diagnosis of select hematological cancers including chronic lymphocytic leukemia (CLL) and acute lymphocytic leukemia (ALL). However, effective use of these tests can be complicated by the need to culture often slowly-dividing cells as well as to assume inclusion of the appropriate probe in a 5-8 probe panel. Although array CGH (aCGH) testing is becoming more common in clinical diagnostic practice, clinical application has largely been limited to diagnosis of constitutional versus acquired abnormalities. Here we describe an aCGH test designed to interrogate all CLL prognostic loci assayed by commercial FISH panels and genomic regions implicated in publications for several hematological disorders, all within a backbone of generic whole genome coverage. The arrays were developed and manufactured in-house and consist of two sets of 887 BAC clones printed in triplicate on a single slide for dye-swapped reactions. We tested the ability of the array to correctly detect and identify chromosomal imbalances using isolated genomic DNA from 21 clinical leukemic peripheral blood samples with known abnormal cytogenetic results by karyotyping, FISH, or commercial research microarrays. Previously identified imbalances were detected by the array in 21/21 samples. In 6 samples, abnormalities not previously documented by cytogenetics or FISH, such as cryptic loss of 14q32.33, were found in addition to the expected changes. By combining the coverage of well-defined loci implicated in hematological disease risk groups with the global genome perspective of an array, this new test offers the most comprehensive and efficient method of genomic imbalance assessment for risk-adapted treatment of leukemia patients.

305/W

Translocation (11;11)(p15;q22) in Acute Myeloid Leukemia - a case report. *S. Gupta[1], Y. Saffari[2], R. Nagwekar[3], R. Forte[4], J. Brody[1], P. Koduru[1].* 1) Department of Laboratory Medicine, North Shore University Hospital, NY; 2) Pathology, Long Island Jewish Medical Center, NY; 3) Pathology, Nassau University Medical Center, NY; 4) North Shore Hematology/ Oncology, PLLC, NY.

A spectrum of cytogenetic aberrations of diagnostic and prognostic implications is associated with acute myeloid leukemia. Molecular analysis of the genes involved in these chromosome abnormalities has led to the understanding of leukemic transformation. 81-year-old female with macrocytic anemia developed fever. Blasts were present in peripheral blood and bone marrow. Immunophenotyping revealed immature myelomonocytic population, positivity for HLA-DR, CD117, CD33, DC13, CD15, partial CD4; negative DC34. Most cells stained positive with myeloperoxidase; chloroacetate esterase and naphthly butyrate esterase positivity was more than 20%. A diagnosis of acute myelomonocytic leukemia was made. Chromosome analysis of bone marrow cells showed 46,XY,t(11;11)(p15;q22)[17]/46,XY,-6,+9,t(11;11)(p15;q22)[3]. Establishing cytogenetic profiles in leukemia is important in making treatment choices and monitoring the response, as well as in search for new targets. Chromosome bands 11p13~15 and 11q22~23 are involved in many recurrent, nonrandom rearrangements both in leukemia and lymphoma. Putative oncogenes and tumor suppressor genes involved in leukemogenic process are discussed.

306/W

Altered expression of *FGF8* in myxoinflammatory fibroblastic sarcoma characterized by a recurrent t(1;10)(p22;q24-25). *K.H Hallor[1], R. Sciot[2], H.C.F Bauer[3], I. Panagopoulos[1], N. Mandahl[1], F. Mertens[1].* 1) Dept. of Clinical Genetics, University Hospital, Lund, Sweden; 2) Dept. of Pathology, Catholic University of Leuven, Leuven, Belgium; 3) Department of Orthopedics, Karolinska Hospital, Stockholm, Sweden.

Recurrent structural chromosomal changes are relatively frequent findings in sarcomas and are thought to play an important role in their development. Myxoinflammatory fibroblastic sarcoma (MIFS) is a low-grade malignant lesion believed to be of fibroblastic origin and is predominantly found distally in the extremities. Its genetic background is largely unknown; one MIFS has previously been reported presenting a complex karyotype with a t(1;10)(p22;q22-24). In the present study, six tumors initially diagnosed as myxofibrosarcoma and dermatofibrosarcoma protuberans were genetically investigated. The tumors were selected on the basis of a recurrent translocation between chromosomes 1 and 10. Histopathologic re-examination showed that the morphology in all cases was compatible with MIFS or hemosiderotic fibrolipomatous tumor. Fluorescence in situ hybridization analysis of the chromosomal breakpoints showed that the *TGFBR3* gene was affected in 1p22, and that the break in 10q24 was located in or near the *MGEA5* gene. Microarray expression analysis and real-time quantitative PCR did not show altered expression levels of *TGFBR3* and *MGEA5*, and no *TGFBR3-MGEA5* fusion transcript could be detected. In contrast, increased expression levels were observed for *NPM3* and in particular *FGF8*, two consecutive genes located adjacent to, and transcribed in the same direction as, *MGEA5*. Thus, our results show that there is a nonrandom clustering of breakpoints in sarcomas with t(1;10), and that deregulation of *FGF8* expression by juxtapositioning to remote regulatory elements might explain the oncogenic consequences of the translocation. In accordance with this, previous studies have shown that increased expression of *FGF8* can stimulate the growth of fibroblasts and altered expression of this growth factor has been implicated in tumor development.

307/W

Genetic and epigenetic evidence for gain of active X-linked genes in ovarian cancer cell lines. *S. Harbord, C.J. Brown, A. Cotton, C. Salamanca, N. Auersperg, W.P. Robinson.* Depts. of Medical Genetics and Obstetrics & Gynaecology, UBC, Vancouver, BC, Canada.

Skewed somatic X-inactivation, X-linked gene over-expression and abnormal X content have all been associated with breast and/or ovarian cancer. Partial or complete reactivation of the inactive X in females may be a step in breast and ovarian cancer by leading to over-expression of a tumour enhancing gene. We examined markers of X reactivation including X-gene dosage, expression, and methylation in 8 ovarian cancer cell lines(OVCAR 2,3,5,8,10; CaOV3; SKOV3; A2780). An immortalized ovarian surface epithelium cell line (IOSE 397) and a 3-X cell line (GMO4626) were used as controls. Combined RNA/DNA FISH was used to quantify the number of inactive Xs compared to total number of Xs within a cell line. There were more than 2 Xs in 5 cell lines and 2 cell lines were highly variable in X content. Three cell lines with more than one X localized XIST to the presumptive inactive X, however the number of inactive Xs was variable. Expression levels of 8 X-linked genes were assessed by real-time PCR. Expression was inconsistent between different genes and among cell lines, ranging from a 2 to 300-fold increase compared to a control. Overall, expression was greatly increased for genes subject to inactivation but not increased in genes that escape inactivation for most ovarian cancer cell lines. Methylation at AR and FMR1 was quantified by a real-time PCR based assay and SNuPE respectively. Methylation was lower than expected for 7 of 8 ovarian cancer cell lines at AR or FMR1, while 3 cell lines had low or no methylation for both genes. In addition, we assayed 2 markers of X reactivation in 2 low passage cultures of normal ovarian surface epithelium from BRCA1 mutation positive breast cancer patients. One sample did not localize XIST to the inactive X and 3 of 5 genes subject to inactivation were over-expressed. Thus, changes to the X chromosome appear in non-cancerous ovarian tissue of BRCA1 heterozygous individuals. In summary, we find evidence for loss of X silencing or gain of an active X in ovarian cancer cell lines and normal ovarian surface epithelium of BRCA1 mutation carriers.

308/W

FISH enhances sensitivity of cytogenetic analysis in evaluation of MDS. *W-T. Hsu[1], K. Szego[1], S. Gregory[2], P. Venugopal[2], H. Fung[2], J. Loew[1], J. Shammo[2].* 1) Pathology, Rush University Medical Center, Chicago, IL; 2) Hematology, Rush University Medical Center, Chicago,IL.

The Myelodysplastic syndromes (MDS) are hematopoietic stem cell disorders, characterized by cytopenia, and risk of progression to acute leukemia. Chromosomal abnormalities are detected in 40-60% of patients with MDS. Cytogenetic findings are critical for diagnosis, prognosis and monitoring therapy. FISH has been reported to detect occult clonal abnormalities in 15-17.8% of karotypically normal patients in some studies, but found to be of limited value in others. In a retrospective study to evaluate whether FISH can be a valuable diagnostic adjunct to conventional cytogenetic (CC) analysis, we identified 41 MDS patients who had both CC and FISH panel tests. Our FISH panel was designed specifically to detect abnormalities in chromosomes 5, 7, 8, 11 and 20. In these patients, clonal chromosomal abnormalities were detected by CC in 20 patients (48.8%), by FISH in 24 (58.5%) and by either CC or FISH in 27 (65.9%). FISH increased the detection rate by 9.7%. Importantly, FISH uncovered occult clonal chromosome abnormalities in 5 of 15 patients (33%) who had either normal karyotype or normal karyotype with non-clonal abnormalities and in 2 of 6 (33%) patients with failed or incomplete CC studies. These 7 patients included 4 with RAEB, 1 with RCMD and 2 with unclassified MDS. In 20 patients with abnormal findings detected by CC, FISH uncovered additional abnormalities in 3 (15%) and changed cytogenetic risk categorization in 1 (5%) patient. FISH failed to show clonal chromosome abnormalities detected by CC in 7 patients, but these abnormalities were not evaluated by this FISH panel. CC also detected a higher percentage of cells with chromosome 5, 8 and 20 abnormalities. These results suggest that FISH increases the rate of detecting chromosomal abnormalities and is a useful adjunct in cases of failed or incomplete CC studies, and in complete CC studies that have failed to reveal clonal abnormalities. In cases with identified cytogenetic abnormalities, FISH was of limited benefit. It is advisable to perform both FISH and CC at diagnosis to establish an accurate baseline.

309/W

Genomic Alterations detected in Colon Cancer Cell Lines by using Array-Comparative Genomic Hybridization. *M.J. Kim[1, 2], S.Y. Park[1, 2], H.J. Hann[1, 2].* 1) Department of anatomy, Ewha womans univ, Seoul, Korea; 2) Ewha Global challenge for Medicine, Seoul, Korea.

Cancer is a kind of genetic disease and cancer development is accompanied by genetic event like losses, gains and amplification of certain chromosome regions or alterations of chromatin structure. Colon cancer is one of the most prevalent cancers and the fourth leading cause of cancer death in Korea. In this study, genomic alterations were analyzed by using array-CGH in colon carcinoma cell lines from Korean, SNU-81, SNU-407 and SNU-1047. Array-based CGH(Array-CGH) can be highly comprehensive, agreeable to very high resolution, sensitive and fast. The method allows investigation of general changes in target oncogene and tumor suppressor genes, which should, in turn, lead to a better understanding of the cancer development. We observed numerous chromosomal imbalances from all cell lines. The common chromosomal gains were observed in 1p36.33 ,1q22, 1q32.1, 2q35, 8p12, 8q22.3, 14q32.33, 16p13.3, and 16q24. Chromosomal losses were found in 4q22.1, 9q13, 14q21.1, 14q32.33, 20p12.1, Xq21.1, and Yq11.223. Also, gains of GON4L, YY1AP1, ELF3, EHF, EIF3SI2, AAMP, PNKD, LMOD1, DCTN6, ODF1, SOX8, ZNF276 and FANCA, with losses of CBWD3 and RBMY2SP were found in all colon cancer cell lines. In conclusion, array-CGH demonstrated the complexity of genetic aberrations in several colon carcinoma cell lines. Chromosomal aberrations identified in this study can provide candidate regions involved in the tumorigenesis and progression of colon carcinomas.

310/W

Results of plasma cell specific FISH analysis of 1,971 patients. *R.A. Knudson, R.P. Ketterling.* Div. of Laboratory Genetics, Mayo Clinic, Rochester, MN.

The plasma cell proliferative disorders (PCPD) are a heterogeneous group of plasma cell dyscrasias which account for approximately 10% of all hematologic malignancies. Subgroups of PCPD include such disparate clinical entities as monoclonal gammopathy of undetermined significance, amyloidosis, smoldering myeloma and plasma cell leukemia. Herein, we report our results on 1971 individual patients referred to the Mayo Cytogenetics Laboratory for a plasma cell-specific FISH assay. The patients had a wide range of reasons for referral encompassing all types of PCPDs. Our homebrew plasma cell FISH assay combines a cytoplasmic immunoglobulin labeled in blue and subsequent FISH analysis for 8 different recurrent abnormalities, including CCND1/IGH translocations, monosomy/deletion of chromosome 13, deletions of 17p, and trisomies of chromosomes 3, 7, 9 and 15. If an IGH rearrangement is detected which does not involve CCND1, we reflex to probes that detect FGFR3/IGH and IGH/c-MAF translocations. A total of 1286 patients (65%) had abnormal results with this targeted FISH assay while the remaining samples had insufficient plasma cells for analysis. Of the 1548 patients that had both conventional chromosome analysis and plasma cell FISH testing performed, 1031 (67%) had abnormal FISH results while 160 (10%) had abnormal chromosome results. Of the 1,286 patients abnormal by FISH, 690 (54%) had a trisomy of at least one chromosome, 627 (49%) had an IGH translocation, 615 (48%) had monosomy/deletion of chromosome 13, 94 (7%) had a deletion of 17p and 62 (5%) had a tetraploid clone. Of those with IGH translocations, 290 (46%) had fusion with CCND1, 112 (18%) with FGFR3, 54 (9%) with c-MAF, and 171 (27%) with an unknown translocation partner. These data fit well with previously established percentages for the common recurrent abnormalities observed in myeloma patients with the exception of a much higher rate of CCND1/IGH fusion. We conclude that a targeted plasma cell specific FISH assay is an important modality for detecting the common genetic abnormalities observed in the PCPD and should be applied to all patients with sufficient plasma cells to determine the prognostic subgroup associated with their genetic signature.

311/W

Array-CGH reveals hidden gene dose changes in children with acute lymphoblastic leukemia and a normal or failed karyotype. *E. Kuchinskaya[1], M. Heyman[2], A. Nordgren[1], J. Schoumans[1], J. Staaf[4], S. Söderhäll[2], D. Grandér[3], A. Borg[4], M. Nordenskjöld[1], E. Blennow[1].* 1) Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; 2) Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden; 3) Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden; 4) Department of Oncology, University Hospital, Lund, Sweden.

Cytogenetic alterations are of major prognostic importance in children with acute lymphoblastic leukemia. Failure to find cytogenetic abnormalities may therefore lead to diagnostic and therapeutic problems. We have used a 33K BAC array with the resolution of 100 kb to identify gene dose changes in 28 children with normal or failed karyotype using G-banding. Interphase FISH with labeled BAC clones from the sites of copy number alterations (CNA) was used to confirm the results. In 22 patients (79%), altogether 137 copy number alterations (CNA) were found, including 69 gains and 68 losses. In general, the losses were smaller than the gains. Array-CGH allowed us to revise the karyotype in 75% of the patients. According to the pattern of molecular cytogenetic and aCGH results, we divided the patients with B-precursor ALL into five groups: high hyperdiploidy (n=4), intrachromosomal amplification of 21q (n=5), ETV6/RUNX1 translocation (n=7), others (n=3), and no CNA (n=5). The aCGH results in patients with T-cell ALL (n=4) were analyzed separately. Tiling resolution aCGH revealed CNAs in almost 80% of the patients with a normal or failed karyotype. In addition to larger abnormalities, most of these patients showed copy number alterations that were below the resolution of G-banding. If aCGH was combined with interphase FISH for the most common, balanced rearrangements, aberrations were found in 90% of the patients. Important information regarding the frequency of genomic imbalances in children with ALL will be gained using this approach, and aCGH will in the future be an important tool to discover hidden and small imbalances that are of importance for the development and progression of ALL.

312/W

Evidence for spontaneous chromosome breakage syndrome in a case of multiple early-onset tumors of the genitourinary tract. *N. Le Meur[1], A. Rossi[1], B. Resch[2], S. Baert-Desurmont[3], T. Frebourg[3].* 1) Laboratory of Cytogenetics, EFS-Normandy, Bois-Guillaume, France; 2) Department of Gynecology, University Hospital, Rouen, France; 3) Department of Genetics, University Hospital, Rouen, France.

A 39 years old patient simultaneously presented a large squamous cell cervical carcinoma, a chromophobe renal cell carcinoma and a low grade vesical tumor. Her father developed an head and neck tumor at the age of 58 and her mother died from lung metastasis of unknown origin at age 59. There was no other familial history of cancer. This remarkable tumor association led us to perform a caryotype because constitutional chromosome 3 translocations have been described in hereditary forms of renal cancer, although of the clear cell type. Unexpectedly, chromosome analysis, performed on peripheral blood lymphocytes, revealed in 53% of the cells, multiple structural chromosomal aberrations including translocations, inversions, deletions and dicentric chromosomes. Most chromosomes were involved in the rearrangements. The level of chromosome breakage was not increased by alkylating agents. Fanconi anemia (FA) is a clinically heterogeneous disorder characterized by congenital malformations, progressive bone marrow failure, and predisposition to malignancies, especially acute myeloid leukaemia and squamous cell carcinoma mostly of the head, neck, and esophagus. Early-onset squamous-cell carcinoma of the lower female genital tract have already been reported in some patients with FA. The association of early-onset multiple primary tumours including squamous cell cervical carcinoma and multiple structural chromosomal aberrations in this patient is strongly suggestive of a Fanconi disease, even in the absence of a typical phenotype and even if the rate of chromosomal rearrangement did not increase in presence of cross-linking agents, considering the possibility of somatic mosaicism. Western blot analysis of FANCD2 ubiquitination is underway. This case report highlights the importance to perform caryotype analysis in patients presenting early-onset primary tumours which cannot be explained by a known mendelian form of cancer.

313/W

Molecular characterization of a new Ewing sarcoma cell line: *EWS-ERG* fusion gene hidden within a complex three chromosomes rearrangement, associated with *RB1* loss and polyploidyzation. *G. Maire[1], J. Bayani[1], C. Pereira[2], C. Brown[3,4], D.H. Gravel[5], J.C. Bell[3,6], J.A. Squire[1,7], M. Zielenska[2,7,8].* 1) Ontario Cancer Institute, toronto, Ontario, Canada; 2) Pediatric Laboratory Medecine and Pathology, The Hospital for Sick Children, Toronto, Canada; 3) Ottawa Health Research Institute, Center for Cancer Therapeutics, Canada; 4) Microbiology and Immunology, University of Ottawa and Orthopaediatric Surgery, Ottawa Hospital and the University of Ottawa, Canada; 5) Pathology and Laboratory Medecine, Ottawa Hospital and University of Ottawa, Canada; 6) Biochemistry, Microbiology and Immunology, University of Ottawa, Canada; 7) Laboratory Medecine and Pathology, University of Toronto, Canada; 8) Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada.

A 25 yo male presented with a very aggressive and metastatic Ewing Sarcoma (ES). Cells cultured from the biopsy transformed into a cell line. The objective was to characterize at the molecular level what were the original abnormalities of this new cell line derived from an unusually aggressive ES tumor. Cultured cells were analyzed by molecular cytogenetics techniques: SKY, FISH, aCGH and by RT-PCR. SKY analysis showed a simple pseudo tetraploid karyotype, with an apparent balanced and reciprocal t(19;22) as the sole structural rearrangement. The breakpoint on chromosome 22 mapped the *EWS* gene, and the RT-PCR for the *EWS-ERG* fusion gene was positive. Further FISH characterization using a collection of 30 BAC, identified a cryptic insertion and inversion between chromosome 21 and 22, resulting in the formation of an in frame fusion of the *EWS* 5'end with the *ERG* 3'end. In addition, aCGH identified a 16Mb deletion which included the *RB1* gene. An analysis of the biopsy prior to the cell line being established confirmed the presence of the rearrangements involving chromosomes 19, 21 and 22, but both *RB1* deletion and tetraploidization were not detected; suggesting acquisition of these aberrations was most likely an *in-vitro* effect. This study allowed us to propose a sequence of molecular evens that may account for the clinically aggressive behavior of this particular ES case.

314/W

Evaluation of N-myc amplification status in 14 neuroblastoma tumours by FISH. *M.J. Marafie[1], R. Mittal[2], S. Abulhasan[1], Z. Mohammed[1], A. Al Adwani[1].* 1) Cancer Genetics, Kuwait Medical Genetics Center, Maternity Hospital, Kuwait; 2) Kuwait Cancer Control Center, Kuwait.

Neuroblastoma is the most frequent malignant solid tumour of early childhood with two thirds of the cases presenting in children younger than 5 years. It arises from embryonal neural crest, including adrenal medulla, paravertebral sympathetic ganglia, and sympathetic paraganglia. Neuroblastoma tumours show a wide clinical and biological heterogeneity, from spontaneous regression forms to cancers with a rapid and fatal progression. Several parameters are used to predict the biological behaviour of an individual tumour more precisely, such as N-Myc oncogene amplification (10 copies), DNA ploidy, deletion or allelic loss of the short arm of chromosome 1, the expression of nerve growth factor receptor encoded by NTRK1 gene, and telomerase activity. These Tumour-derived biomarkers are suggested to have the key role in determining the aggressiveness and progression of the tumours. However, N-myc oncogene amplification is considered the most important factor to evaluate survival and therapeutic choices in these patients, more intensive than usual chemotherapeutic regimens are used for patients with aggressive tumours. We investigated the N-myc amplification status of neuroblastoma tumours derived from 14 patients, using fluorescence in situ hybridization. Eight samples showed N-myc amplification, all came from patients with high risk stage 4 neuroblastoma. Those who received special chemotherapeutic regimen, responded well to treatment and are up to now with good prognosis. FISH is a sensitive technique that facilitates characterization of neuroblastoma tumours and aids in improving the clinical management of particular patients.

315/W

Two cases of deletions of the derivative chromosome 9 in CML. *T.A. Mercado[1], A. Zaslav[1], S. Richard[2], D. Tully[1], E. Knorr[1], M. Dahir[1].* 1) Cytogenetics, SUNY, Stony Brook, Stony Brook, N.Y; 2) Blood and Marrow Stem Cell Cell Transplantation Program, SUNY Stony Brook, Stony Brook, N.Y.

Ph results from a reciprocal (R) translocation (T) of chromosomes 9 and 22. The BCR-ABL gene is formed on the der(22) and ABL-BCR on the der(9). FISH identified unexpected deletions (D) of the T product in 10-15% of patients (PTS) with CML. Studies have demonstrated atypical (AT) abnormal (AB) findings were associated with a more rapid progression (P) to blast crisis and shorter survival (OS) time. We report 2 cases of CML with a D of ABL-BCR on 9. PTS were male (PT 1 25y, PT 2 44y), diagnosed with chronic CML. PTS were placed on hydroxyurea and allopurinol awaiting imatinib therapy. PTS were evaluated using standard cytogenetic & molecular techniques: PT 1: 24H BM; PT 2: BM & unstimulated blood (UB). The T was seen in all cells of both PTS. PT 2 also had a del(6)(q21) in 6/20 cells (BM & UB). FISH using the BCR/ABL DF DC probe (200 nuclei) revealed an AT AB signal pattern of 2O:1G:1F: PT 1:194/200; PT 2:121/200 BM; 157/200 UB. FISH on 10 G-banded metaphases: PT 1: 6 BM cells; PT 2: 9 UB cells. All cells showed the AT AB pattern. Evidence showed that DS occur at the time of the Ph T and the recombination that generated the RT can also produce large DS. It has been demonstrated that DS on the 9 may be a significant prognostic indicator (Lee,Y et.al., 2006, Ca Genet Cytogenet 166(1):65; Huntly,B et.al., 2003, Blood 102(4):1160; 102(6):2205). Early studies were based on the der(9) PT treatment with hydroxyurea or interferon-based regimens. Data on D status in PTS receiving imatinib, although preliminary, appear to indicate that PTS with the DS have shorter P-free S in both chronic &/or advanced phases of CML. The PTS reported here have AT DS on the der(9). Since they are newly diagnosed and in the preliminary stages of treatment they will be monitored as to disease P and OS. It is possible that these DS are associated with the loss of one or more tumor suppressor genes within the deleted region on the der(9). This may be related to accelerated P to blast crisis in these PTS. These findings will be used to assist in determining prognosis and treatment for PTS with AT DS.

316/W

Common leukemia-associated genetic alterations in prenatal samples. *D. Mercer[1], X. Hu[3], M.M. Li[1,2,3].* 1) Hayward Genetics Center, Tulane Univ Medical Sch, New Orleans, LA; 2) Department of Pediatrics, Tulane Univ Medical Sch, New Orleans, LA; 3) Louisiana Cancer Research Consortium, New Orleans, LA.

Leukemia-associated genetic alterations play important roles in leukemogenesis. They also serve as biological markers in the diagnosis, prognosis, treatment, and follow-up of hematopoietic malignancies. We previously reported on the presence of some of these genetic alterations in the peripheral blood of healthy individuals when evaluated with nested RT-PCR. We then sought to determine if these aberrations arise early in human development by performing nested RT-PCR on cultured amniocytes or chorionic villi. We studied MLL partial tandem duplications (PTDs), BCR/ABL p190, BCR/ABL p210, and MLL/AF4 rearrangements in 30 prenatal samples. All 30 samples (100%) showed at least one MLL PTD rearrangement. Sequencing showed that the most common exon fusions were 9/3 (30 samples), 9/4 (20 samples), and 11/3 (8 samples). Genomic PCR was performed on DNA available from 16 of these samples, in which MLL PTDs were detected in the genomic DNA of 15 samples. Quantitative Real-time PCR was performed to assess the copy number of MLL PTD transcripts. The prenatal samples tested contained between 1 in 5,000 and 1 in 10,000 MLL PTD transcripts, a range that is indistinguishable from minimal residual diseases. All 30 samples (100%) were also positive for the BCR/ABL p190 e1a3 rearrangement, while 8 samples (27%) were positive for a BCR/ABL p210 rearrangement, and 20 were positive for an MLL/AF4 rearrangement (67%). These data demonstrate that many leukemia-associated genetic alterations are present in early fetal development and occur more often than what has been observed in peripheral blood or bone marrow from adults, suggesting that genetic alterations take place during cell division and present more frequently in fast growing tissues. Our data also further emphasize that serial quantitative monitoring of patients with minimal residual disease is much more informative than qualitative PCR.

317/W

Epigenetic regulation of expression of Septin 9 isoforms in cancer cells. *C. Montagna[1], D. Connolly[1], S. Nguyen[1], M. Suzuki[1], K. Nagata[2], N. Suhr[1], J. Glass[1], J.M. Greally[1], S.B. Horwitz[1], P. Verdier-Pinard[3]*. 1) Albert Einstein College of Medicine, Bronx, New York 10461, USA; 2) Aichi Human Service Center, 713-8 Kamiya-cho, Kasugai 480-0392, Japan; 3) Faculté de Pharmacie, 13005 Marseille, France.

Septin 9 is a cytoskeleton-associated protein whose function in normal and cancer cells remains largely unknown. Our previous comparative cytogenetic analysis performed on a variety of mouse models for breast cancer revealed that amplification of the Sept9 locus occurred in the form of double minute chromosomes and resulted in Sept9 over-expression. The Sept9 locus is also amplified and over-expressed in human breast and ovarian tumors. Some septin genes can generate multiple splice variants for which the regulation of expression and functional significance are poorly understood. 18 possible transcripts can be encoded at the Sept9 17q25.3 gene locus. We identified CG clusters mapping to isoform transcription start sites prompting the hypothesis that methylation at specific CG di-nucleotides is one of the mechanisms involved in the regulation of isoform expression. A synergistic approach combining proteomic, genomic and epigenetic analyses was implemented to decipher the mechanism regulating the expression of the multiple Sept9 isoforms. Treatment of A549, a lung cancer cell line, and of MDA-MB-231, a breast cancer cell line, with compounds interfering with DNA methyltransferase activity had profound effect on Sept9 isoform expression. This was detected at the protein level by 2D Western blotting and by real time PCR where an up-regulation of pan-Sept9 mRNA levels was detected. Pyrosequencing analysis is being performed to quantify differential levels of methylation in untreated versus treated cell lines. Consequences of this differential isoform expression on Sept9 cellular localization are also being investigated and identification and quantification of the isoforms differentially expressed by quantitative capillary PCR and mass spectrometry are ongoing. These experiments will generate insights into the epigenetic regulation of Sept9 isoform expression and its potential role in cancer development.

318/W

Secondary cytogenetic changes accompanying the t(2;7)(p11-12;q21-22) of chronic lymphoproliferative disease: implications for mechanisms underlying disease progression. *S. Moore[1], N. Wickham[2], R. Fraser[1], J. Suttle[1], D. Kotasek[2], T. Hillier[1]*. 1) Cancer Cytogenetics, I.M.V.S., Adelaide, SA, Australia; 2) Adelaide Cancer Centre, Ashford, SA.

Initially, translocations involving the CDK6 gene at 7q22 were considered to identify a specific subgroup of SLVL/SMZBL. It is now apparent that they also occur in CLL, with approximately equal frequency. These translocations result in over expression of CDK6,which is a gene involved in cell cycle progression through G1. The translocation breakpoints have been shown to cluster upstream of CDK6 and to involve the kappa immunoglobulin light chain locus at 2p12. 12 patients with CLD characterised by t(2;7) have been reported to date (1 B-PLL, 6 CLL and 5 SLVL/SMZBL). We now describe a 67 year old man who has low grade lymphoma characterised by 46,XY,t(2;7)(p12;q22),der(8)t(8;12)(p11;q13).

Over expression of CDK6 by translocation is likely to be insufficient, on its own, to cause aggressive disease since the cases reported so far have fairly indolent disease unless accompanied by additional cytogenetic changes.

Of the 13 patients now recognised with t(2;7), 8 showed additional cytogenetic changes. 6 patients showed changes in common: loss of 8p in 5, loss of 17p in 4 and trisomy 12 in 4. Interestingly, an unbalanced translocation involving 8p and 17p provides the mechanism for loss of these regions in 2 patients and unbalanced translocations between 8p and 12q resulted in loss of 8p and gain of 12q in 2 patients. The likely tumour suppressor gene on 17 is the p53 gene. Trisomy 12 is a common finding in CLL and has also been reported in the karyotypes of patients with various forms of lymphoma, although the gene(s) that contribute to oncogenesis have not yet been identified. The tumour suppressor(s) on 8p are unknown. Characterisation of the minimal regions of deletion on 8p and gain on 12q may help to identify patients who have a more aggressive disease and who may benefit from some of the newer therapies that are being developed to target cyclin-dependent kinases. To this end we are using BAC FISH to explore the breakpoints of the t(8;12) in our patient.

319/W

ANEUPLOIDY OF CHROMOSOME 17 AND TP53 GENE DELETION IN GASTROINTESTINAL TUMORS OF A COLOMBIAN COHORT. *C.M. Muñetón-Peña[1], G.C. Ramírez-Gaviria[1], J.C. Herrera-Patiño[1], L.F. Isaza-Jimenez[2], F. Quintero-Rivera[3], G. Vásquez-Palacio[1]*. 1) Unidad de Genetic Medica, Facultad Medicina Universidad Antioquia, Medellin, Antioquia, Colombia; 2) Departamento de Cirugía, Facultad de Medicina, Universidad de Antioquia, HUSVP, Medellín, Colombia; 3) Department of Pathology & Laboratory Medicine, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Gastrointestinal cancer is one of the most common malignancies in Colombia. The development and progression of gastrointestinal tumors is generally driven by an accumulation of genetic alterations. Among the alterations, mutations in the TP53 gene or deletion on chromosome 17p13.1 seem to be the key factors in the development of gastrointestinal cancer. Our aim was to evaluate aneuploidy of chromosome 17 and TP53 gene deletions in primary gastrointestinal tumors samples by dual-color FISH. 15 primary gastrointestinal tumor samples were analyzed from different tissues: stomach n=5; esophagus n=2; colon n=6; and rectum and duodenum n=1, respectively. Samples were minced and enzymatically disaggregated with 0.2% collagenase to obtain tumor cells suspension. Dual-color FISH assays were performed using direct fluorescent labeling probes for the centromere (CEP) of chromosome 17 and LSI TP53 gene. Hybridization signals were counted in 100 interphase nuclei. Aneuploidy (monosomy) of chromosome 17 was found in 33.3% (5/15) of the samples. Most of tumor samples exhibited heterogeneous clones that were monosomic, disomic, trisomic and occasionally tetrasomic. The TP53 gene deletion was found in 93.3 % (14/15) of the analyzed samples. Only 1 sample was normal for copy number of chromosome 17 and TP53 gene. 14 out of 15 tumors samples showed an advanced stage of tumorigenesis. These findings demonstrate a low frequency of aneuploidy of chromosome 17; however, we found a high frequency of TP53 deletion in the group of samples with advanced stages and confirmed that deletion in 17p13.1 region is common in gastrointestinal cancer, especially in advanced adenocarcinoma, and that it might play an important role in tumor progression. FISH analysis is a useful tool to simultaneously detect numerical and structural chromosome abnormalities in tumor cells.

320/W

High-resolution analysis of Segmental DNA Changes in various cancer tissues. *Y. Murayama[1], S. Ozawa[2, 3], S. Asakawa[1], Y. Saikawa[2], H. Hasegawa[2], H. Jinno[2], K. Aiura[2], A. Takayanagi[1], M. Maekawa[4], Y. Kitagawa[2], M. Kitajima[2, 6], N. Shimizu[1, 5]*. 1) Dept. of Molecular Biology, Keio University School of Medicine, Shinjyuku, Tokyo, Japan; 2) Dept. of Surgery, Keio University School of Medicine, Shinjyuku, Tokyo, Japan; 3) Department of Surgery, Banbuntane Houtokukai Hospital, Fujita Health University, Nagoya, Japan; 4) GSP Lab. Inc., Kawasaki, Kanagawa, Japan; 5) The Leading Institutes of Keio University, GSP Center, Tsukuba, Ibaraki, Japan; 6) Tokyo Mita Hospital, International University of Health and Welfare, Tokyo, Japan.

We have made original BAC-microarray using the Keio BAC-library that was used extensively for genomic DNA sequencing during the human genome project. The Keio BAC-microarray consisted of 7,718 DNA segments of average size 150 kb in triplicates and those DNA segments covered over 1/3 of the human genome in the interval of one BAC clone every 400 kb. The chromosomal position of each BAC DNA is located on the updated genome sequence data (Build36), and known genes present in the corresponding DNA region can be readily identified by using home-made computer software. We employed the Keio BAC-microarray to detect segmental DNA copy number changes in various cancer tissues. We have analyzed 80 among 200 cancer samples so far collected from esophagus, breast, colorectal and gastric origins. In fact, we detected copy number changes of particular DNA segments in those cancer tissues, in which some of known oncogenes and tumor suppressor genes are identified. We detected copy number changes at a locus of 9q33 in a colorectal cancer, where a cancer related gene was identified. Further studies on these 2,000 cancer samples would provide new information which should be inevitable to establish new marker genes for the diagnosis of each cancer type and discovery of therapeutic agents.

321/W

A comparison of the proximal promoter regions of the *PAX3* and *PAX7* genes. *E. Möller, M. Isaksson, N. Mandahl, F. Mertens, I. Pangopoulos.* Dept. of Clinical Genetics, University Hospital, Lund, Sweden.

The *PAX3* and *PAX7* genes are rearranged through the common chromosomal aberration t(2;13)(q35;q14) and less frequent variant t(1;13)(p36;q14), respectively, in the pediatric soft tissue tumor alveolar rhabdomyosarcoma (ARMS). The resultant hybrid *PAX3-FOXO1A* and *PAX7-FOXO1A* genes are expressed in ARMS and encode chimeric transcription factors that are more potent than the wildtype transcription factors. Previous studies have suggested that the expression of *PAX7-FOXO1A* is copy-number dependent whereas that of *PAX3-FOXO1A* is not, and it has been suggested that this may be due to a weaker *PAX7* promoter compared to *PAX3*. The aim of the present study was to compare the abilities of the *PAX3* and *PAX7* proximal promoters to drive *Photinus Pyralis* (firefly) luciferase expression. The *PAX3* and *PAX7* promoter fragments were analyzed with the dual-luciferase reporter assay using three vector systems, pGL3-Basic, pGL4.10[*luc2*] and pFhRL. The following eight cell lines were included in the study: HEK293, NIH3T3, HeLa, embryonal rhabdomyosarcoma cell line RD and alveolar rhabdomyosarcoma cell lines RH-30, SJCRH30, RH-41 and RC2. The *PAX3* promoter fragment was found to be capable of more efficient transcriptional activation than that of *PAX7*, irrespective of vector system or cell line used. Our findings are consistent with the notion that an amplification event might be required for the *PAX7-FOXO1A* chimeric transcript to reach a critical expression level for oncogenic activity.

322/W

MLL amplification is a distinct biological entity in patients with MDS/AML carrying complex chromosomal changes and predicts poor prognosis. *V. Nandula[1,3], E. Ritchie[4], D. Savage[2], B. Alobeid[1], G. Bhagat[1,3], V.V. Murty[1,3]*. 1) Departments of Pathology; 2) Medicine; 3) Institute for Cancer Genetics, Columbia University Medical Center, New York, NY:10032; 4) Department of Medicine, Weill Cornell Medical Center, New York, NY:10021.

Acute myeloid leukemia is a disease that is heterogeneous in morphology and prognosis. Complex karyotypes account for 10-20% of all the AML cases and in 50% of the therapy related AML and MDS cases, increasing with age. Despite intensive treatment their median overall survival is less than 6 months. MLL amplification has been frequently reported in MDS/AML patients. The aim of the present study is to identify specific karyotypic and molecular alterations among this subset of patients and correlate the changes with treatment outcome. MDS/AML cases (N=72) with complex chromosomal rearrangements ascertained from 1998 to 2004 were included in the study. Of these, 12 cases had material available for further evaluation. Using molecular cytogenetic methods (FISH and SKY) we studied MLL status along with the frequently reported losses (5q, 7q, 12p and 17p) and gains (8, and 21). Eight of the twelve cases (65%) had MLL amplification (copy number ranging from 6-20copies) while 50% of these cases had ATM gene co amplified with the MLL locus. All cases had loss of chromosome 5q. 50% of the cases had gains of chromosome 8. 17p loss was seen in 50% cases. All the cases that had 17p deletion had over expression of TP53. 12p deletions were found in 40% of the cases. All the cases had a very less overall survival from few days to few months. The present study with losses of 5q, 12p and 17p and gains of MLL form a morphological-cytogenetic entity and identifies a subset of MDS/AML patients with poor prognosis.

323/W

Positional dislocation of an intact RARα due to inv(17)(p12q22) in a case of acute myeloid leukemia. *K.H. Ramesh[1], D. Wei[1], M. Zohouri[1], H. Ratech[2], R. Shaknovich[2], A. Verma[3], L.A. Cannizzaro[1].* 1) Div. Cytogenetics, Dept of Pathology, Montefiore Med Ctr, Albert Einstein Col Medicine, Bronx, NY; 2) Division Hematopathology, Dept of Pathology, Montefiore Med Ctr., Albert Einstein Col Medicine, Bronx, NY; 3) Department of Oncology, Montefiore Med Ctr., Albert Einstein Col Medicine, Bronx, NY.

A 54-year-old female with a history of metastatic infiltrating mammary tubulo-lobular carcinoma with perineural invasion and micro calcification now presented with pancytopenia. Flow cytometry of the bone marrow revealed 60% CD12+, CD33+, CD117+, CD34+, MPO+ myeloblasts with aberrant expression of CD2. The bone marrow biopsy showed hypocellular marrow (0-10%) containing approximately 20% scattered blasts. The histochemical analysis performed on the paraffin sections of the bone marrow biopsy revealed approximately 20% CD34+ and CD117+ hematopoietic precursors in a hypocellular marrow. Based on these results a diagnosis of acute myeloid leukemia was confirmed. Chromosome and FISH studies were also performed on the bone marrow. Initial interphase FISH analysis with the panel of probes for AML did not reveal any abnormalities including alterations of RARα. However, chromosome analysis revealed an acquired derivative chromosome 17. Further characterization of the der(17) by additional DNA probes localized to the short arm of chromosome 17 along with the RARα probe revealed, that this was an inv(17)(p12q12). A final cytogenetic diagnosis of: 46, XX,inv(17)(p12q22).ish inv(17)(p12q22)(wcp17+,LIS1+,p53+, RARα+,D17Z1+,D17S928) was made. This result confirmed the dislocation of an intact RARα from its original 17q21 position to 17p12 in the abnormal bone marrow cells of this patient. Further molecular studies may elucidate if the presence of RARα in the short arm of chromosome 17 proximal to p53 would have any effect on the prognosis.

324/W

DETECTION OF PHILADELPHIA CHROMOSOME IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) BY CONVENTIONAL AND MOLECULAR TECHNIQUES. *G.C. Ramirez-Gaviria[1], C. Aya[1], M. Diosa[1], J.L. Ramírez-Castro[1], F. Quintero-Rivera[2], G. Vásquez-Palacio[1].* 1) Medical Genetics Unit, Antioquia Univ, Antioquia, Colombia; 2) Department of Pathology & Laboratory Medicine, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

CML and ALL are myeloid and lymphoproliferative disorders associated to the Philadelphia chromosome (Ph+),which plays an important role in their pathogenesis. The gold standard for its detection is conventional cytogenetics (CC) on bone marrow or peripheral blood. However interphase D-FISH and RT-PCR are molecular techniques that offer specific alternatives mainly in patients on chemotherapy or bone marrow transplantation (BMT). 23 patients with CML and ALL were study by CC , D-FISH and RT-PCR in order to determine the diagnosis and monitoring the response to Glivec® therapy (GT) or BMT. Samples were processed using standard protocols. Of the 17 patients with CML, 9/17 were Ph(+) at diagnosis 62% with three techniques, and 38% only by RT-PCR. 1/17 was Ph(-) by CC and RT-PCR. 44% of patients showed other chromosomal abnormalities by CC (clonal evolution). Of the 4/17 patients evaluated for monitoring of GT: 25% were Ph(+) with all techniques and 75% only by FISH and RT-PCR (b2a2 transcript). In 2/4 patients 9q34 deletion and an extra Ph+ was observed by CC and FISH respectively. Finally, of the 3/17 patients post-BMT: 67% showed transcripts p210 by RT-PCR but negative BCR-ABL fusion by FISH and only 1/3 was Ph(-) by both techniques. Of the 6 patient with ALL: 86% were Ph(+) by RT-PCR but Ph(-) by FISH and CC, however, CC revealed aneuploidy. 14% were Ph(-) by all tecqniques. Only one patient on Glivec exhibited a complex karyotype without Ph+,but showed e1a2 transcript by RT-PCR. In this study we observed a good association (62%) among the results obtained with three different techniques. CC and D-FISH are less sensitive compared to RT-PCR, but important to reveal other chromosomal aberrations which can determine the prognosis in patients with CML and ALL. These results support the fact that all three techniques are an excellent complement for determining diagnosis and monitoring in those patients.

325/W

Identification and characterization of a *NUP98-PHF23* fusion gene in acute myeloid leukemia. *J.C. Reader[1], J.S. Meekins[2], I. Gojo[3,4], Y. Ning[1,2].* 1) Program in Human Genetics; 2) Dept. of Pathology; 3) Dept. of Hematology-Oncology, Univ of MD School of Medicine, Baltimore, MD; 4) Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD.

NUP98 is a promiscuous fusion partner gene linked to hematological malignancies. We have recently identified a cryptic 11;17 translocation in an acute myeloid leukemia (AML) patient creating a novel in-frame fusion between *NUP98* exon 13 with *PHF23* exon 4. *NUP98*, which encodes a nucleoporin, has been involved in more than 20 different fusions. *PHF23* is an uncharacterized gene encoding a protein containing a plant homeodomain (PHD) which is found in proteins that mediate chromatin remodeling. The fusion partners of *NUP98* form two distinct groups: homeobox (*HOX*) genes and non-homeobox (non-*HOX*) genes. The non-*HOX* fusion partner genes, which include *NUP98-PHF23*, are diverse in function and are only related by having the capacity to form coiled-coil domain(s). Since the majority of the research has focused on the mechanism of *NUP98-HOX* genes in leukemogenesis, we are interested in further characterizing this novel fusion gene in the non-*HOX* group. In order to test if *NUP98-PHF23* is able to induce oncogenic transformation we have cloned the full length (FL) fusion gene and two mutant constructs into expression vectors. The mutant constructs, which have the PHD domain (ΔPHD) or the PHD and coiled-coil domains (ΔCoil) removed, will be used to determine which domains, if any, are important for transformation. The FL and ΔPHD constructs were used in an anchorage independent growth assay with preliminary results suggesting that the FL and ΔPHD constructs are both capable of inducing anchorage independent growth in NIH3T3 cells; however, the results of this experiment and the ΔCoil construct remain to be confirmed. The constructs will also be used to test their oncogenic potential in blocking differentiation in myeloid cells. Future directions include localization studies, identifying potential interacting co-factors, and determining whether *NUP98-HOX* and *NUP98*-non-*HOX* have a shared mechanism in leukemogenesis which, in turn, could lead to new potential therapeutic targets.

326/W

Development of a Clinical Biomarker Assay for the t(4;14) Translocation Associated with Multiple Myeloma. *M.R. Reed[1], J.A. Roseberry Baker[2], G. Zhao[3], C. Lopez-Correa[1].* 1) Integrative Biology, Eli Lilly and Company, Greenfield, IN; 2) Drug Disposition, Eli Lilly and Company, Indianapolis, IN; 3) Angiogenesis-Tumor Microenvironment Biology, Eli Lilly and Company, Indianapolis, IN.

Fifteen percent of Multiple Myeloma cases are associated with a translocation between chromosomes 4 and 14, and the patients carrying this genetic aberration typically have a poor prognosis. The t(4;14) translocation moves the FGFR3 gene from chromosome 4 to the vicinity of the IgH locus on chromosome 14 resulting in ectopic expression of FGFR3. It is thought that the transcriptional activation of FGFR3 resulting from its juxtaposition to the strong enhancers of the IgH locus may be one of the oncogenic events leading to the development of Multiple Myeloma. Since only fifteen percent of Multiple Myeloma patients bear the t(4;14) translocation, a clinical assay for this genetic biomarker is needed to identify which individuals have the translocation and will, therefore, benefit from FGFR3 treatment. We have assessed two techniques for their suitability to be translated into the clinic as biomarker assays for the t(4;14) translocation, RT-PCR and FISH. We tested five Multiple Myeloma cell lines, three with the t(4:14) translocation and two without, in each assay. Both assays were able to clearly distinguish Multiple Myeloma cell lines carrying the t(4;14) translocation from those without this translocation. However, variability in the RT-PCR assay caused by the fact that the translocation breakpoints span a 60 kb region lead us to the conclusion that it is not suitable for a clinical setting, while the FISH assay, which lacks this variability, could be more reliably translated into the clinic.

327/W

Validation of micro-array comparative genomic hybridization (aCGH) from cancer cell lines by fluorescence in situ hybridization (FISH) to provide biomarkers for pharmaceutical development. *J.A. Roseberry Baker[1], E.M. Felke[1], R. Gupta[3], L. Gautier[3], Y. Xiang[3], C. Lopez-Correa[2].* 1) Department of Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana; 2) Department of Functional Genomics, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, Indiana; 3) Lilly Systems Biology, Lilly Research Laboratories, Eli Lilly and Company, Singapore.

Cancer cell lines are an important tool in the discovery of biomarkers and for drug target validation. Array comparative genomic hybridization (aCGH) can be useful for a high throughput characterization of cancer cell lines in which large amounts of data can be generated focusing mainly on amplifications and deletions in the genome. However, cancer cells can have a variety of genetic variations in addition to amplifications and deletions that make aCGH data difficult to interpret. This can include large chromosomal rearrangements and polyploidy. Further limitations to current aCGH technology include limited resolution across the genome, variability across binding strengths for different probes and difficulty with calibration for precise copy number detection. We have demonstrated that FISH can enhance the powerful method of aCGH analysis by overcoming some of these limitations. To exemplify the power of this approach, the aCGH data implied that there was amplification of the ERBB2 gene in three different cell lines. FISH, using a commercially available probe for this region, indicated that the amplification in each cell line was to different levels. In a second example, aCGH data suggested that the CDKN2A gene was deleted in three cell lines. The FISH technique was able to show that all three cell lines were homozygous for this deletion. In many cases these cell lines were polyploid, yet aCGH in combination with FISH was able to quantitate the aforementioned genetic anomalies, which in the past could only be estimated. Thus, the use of aCGH together with FISH can provide significant support to characterization of cancer cells lines (or tumor samples) that can be used as biomarkers or target validation for development of cancer treatments.

328/W

Abnormal karyotype at relapse of Acute Myeloid Leukemia (AML) patients with normal primary diagnostic karyotype. *S.N.J. Sait[1], E.S. Wang[2], M. Barcos[3], A.W. Block[1], M. Wetzler[2], G. Deeb[3].* 1) Clinical Cytogenetics Laboratory, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY; 3) Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY.

Karyotypic abnormalities are the most important independent prognostic factor in acute myeloid leukemia (AML) and predict for treatment response and overall survival following chemotherapy and stem cell transplantation. At Roswell Park Cancer Institute, we examined a cohort of 12 de novo AML patients with an apparently normal karyotype at diagnosis and abnormal karyotype at relapse. According to FAB morphological classifications, five cases were M2, five M1, one M4, and one M5a. The patients' median age was 63 years (range 21 to 81 years); 6 were males and 6 females. Chromosomal abnormalities included 3 with unbalanced structural rearrangements; 3 with balanced rearrangements; 5 with complex karyotypes and 1 with numerical changes. Rearrangements observed at relapse did not include those commonly observed in patients with AML at initial diagnosis, except for one patient with trisomy 8. Aberrations commonly associated with secondary AML i.e. -5/5q-, -7/7q- and 11q23 abnormalities were also not observed. FLT3 mutation analysis performed on seven patients in relapse was positive in six cases. Patients had a median overall survival time of 15 months with the median time to relapse being 12 months and median survival after relapse of 4 months. All patients died of refractory disease. The longest overall survival time observed (49 months) was seen in the patient with trisomy 8. Thus, karyotypic instability may play a role in the development of refractory disease in AML. Defining the karyotypic abnormalities at relapse may contribute to the risk stratification of AML patients with a normal karyotype at diagnosis.

329/W

AN UNUSUAL CHROMOSOMAL CHANGE IN A PATIENT WITH MULTIPLE MYELOMA. *H.O. Shah[1,2], N. Chen[1], Y. Zhong[1], R. Nagwekar[1], H. Yu[1], J. Lin[1,2].* 1) Department of Pathology, Nassau University Medical Center, East Meadow, NY; 2) Health Science Center, State University of New York, Stony Brook, NY.

Multiple myeloma (MM), a mature B-cell lymphoproliferative neoplasm, is characterized by clonal proliferation of malignant plasma cells in the bone marrow with monoclonal gammopathy and clinically manifested by bone destruction, anemia, immunosuppression and renal failure. The etiology of MM is largely unknown and the role of genetic abnormalities is still uncertain. The primary chromosomal translocations in MM involving 14q32 (*IgH* locus) include t(11;14)(q13;q32), t(4;14)(p16.3;q32.3), t(6;14)(p25;q32) and t(14;16)(q32.3;q23), of which, t(11;14)(q13;q32), being most common, results in up-regulation of cyclin D1. Other translocations (secondary) or abnormalities including t(8;14)(q24;q32), 1q21 aberrations, 13q14 and 17p13 deletions have been associated with disease progression, poor prognosis and response to chemotherapy. Clinically, t(4;14), t(14;16) and 17p13 deletion are associated with poor prognosis, while 13q14 deletion is linked with intermediate prognosis, and all others correlate with good prognosis. We present here a 50 year-old Hispanic male with initial presentation of acute kidney failure. CT abdomen/pelvis revealed a hypodense mass causing collapse and destruction of L4 vertebra and other multiple lytic bone lesions. Kidney biopsy showed kappa-light chain cast nephropathy. Bone marrow aspirate and biopsy revealed that diffuse infiltrate of plasma cells with diffusely positive for CD138 and kappa-light chain. Flow cytometry confirmed plasma cell neoplasm with kappa-light chain restriction. Chromosomal study on bone marrow aspirate observed a *de novo* Robertsonian translocation with the karyotype: 45,XY,der(13;14)(q10;q10). Finding of chromosomal abnormality with a balanced rearrangement in this case is unusual in MM patients. This chromosomal structural change may affect the *IgH* locus and related oncogenes on chromosomes 13 and 14, which may trigger the development of MM and render a poor prognosis in this patient. Routine cytogenetic study in newly diagnosed MM patients may provide clinically useful information for predicting prognosis and therapeutic response.

330/W

Loss of maternal alleles on chromosome 11 is associated with aggressive prostatic rhabdomyosarcoma in two patients with Costello Syndrome. *K. Sol-Church[1], D.L Stabley[1], K. Conard[2], L. Nicholson[3], J. Sanford Biggerstaff[4], W. Liu[4], J. Campbell[5], W.H. Meyer[5], K.W. Gripp[3].* 1) Center for Pediatric Research; 2) Department of Pathology and; 3) Medical Genetics, Al duPont Hospital for Children, Wilmington, DE; 4) Sacred Heart Medical Center Cytogenetics Lab, Spokane, WA; 5) OU Health Sciences Center Oklahoma City, OK.

Costello syndrome (CS) is a rare autosomal dominant, multiple congenital anomaly syndrome comprising failure to thrive, short stature, mental retardation, congenital heart disease, and a predisposition for embryonal rhabdomyosarcoma (ERMS). We report on the molecular evaluation of two CS pts with ERMS exhibiting LOH for chromosome 11. **Case 1**: A male with polyhydramnios, prematurity, feeding difficulties and short stature received growth hormone from age 5 to 12 years. A pelvic ERMS involving the prostate and bladder occurred at age 16 years. While the pt had been previously diagnosed with Noonan syndrome, molecular analysis revealed a heterozygous *HRAS* G12S mutation diagnostic of CS. **Case 2**: A male with a history of polyhydramnios, severe feeding difficulties, developmental delay and typical facial features for CS had a germline *HRAS* G12S mutation. He presented at age 18 months with abdominal distension and bilateral hydronephrosis due to outflow tract obstruction. CT scan revealed a large pelvic ERMS, later confirmed by needle biopsy. While normal cells derived from these two pts display biallelic *HRAS* expression, tumor cells show loss of maternal markers located on both arms of chromosome 11 and monoallelic expression of the mutated *HRAS* gene. Besides LOH for chromosome 11 the tumor biopsies display allelic imbalance for markers vWA (12p), FGA (4q), Amelogenin, D5S818, D7S820, D8S1179, D13S317 D18S51, and D21S11. **Conclusion**: LOH for 11p13-15 resulting in overexpression of the paternal allele has been suggested as an initiating event for ERMS. This implies a decreased ERMS risk for CS pts carrying a maternally derived mutation. While our cohort study currently contains too few pts with maternally derived mutations to address this issue, determination of parental *HRAS* mutation origin may further define the risk for ERMS in CS.

331/W

Identification of Gain 1q and 12 as the most common karyotypic changes in Wilms tumor: Analysis of 36 patients. *S. Subramaniyam[1,4], S.V. Nandula[1,4], J. Kandel[2], W. Middlesworth[2], D. Yamashiro[3,4], B. Tycko[1,4], V.V. Murty[1,4].* 1) Departments of Pathology; 2) Surgery; 3) Paediatric Oncology; 4) Institute for Cancer Genetics, Columbia University Medical Centre, New York, NY:10032, U.S.A.

Wilms tumor (WT) is the most common pediatric renal malignancy with an incidence about 1 in 10,000. Conventional treatment protocols results in 85-90% survival in these patients, but still a group of patients relapse indicating a need for intensive salvage regimens to improve their survival rates. Data from published literature indicates that cytogenetic and molecular genetic abnormalities have significant role in predicting prognosis of WT patients, for instance 1q gain in favorable histology predicts significant risk of relapse. The aim of the present study is to identify cytogenetic changes and assess their role in prognostic outcome. Forty-two tumor specimens from 36 patients ascertained from 1998 to 2005 were analyzed by conventional G-banding karyotype and Spectral karyotyping (SKY). Thirty-four tumors had an abnormal karyotype, histologically 3 cases had anaplastic changes (unfavorable histology) and 23 were classified into favorable histology group. Eleven cases had metastatic tumors infiltrating various organs. Combined analysis of Cytogenetics and SKY revealed that gains of chromosome 1q in 13/34 (38.2%) tumors with frequent breakpoints localized to 1q11-12 in 9 (69.2%). Whole chromosome gains were seen for chromosomes 8 in 9 (26.5%) tumors, 12 in 20 (59%) tumors and 13 in 9 (26.5%) tumors. Losses of chromosome 7p and 16q were seen in 8 (23.5%) and 9 (26.5%) tumors, respectively. All cases were treated and followed up according to standard protocols. Among them 6/13 (46%) cases with 1q gain, 6/20 (30%) with +12, 4/8 (50%) with 16q loss, 4/8 (50%) with 7p loss were either relapsed or died of disease (DOD). The data from the present study provides evidence in support of the hypothesis that chromosome 1q+, +12, 7p- and 16q- plays a role in WT tumorogenesis and predicts poor outcome.

332/W

Exclusion of APC and VHL gene deletions by array based comparative hybridization in two patients with microscopically visible chromosomal aberrations. *G.A Toruner[1,3], R.J Wallerstein[2,3], S. Sklower Brooks[4], D.L Streck[1,3], R. Kuravthi[2].* 1) The Center for Human and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ 07103, USA; 2) Genetics and Genetic Counseling Program, Hackensack University Medical Center, Hackensack, NJ 07601; 3) Department of Pediatrics, UMDNJ-New Jersey Medical School, Newark, NJ 07103; 4) Department of Pediatrics and Obstetrics, Gynecology and Reproductive Sciences UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08901.

Karyotyping is a major component of the genetic work-up of patients with dysmorphism. Cytogenetic aberrations, which are close to a known tumor suppressor gene, raise important clinical issues, since deletion of that tumor suppressor gene can cause genetic predisposition to cancer. We present two cancer-free dysmorphic patients with karyotypes of del 46,XX, del (5)(q15q22.3) and del 46,XX,del(3)(p25.2-pter). These deletions are close to the APC and VHL genes that confer susceptibility to Familial Adenomatous Polyposis (OMIM #17510) and Von-Hippel-Lindau syndrome (OMIM #193300) respectively. The array-CGH analysis using a custom Agilent 44K oligonucleotide array demonstrated an interstitial 20.7 Mb deletion on 5q (chr5: 89,725,638-110,491,345) and a terminal 9.45Mb deletion on 3p (chr3:pter-9,450,984). According to the March 2006 human reference sequence, the APC gene is located at chr5: 112,101,483-112,209,835 and the VHL gene is located at chr3: 10,158,319-10,168,746. These results indicate that the APC gene is 2,300 kb and VHL gene is 700 kb away from the respective deletion. Southern blot analysis for APC and VHL genes were negative, consistent with array-CGH findings. These results demonstrate the power of array-CCH for the assessment of potential tumor suppressor gene involvement and cancer risk in patients with microscopically visible deletions in areas near tumor suppressors.

333/W

Characterization of a complex karyotype in a patient with primary plasma cell leukemia using multicolour spectral karyotyping. *E. Van Assche[1], Z. Berneman[2], A. van de Velde[2], M. van der Plancken[2], K. Vermeulen[2], H. De Raeve[2], R. Van Luijck[1], S. Scheers[1], B. Blaumeiser[1], J. Wauters[1].* 1) Dept Medical Genetics, Univ Hospital Antwerp, Edegem, Belgium; 2) Dept Hematology and Hemostasis, Univ Hospital Antwerp, Edegem, Belgium.

Primary plasma cell leukemia (PCL) is a rare neoplastic disorder that usually carries an aggressive course with a rapidly fatal outcome. We report on a 51-year-old patient who developed primary PCL. In agreement of previous reports (Saccaro et al., 2005; Avet-Loiseau et al., 2001) chromosomal analysis of bone marrow of this PCL case showed a complex chromosomal abnormalities. As GTG-banding was not able to resolve all karyotypic changes multicolour spectral karyotyping (SKY) was done. Using this molecular cytogenetic approach the karyotype can be described as: 48,XX,t(1;10)(p11;q26),+der(1)t(1;10)(p11;q26),del(6)(q?), +7,t(13;16)(q22;q?),t(14;16)(q32;q?). Only few cytogenetic analyses of patients with primary PCL have been published, especially the translocations involving the immunoglobulin heavy chain locus at 14q32, specifically t(11;14) and t(14;16) have been reported in 80% of patients with PCL. In our patient the t(14;16) could not be observed by conventional cytogenetics, only by using SKY the abnormality became visible. As mentioned by other authors (Saccaro et al., 2005; Avet-Loiseau et al., 2001; Hayman et al., 2001; Mateo et al., 2005), the karyotype of our PCL patient showed also abnormalities of chromosome 13, and abnormalities of chromosome 1. An overview of literature of other PCL cases will be given. Flow immunophenotypic studies were also performed but were not typical for PCL.

334/W

The clinical significance of Y chromosome loss in hematologic disease. *D.L. Van Dyke, A.E. Wiktor, J.M. Hodnefield, C.A. Hanson.* Dept Lab Medicine & Pathology, Mayo Clinic, Rochester, MN.

In 1972, Pierre & Hoagland (Cancer 30:889) wrote that Y chromosome loss is a common event, correlated with advancing age, and should not be considered evidence of a specific disease state. Loss of the Y chromosome as the sole cytogenetic change is more common in older men, and the size of the 45,X,-Y cell population probably increases gradually with age. This natural phenomenon challenges our ability to distinguish between a normal and a potentially disease-associated 45,X,-Y clone. Wiktor et al (Genes Chrom Ca 27:11, 2000) observed a difference between the disease and control populations only when the percentage of -Y was greater than 75%. To examine this finding further, we identified 129 Mayo Clinic patients seen from March 1995 to May 2006 whose bone marrow karyotype revealed >75% of cells with Y loss as the sole cytogenetic change. Of these 129, three were under age 50 years and 61 were age 75 years or older. There was a similar age distribution for the 51 cases with 100% -Y metaphases as compared to the entire group. A hematopathology review of the bone marrow was done in each case. There were 43 samples (33%) with no evidence of hematologic disease, including 9 of the 51 cases (18%) with 100% Y loss. For 23 of 30 patients (77%) with lymphoproliferative disease or lymphoma, the proportion of malignant cells in the bone marrow aspirate was negligible and cannot account for the high proportion of -Y cells. The clear lack of association between Y loss and the disease clone in 66 of the 129 patients (51%) makes an association between -Y and disease seem unlikely for the remaining 56 patients with myeloid disease. It is possible that the association is merely coincidental because loss of the Y and risk of hematologic disease both increase with age. A hematologic disease may just as easily arise in a normal cell or in a cell that happens to be -Y. This is consistent with the typically similar risk categorization of normal and -Y karyotypes in AML (Slovak et al., Blood 96:4075, 2000). In exceptional cases where Y loss waxes and wanes with recurrence and remission of disease, the disease-causing mutation(s) are likely to be submicroscopic.

335/W

Non-random telomere length changes associated with specific chromosome ends in chronic myeloid leukemia (CML). *J. Yan[1], O. Samassekou[1], A. Ntwari[2].* 1) Dept Pediatrics, Univ de Sherbrooke, Sherbrooke, PQ, Canada; 2) Dept Mathématique, Univ de Sherbrooke, Sherbrooke, PQ, Canada.

Critically shortened telomeres can lead to chromosome instability and thereby promote tumor development. However, most conclusions regarding the telomere shortening were from studies on general or average telomere length. It remains unknown if there are any chromosome-specific telomere length changes associated with the diseases and which chromosome-specific telomere changes play a role in chromosomal instability and rearrangement in cancer. Using quantitative FISH (Q-FISH), we recently demonstrated that the telomere lengths at some specific chromosome ends altered more frequently than others. Particularly, our results showed that the telomere at short arm of the X chromosome (Xp) was significantly lengthened in cells from chronic myeloid leukemia (CML) cases that carry a chromosome 9 and 22 translocation [t(9;22)(q34;q11.2)] as the sole detectable abnormality. The longest telomere on Xp reached about 300 kb that was detected by fiber FISH. This finding of a dramatically lengthened telomere at a specific chromosome from leukemia cells has not yet been reported in the literature. Therefore, the precise measurement of the telomere length would greatly increase the study value in the chromosomal stability. Based on our recent findings, we hypothesize that chromosome-specific telomere lengthening in cancer cells may be the one of the earliest events that will facilitate cell proliferation and thus can be used as a marker to monitor and evaluate the evolution of a cancer at different clinical stages.

336/W

Constitutional Cytogenetic Analysis in Men with Familial Testicular Germ Cell Tumor. *C.M. Mueller, L. Korde, J. Peters, M.H. Greene.* Clinical Genetics Branch, DCEG, NIH, NCI, Rockville, MD.

Testicular germ cell tumor (TGCT) is the most common malignancy in young men. Familial clusters of TGCT, epidemiologic studies demonstrating that family and personal history of TGCT increase disease risk, and the association of TGCT with congenital anomalies all suggest the existence of an inherited predisposition to TGCT. Unfortunately, unraveling the genetic basis of familial testicular cancer through traditional linkage studies has been difficult, in part because families with many affected individuals are exceedingly rare. Several somatic cytogenetic abnormalities have been associated with TGCT, notably isochromosome 12p which is frequently identified in tumor tissue, and the germline chromosome abnormality 47,XXY (Klinefelter syndrome) which is associated with increased risk of mediastinal germ cell tumors. Although somatic and germline cytogenetic abnormalities helped to localize other hereditary cancer syndrome genes (e.g., retinoblastoma, Wilms tumor, familial adenomatous polpyposis), only one previous conventional karyotype analysis has been performed in men with TGCT, with no germline chromosomal rearrangements detected among the 12 subjects studied.

As part of a multidisciplinary familial TCGT study, we performed conventional karyotype analysis and spectral karyotyping (SKY) on peripheral blood lymphocytes from twenty-six affected men from 15 multiple-case TGCT families, with the goal of identifying candidate loci of as-yet-unidentified testicular cancer susceptibility genes. We detected the paracentric inversion 46,XY,inv(7)(q21.2q32) in one subject with an affected father, but this clonal abnormality was inherited from his phenotypically normal mother. Neither this chromosome 7 inversion, nor its related breakpoints, has been associated with any phenotypic abnormalities. We have concluded that this cytogenetic abnormality is not associated with an inherited predisposition to TGCT. Future studies using higher resolution tools, such as array-based comparative genomic hybridization (CGH), may be useful in the attempt to identify high-penetrance TGCT susceptibility genes.

337/W

Clinical, cytogenetic and molecular profiling of chromosome breakage disorders in patients of Indian origin. *R. Shukla[1], R.A. Gatti[2], M. Kabra[1].* 1) Dept Pediatrics, Genetics Unit, AIIMS, New Delhi, India; 2) Dept Molecular Pathology and Laboratory Medicine, UCLA School of Medicine, UCLA, Los Angeles, CA, USA.

Chromosome breakage disorders (CBS) are a class of disorders characterized by increased frequency of chromosome damage in the cells of the patient, either spontaneously or following exposure to various DNA damaging agents. All disorders of CBS show common clinical features of disturbance of growth,and development, defects of the immune system and bone marrow function and predisposition to develop malignant tumors.The diseases of this group are all autosomal recessive, having diverse eitology and clinical manifestations. the diseases include, Fanconi anemia (FA), Ataxia telangeictasia (AT), Xeroderma pigmentosum (XP), Bloom syndrome (BS), Cockayne syndrome (CS), trichothiodystrophy (TTD), and Nijmegen breakage syndrome (NBS). At the All India Institute of Medical Sciences, New Delhi, India, we have been maintaining a registry of CBS patients since 2004 and offering clinical, cytogenetic and molecular diagnosis and genetic counselling to affected families. Various strategies are being used to confirm the clinical diagnosis in patients suspected with a CBS. Cytogenetic stress test using mitomycin (C) has been used for the diagnosis of FA. Radiosensitivity assay, haplotype analysis and sequencing has been used to confirm diagnosis of AT, FA, XP. The CBS registry has data on clinical, cytogenetic and molecular features of over 100 patients, the details of which will be discussed.

338/W

The four and a half LIM domain protein 2 (FHL2) interacts with CALM and is highly expressed in AML with complex aberrant karyotypes. *Z. Pasalic, B. Tizazu, M. Mulaw, L. Fröhlich-Archangelo, A. Krause, P. Greif, S. Bohlander.* GSF- MEDIII, KKG-Leukemia, Munich, Germany.

The CALM/AF10 translocation t(10;11)(p13;q14) is found in acute myeloid leukemia (AML), T-cell acute lymphoblastic leukemia (T-ALL) and malignant lymphoma. The CALM/AF10 fusion gene has been shown to cause aggressive biphenotypic leukemia in a murine bone marrow transplant model. The CALM (Clathrin Assembly Lymphoid Myeloid leukemia gene) gene product is a clathrin assembly protein which plays a role in clathrin-mediated endocytosis and trans Golgi network trafficking. AF10 is a putative transcription factor likely involved in processes related to chromatin organization. To learn about the function of the CALM/AF10 fusion protein, we searched for protein interaction partners of CALM using a yeast-two-hybrid (Y2H) assay and identified FHL2 as a putative CALM interacting partner. The CALM FHL2 interaction was confirmed by GST pull-down and CoIP experiments. In co-localization studies a translocation from cytoplasm to the nucleus is seen. Expression analysis (Affymetrix based) in different AML subtypes showed a significantly higher expression of FHL2 in AML with complex aberrant karyotypes compared to AML with normal karyotypes or balanced chromosomal translocations like the t(8;21), inv(16) or t(15;17). FHL2 is a TP53 responsive gene known to interact with proteins in both nucleus and cytoplasm and it can function as a transcriptional cofactor. Known FHL2 interactors include TP53, BRCA1, PLZF (promyelocytic leukemia zinc finger protein), the proto oncogene SKI1 and beta-catenin. High expression of FHL2 in breast cancer has recently been shown to be associated with an adverse prognosis. Reporter gene assays using a GAL4-DNA binding domain FHL2 fusion protein and a GAL4 responsive luciferase reporter were able to demonstrate a transcriptional activation function of FHL2. In independent experiments with CALM and CALM/AF10, a synergism occurred implying that it is conceivable that FHL2 is playing an important role in CALM/AF10-mediated leukemogenesis by tethering the CALM/AF10 fusion protein to various nuclear transcription factor complexes.

339/W

Comparative Evaluation of Conventional Cytogenetics, Flow Cytometry, FISH and Array-CGH in Chronic Lymphocytic Leukemia (CLL) Patients. *A. DWIVEDI[1,2], A. EHSAN[1], R.S. ROBETORYE[1], S.R. GUNN[1], S.G. ADHVARYU[1,2].* 1) Clinical & Molecular Cytogenetics Laboratory,Department of Pathology, UTHSCSA, San Antonio, TX 78229-3900; 2) Department of Pathology, UTHSCSA, San Antonio, TX 78229-3900.

Chronic Lymphocytic Leukemia (CLL) comprises a heterogeneous group of disease representing approximately 70% of lymphoid leukemias and 25% of all leukemias. CLL primarily affects people above 60 years of age and is characterized by mature B- lymphocytosis followed by progressive lymphadenopathy and bone marrow failure. In recent years, there has been increased emphasis on early and accurate diagnosis of CLL patients. In this context, a number of tests are employed for the detection of diagnostic / prognostic markers. Conventional cytogenetics (CC) plays an important role in identifying clonal anomalies in about 50% of CLL cases. During the last decade, fluorescence in-situ hybridization (FISH) tests have proven to be more sensitive (~80%) and specific in the detection chromosomal abnormalities not identified by CC. The most common deletion, del(13)(q14.3) is considered to be clinically favorable when present as the sole abnormality. On the other hand, trisomy12 and deletions of 17p and 11q are associated with more aggressive disease. Recently, array-based comparative genomic hybridization (array-CGH) has emerged as a powerful diagnostic tool for the high throughput, high resolution whole genome analysis of recurrent genomic imbalances like micro-deletions and amplifications. Flow cytometry (FC) is also used for the identification of surrogate prognostic cell markers like CD38 and ZAP-70 in cases of B-CLL. We present here a comparative study encompassing CC, FISH, FC and array-CGH on the bone marrow specimens of CLL patients. The salient findings of our study are: (1) CC demonstrated both normal as well as complex karyotypes. (2) FISH analysis confirmed trisomy 12 and deletions of 13q, 17p and/or 14q32. (3) Array-CGH detected additional genomic losses in chromosomes 1, 6, 13, 14 and gains in chromosomes 2, 5, 7, 10, 11 and 12. (4) FC results were variable for CD38 and ZAP70. Analysis of data with significance and limitations of each technique will be presented.

340/W

Cytogenetic analysis of 135 myelodysplastic syndrome patients. *E. Pariltay[1], A. Alpman[1], E. Karaca[2], B. Durmaz[2], O. Cogulu[2], F. Ozkinay[2].* 1) Medical Genetics, Ege University Faculty of Medicine, Izmir, Turkey; 2) Department of Pediatrics, Division of Genetics and Teratology, Ege University Faculty of Medicine, Izmir, Turkey.

Myelodisplastic syndromes (MDS) represent heterogeneous group of disorders with a variety of features including peripheral cytopenia, characteristic morphological findings and cytogenetic abnormalities in bone marrow. Clonal chromosomal aberrations may be found in about 40 to 50% of all MDS patients. In this study we retrospectively evaluated the cytogenetic findings in a series of 135 MDS patients that karyotype analysis were performed between 1998 and 2007. Among 124 cases of MDS that were cytogenetically analyzed successfully, abnormal karyotype was found in 33 cases. The abnormal karyotypes were determined to be 20 numerical, 12 structural changes and 1 both numerical and structural. The most common chromosomal aberration was found to be loss of Y chromosome (n=5) which is followed by mosaic trisomy 21 (n=3). The structural findings included deletions (chromosome 5q, 7q, 9q, 12p, 13q, 17p and 22q) and translocations [t(1,3)(p10;q10), t(10;17)(q24.3;q11.2), t(3;11)(p12;p15), t(8;20)(p21;q21), t(7;14)(q10;p10)]. The evaluation of the cytogenetic findings in this relatively large, single-institution study will likely facilitate further studies to characterize and to document rare and primary cytogenetic changes associated with MDS.

341/W

Evaluation of cytogenetic markers in peripheral blood lymphocytes of asbestos exposed workers. *S. Yadav[1], A.B. Pant[1], M. Yunus[2], Q. Rahman[1].* 1) Industrial Toxicology Research Centre, M.G. Marg, Lucknow 226 001, India; 2) Babasaheb Bhimrao Ambedkar University, Rae Bareli Road.

Asbestos is an established genotoxicant known to give rise to DNA damage and chromosomal aberrations but the exact mechanisms for the genotoxicity are still unknown. The aim of this study was to investigate the genotoxic risk to workers occupationally exposed to asbestos in milling and grinding area (unorganized unit of Beawar and Deogarh, Rajsthan, India). We studied cytogenetic biomarkers associated with asbestos exposure. During the study micronucleus (MN) formation and chromosomal aberrations (CA) in 63 male workers were analyzed. Fluorescent in situ hybridization (FISH) was also carried out to see clastogenic and aneugenic nature of asbestos induced damage. Confounding factor such as smoking found to modulate genetic damages with asbestos exposure. Therefore, to determine whether cigarette smoke has any modulatory effect on toxicity of asbestos exposure, we divided our study in two categories, exposed smokers and exposed non-smokers. The results were compared with a control group of 16 healthy male individuals without exposure to any known genotoxic agents. In result the mean frequencies of MN and CA were significantly higher in workers (in both groups- exposed smokers and exposed non smokers) than in the control group. In addition, prominent clastogenic nature of the genetic damage was reported. The data obtained from this study clearly showed cytogenetic damages in the lymphocytes of asbestos exposed workers and also reported synergistic effect of cigarette smoke. This genetic damage might be attributed to the cumulative effects of several substances due to chemical complexity of the asbestos and cigarette smokes that contains several genotoxicants. Study suggested that smoking makes genetic material/ apparatus of the cells more vulnerable to the deleterious effects of asbestos in exposed population.

342/W

Serendipitous detection of a constitutional microdeletion 16p13.11 in a patient with AML-M4eo. *H. Bruyere[1,2], C.J. Jensen[1], K.W. Song[2,3].* 1) Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver, BC, Canada; 2) University of British Columbia, Vancouver, BC, Canada; 3) Division of Hematology, Leukemia/Bone Marrow Transplantation Program, Vancouver General Hospital and BC Cancer Agency, Vancouver, BC, Canada.

A 35-year-old male patient was diagnosed with AML-M4eo. His karyotype revealed a pericentric inversion 16 in 23/25 metaphases. FISH, performed with a break-apart MYH11 probe located at 16p13.11, showed an unexpected signal pattern. In 78% of the interphase nuclei, one green signal and one red signal separated from each other were detected. The fusion signal corresponding to the normal homologous chromosome 16 was missing. In 18% of the nuclei, only one fusion signal was seen. On the 15 metaphases analyzed, one green signal and one red signal separated from each other were detected with no evidence of a fusion signal. Five metaphases from a peripheral blood specimen were apparently normal at 500-550 band resolution. Fish with the MYH11 probe showed only one normal (fused) signal. Therefore, a constitutional microdeletion at 16p13.11 was diagnosed. Two copy-number-variation databases were searched for a MYH11 copy number's alteration and revealed the loss of one copy of the MYH11 gene in 6/364 control individuals. The patient is reportedly phenotypically normal. He achieved remission after induction therapy. Further characterization of the microdeletion is pending. The MYH11 probe designed for the diagnosis and follow-up of patients with an inv(16)(p13q22) or t(16)(p13;q22) may be located at a site of a genome copy number polymorphism.

343/W

Sequential phenotype-genotype analysis improves detection of residual disease in "high risk" B-cell ALL. *E.L. Enriquez[1], V. Bedell[1], Y. Kim[2], Q. Huang[2], S.J. Forman[3], M.L. Slovak[1].* 1) Cytogenetics, City of Hope, Duarte, CA; 2) Anatomic Pathology, City of Hope, Duarte, CA; 3) Hematology & Hematopoietic Cell Transplantation, City of Hope, Duarte, CA.

Acute lymphoblastic leukemia (ALL) is a hematological disorder characterized by uncontrolled proliferation of immature white blood cells. In B-cell ALL, the presence of t(9;22) or MLL rearrangements is a poor prognostic indicator. Consequently, early detection of MRD is critical for appropriate diagnostic and therapeutic decisions. Current methods for MRD measurements are based on morphologic analyses, flow cytometry, molecular studies and cytogenetics/FISH. While high abnormality rates ease disease detection through standard morphologic and cytogenetic analyses, the presence of affected cells at low quantities hampers diagnosis of disease. We analyzed 78 samples collected from 38 pts using a sequential IHC(phenotype)/FISH (genotype) approach to classify B-cells with rearrangements of BCR/ABL or MLL. Cytospin slides, made from residual bone marrow, were stained with a monoclonal CD19 antibody and scanned on a Bioview Analyzer to target the B-cell population. The slides were subsequently hybridized with FISH probes specific for the genotypic rearrangements mentioned above, with only antibody-targeted cells analyzed. Disease was detected in 33 samples (42%), a finding comparable to the percentage identified by pathology. In samples with positive FISH results, T-FISH outperformed or was comparable to standard FISH in detecting disease in 31 (94%) samples. In two (6%) samples, abnormal cells were not CD19 positive and thus not detected until followup with area scans, which revealed low-level positivity in a subset of progenitor B-cells. Additionally, T-FISH detected residual disease in seven (9%) samples that were negative by other conventional methods; 100% concordance was observed in the four samples that had concurrent Q-RT-PCR results. Cell culture experiments demonstrated that T-FISH consistently identified abnormalities at dilutions of 10^{-3}. These observations suggest that antigen-targeted FISH is an effective way to increase the sensitivity of the assay in detecting residual "high risk" ALL.

344/W

Sequential phenotype/genotype FISH assay targeting rare tumor cells using archived bone marrow smears and paraffin embedded tissue sections. *V. Bedell[1], S.J. Forman[2], K. Gaal[3], V. Pullarkat[2], L.M. Weiss[3], G. Somlo[2], S. Wilcyznski[3], M.L. Slovak[1].* 1) Cytogenetics, City of Hope, Duarte, CA; 2) Hematology & Hematopoietic Cell Transplantation, City of Hope, Duarte, CA; 3) Anatomic Pathology, City of Hope, Duarte, CA.

Interphase fluorescent in situ hybridization (FISH) is the standard cytogenetic assay to detect specific clonal karyotypic aberrations in formalin-fixed paraffin-embedded tissue (PET). Direct correlation with immunophenotype or morphology in focal disease or infrequent cell types is rarely performed because the procedure is labor-intensive and usually requires extensive troubleshooting. We examined various archived leukemic bone marrow smears and paraffin embedded tissue, many of which were second opinion or consult cases, either to help the clinician have a better picture of the evolution of a patient's hematologic malignancy or to assess a specific genetic target, such as IGH@ or ERBB2, in samples with low tumor burden. We present a sequential FISH-based technique that utilizes the identical bone marrow smears or PET sections evaluated by a pathologist to target rare tumor cells; specifically plasma cells, mast cells, or leukemic blasts in 11 archived bone marrow smears and CD20 positive cells in 21 lymphomas. Thirty-two breast tumors were also analyzed. Successful hybridization was achieved in 62/64(97%)samples. The two unsuccessful samples were not evaluable by our current automated system configuration. Seven blinded control sections were all concordant (5 negative/2 positive). Cutoff limits were determined independently for each FISH probe used, and ranged from 2-5%. The method was applicable with various probes, both commercially available and "homebrew", in specimens ranging in age from one month to 14 years. The methodology is straightforward, using uncomplicated pretreatment and hybridization conditions. Basic equipment, attached to an automated image analyzer with image capture software, records the location of targeted cells for genotypic/phenotypic correlation. This assay is reliable and reproducible on test samples regardless of specimen age, probe design, tissue type or referring institution.

345/W

DETERMINATION OF THE INDUCED APOPTOSIS BY CYCLOPHOSPHAMIDE IN CULTURES OF HUMAN LYMPHOCYTES BY ASSAY COMET. *G. Razo-Aguilera, R. Baez-Reyes.* Deparment of Genetics, National Institute of Perinatology, Mexico City, MEXICO.

Apoptosis is a process of cell death that differs morphologically of the classic necrosis, because the presence of cell shrinkage, fragmentation of the DNA and formation of apoptotic bodies. Apoptosis is an intrinsic part of the development program for some cellular types; however it can also be induced by various toxic agents. The objective of this work was determine if the "comet assay" is a sensitive method to evaluate apoptosis, also, to determine the cyclophosphamide potential as an apoptotic inducer. We made cultures from lymphocytes of 20 healthy subjects (10 women and 10 men), and treated them with 10 and 100.

346/T

Leptin Gene Polymorphism Is Associated with Breast Cancer In Obese Postmenopausal Women. *B.A. Bhavani[1], M. Madhupoornima[2], Ammena[3].* 1) Genetics, kasturba gandhi degree college, Secunderabad, Andhra pradesh, India; 2) 2. Department of Biochemistry, Bhavan's degree and PG College for women, secunderabad, Andhra Pradesh, India; 3) 3. Department of Biotechnology, Lyola degree and PG college, Secunderabad Andhra Pradesh, India.

Leptin is an adipocyte-derived hormone that regulates food intake and energy expenditure. Recent functional studies have suggested a direct effect of leptin on menopausal status and breast cancer. In this study we examined the genetic association of the leptin gene polymorphism with obesity, postmenopausal status and risk for breast cancer in postmenopausal woman. A highly polymorphic tetranucleotide repeat polymorphism in the 3'-flanking region of the leptin gene was examined. The alleles of the polymorphism consisted of two groups with different size distributions: a shorter one (class I) and a longer one (class II). The frequency of class I/class I genotype was much higher in postmenopausal woman than in premenopausal woman. (13.5% vs. 3.4%; P = 0.0027). Further postmenopausal woman with breast cancer were observed to have high frequency of class I/class I genotype as compared to postmenopausal woman without breast cancer (OR- 2.56, CI 1.256-4.892, p<0.05). Significant difference in body mass index was observed with different genotypes in postmenopausal woman (p<0.05). The leptin gene polymorphism was associated with obesity and postmenopausal status. These data together with recent functional data on the direct effect of leptin onmenopausal status suggest that the leptin gene and its product, leptin, are an attractive target for studies on the mechanisms of menopausal changes and for the development of methods for the prediction, prevention, and therapy for menopausal changes and breast cancer.

347/T

Accurate prediction of *BRCA1* and *BRCA2* heterozygous genotype using expression profiling of lymphocytes after irradiation induced DNA damage. *Z. Kote-Jarai[1], S. Jugurnauth[1], L. Matthews[2], I. Giddings[2], E. Bancroft[3], Carrier Clinic Collaborators[3], P. Agius[4], M. Girolami[4], C. Campbell[5], R. Eeles[1,3].* 1) Translational Cancer Genetics, Inst Cancer Research, Sutton, Surrey,UK; 2) Molecular Carcinogenesis, Inst Cancer Research, Sutton, Surrey,UK; 3) Royal Marsden NHS Foundation Trust, London, UK; 4) Computational Intelligence Unit, University of Bristol,UK; 5) Bioinformatics Research Centre, University of Glasgow,UK.

Germline mutations in *BRCA1* and *BRCA2* genes predispose women to an increased risk of breast/ovarian cancer. Both genes have important roles in DNA damage repair and are implicated in gene expression regulation. We have previously shown that normal fibroblasts from mutation carriers can be distinguished from non-carriers following radiation-induced DNA damage. In this new study we used lymphocytes to find out if these also show differential response to induced DNA damage and if expression profiling using microarray technology could be used to accurately predict the BRCA genotype. Short-term lymphocyte cultures were established from fresh blood samples from 20 *BRCA1* and 20 *BRCA2* mutation carriers and 10 negative controls (individuals tested negative for the mutation present in the family). Lymphocytes were subjected to 8 Gy ionizing irradiation to induce DNA damage and RNA was extracted one hour post-irradiation. For expression profiling genome-wide (30 K) spotted cDNA microarrays manufactured by the Cancer Research UK Microarray Facility were used. We applied Support Vector Machine (SVM) classifier with statistical feature selection to determine the best feature set for predicting *BRCA1* and *BRCA2* heterozygous genotype. We also investigated the prediction accuracy using a non-probabilistic classifier (SVM) and a probabilistic classifier (a Gaussian Process Classifier:GPC). In general we achieved high accuracy (96%) in predicting the mutation carrier status. We will present the detailed outcome of using SVM and GPC in the task of distinguishing between the 3 classes, *BRCA1* and *BRCA2* mutation carriers and non-carriers, and evaluate if this microarray technology can be used to facilitate the clinical detection and classification of mutations.

348/T

Analysis of the Myb Oncogene and its Cooperation in Nf1 Leukemogenesis. *A.G. Hadjipanayis[1], B. Sack[1], J. Walrath[1], J. Zucali[1], V. Kelley[1], J. Guerts[3], D. Largaespada[3], S. Kogan[4], J. Resnick[1], K. Shannon[2], P. Wallace[1].* 1) Molecular Genetics, University of Florida, Gainesville, FL; 2) UCSF Pediatrics, San Francisco, CA; 3) University of Minnesota; 4) UCSF Laboratory of Medicine, San Francisco, CA.

Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative disease characterized by leukocytosis, monocytosis, thrombocytosis, splenomegaly, and lymphadenopathy. Children with neurofibromatosis (NF1), a dominant tumor syndrome, are at a 200-500-fold increased risk for JMML. Twenty percent of children with JMML have NF1, which may not be clinically evident at time of diagnosis. Tumor cells show loss of the normal NF1 allele, fulfilling the two-hit mechanism. Based on this and other research, it has been hypothesized that somatic mutations at other genes are necessary to cause JMML to progress to a more acute form. To search for such loci, the Nf1Fcr mouse knockout mutation was crossed onto the BXH2 mouse background, which develops AML due to retroviral mutagenesis. Nf1Fcr/BXH2 mice developed acute myeloid leukemia (AML) at an earlier onset, and common sites of viral integration were mapped in the tumors. The first locus to be analyzed is termed Epi1, and these integrations were downstream of the Myb oncogene. We hypothesized that this causes increased Myb function. To test this, we constructed a mouse reconstitution model that over-expresses Myb simultaneous with inactivation of Nf1 in bone marrow used these cells to engraft lethally irradiated mice. These mice have leukocytosis and are dying with evidence of myeloid dysplasia and organ infiltration. We are analyzing tissues and data to determine whether this is an acute disease, and whether Myb hyperactivation accelerates onset of the disease compared to Nf1 mutant only. In addition, bone marrow cells (presumably tumor) from the Myb/Nf1 mice have been engrafted into new sub-lethally irradiated recipient mice. If these mice develop disease, then Myb in fact cooperates with Nf1 in acute myeloid leukemia. The experiments will be finished soon, and we will present our complete data analysis.

349/T

Investigation of the Association between Trinucleotide Repeat at the First Exon of Androgen Receptor Gene and A/G Polimorphism in Prostate Specific Antigen Gene Promoter Region. *D. Alptekin[1], M. Izmirli[2], Y. Bayazit[3], H.U. Luleyap[1], B. Soyupak[3], Z. Tansu[3].* 1) Department of Medical Biology, Cukurova University, Medical Faculty, Adana, Balcali, Turkey; 2) Department of Radiology, Cukurova University, Medical Faculty, Adana, Balcali, Turkey; 3) Department of Urology, Cukurova University, Medical Faculty, Adana, Balcali, Turkey.

Number of polymorphic repeats (CAG-Poigulutamine, GGN-Polyglycine) at the first exon of androgen receptor (AR) gene is known to be related with prostate cancer. Moreover, it is also associated with the A/G polymorphism at the ARE I region of Prostate Specific Antigen (PSA) gene. This study aims to search if the malformations in AR and PSA gene found in the individuals with prostate cancer are also detected in the patients' son. To estimate this 10 ml blood samples were collected from the patients with prostate cancer and this sons. The DNA was isolated and amplified using PCR. The products of PCR were separated in agarose gel electrophoresis and the number of trinucleotide repeats was determined. The amplified PCR product is digested with Nhe I enzyme to identify AA, AG and GG genotypes for PSA gene A/G polymorphism. Vast majority of the individuals with prostate cancer manifested less than 20 CAG trinucleotide repeats (75.8 %) while their sons had low number of the repeat number below 20 (11.1 %). Number of GGN trinucleotide repeats was less than 20 (67.2 %) in most of patients, however, unlike CAG, the repeat number was not diminished in their sons. PSA gene A/G polymorphism was remarkably high in AA homozygote (41.4%) and AG heterozygote (44.8 %) in the patients with prostate cancer. Their sons also manifested high AA homozygote (38.9 %) and AG heterozygote (44,4 %) forms. Taken together, we report AA and AG polymorphism is associated with prostate cancer, and their sons comprise the risk group. Therefore the individuals with prostate cancer above the age of 40 and their sons are recommended for annual medical check up.

350/T

Juvenile Hyaline Fibromatosis, a Genetic Disease. Three Mexican Cases Report. *J. Aparicio[1, 10], P.M. Barrientos[2], M.V. Chong[3], H.M.A. Garrido[3], L.A. Luis[4], H.G. Lopez[4], M.L. De la Torre[5], R.N. Balbuena[6], H.M.L. Hurtado[7], S.S. Monroy[1], O.N.C. Gil[8,10], B.W. San Martin[9], H.F. Lara[9].* 1) Genetics; 2) Endocrinology; 3) Oncology; 4) Hematology; 5) Pediatric surgery; 6) Dermatology; 7) Cytogenetics; 8) Estomatology; 9) Pathology, Hospital para el Nino Poblano; 10) Estomatology, Benemerita Universidad Autonoma de Puebla, Mexico.

INTRODUCTION. Juvenile hyaline fibromatosis (JHF) is a rare genetic disease of the connective tissue. It is characterized by papulonodular skin lesions, soft tissue masses, gingival hypertrophy, osteolytic bone lesions and flexion contractures of the large joints. JHF is caused by mutation in the gene encoding capillary morphogenesis protein-2 (CMG2, or ANTXR2) on chromosome 4q21. JHF is also considered an autosomal recessive genetical condition. CLINICAL CASES. In this study three male patients, 1 , 7 and 8 years old were studied. All the patients with characteristic clinical features of JHF as nodular/papular skin lesions and gingival hypertrophy. The skin lesions was observed on the hands, scalp, ears, and around the nose and head. It require recurrent excision. Progressive joint contractures and osteopenia was also showed and severe limitation of mobility was observed. CONCLUSION. The three patients presented multiple subcutaneous tumors, particularly of the scalp and slowly growing, causing deformities, gingival fibromatosis and large tumors were found on the scalp and whitish nodules on the nape and sides of the neck. Hypertrophic gingivae and tumors at both commissures of the lips were found. The diagnosis is confirmed by demonstration of hyaline deposition in the dermis, since pathology studies showed a mesenquimotosa lession with a lower celullarity made of fibroblastic cells with a high hyaline matriz. Celular atipia nor mytosis was idenftified. Therefore, histologically I was demonstrated an abundance of homogeneous, amorphous, acidophilic ground substance. Early diagnosis and treatment is important for a better function and quality of life. REFERENCES. 1.Aldred, M. J.; Crawford, P. J. M. : Juvenile hyaline fibromatosis. Oral Surg. Oral Med. Oral Path. 63: 71-77, 1987. 2. Bedford, C. D.; Sills, J. A.; Sommelet-Olive, D.; Boman, F.; Beltramo, F.; Cornu, G. : Juvenile hyaline fibromatosis: a report of two severe cases. J. Pediat. 119: 404-410,1991.PubMed ID : 1880654. 3. Breier, F.; Fang-Kircher, S.; Wolff, K.; Jurecka, W. : Juvenile hyaline fibromatosis: impaired collagen metabolism in human skin fibroblasts. Arch. Dis. Child. 77: 436-440, 1997. PubMed ID : 9487969 jmapar@prodigy.net.mx.

351/T

Serum proteins of tumor free and tumor bearing Xiphophorus. *A. Islam.* Ophthalmology, Schepens Eye Resaerch Institute, HMS, Boston, MA.

Comparative blood serum protein analysis of tumor bearing and tumor free Xiphophorus helleri was investigated by using native-PAGE (Poly Acrylamide Gel Electrophoresis). For comparative analysis, the serum proteins e.g. albumin, transferrin, globulins, and lipoproteids bands were analyzed. These 4 proteins spectra have a diversification in constructions, which showed in different polymorphic peaks in between the normal or tumor free (tf) and tumor bearing (tb) Xiphophorus species. The globulin and transferrin fractions have distinct polymorphism in the affected and non-affected animals. The serum proteins analysis of tf and tb Xiphophorus showed that the globulin fractions were depressed in normal and have found declining peaks in the acute and tumor bearing melanoma formed fish. Tumor free fish have up rising peaks of globulin and transferrin spectra in comparison to Xtb type. The albumin peaks of both tf and tb fish showed more or less similar peaks with a molecular weight of 65 kDa. In tumor bearing species, lipoproteids peaks were variable and higher than the tumor free Xiphophorus (Xtf). The protein variations between tf and tb animals which were in inbred lines interpret different aspects and functions of blood serums. The result could be an ideal material to compare and identify immunity and physiology of diseased and non-diseased populations in relation to different proteins combinations. The protein markers of Poeciliidae could be used as a cancer marker for human.

352/T

Depletion of Bypass DNA polymerases leads to genomic instability after treatment with DNA interstrand crosslinking agents. *K. Riggan, A. Hemphill, M. Al-Dhalimy, S.B. Olson, R.E. Moses.* Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR.

DNA interstrand crosslinks (ICLs) are potent forms of DNA damage, inhibiting replication and transcription. Yeast mutants of Rev3, the catalytic subunit of the bypass polymerase Polζ , are sensitive to ICL agents. We tested whether depletion of human Rev3 would recapitulate that sensitivity to ICL agents. Depletion of Rev3 produces genome instability as manifested by increased radial formation with ICLs. GM639 immortalized fibroblasts were sensitized to Mitomycin C (MMC) by depletion of Rev3, as shown by decreased survival. Rev1 is an inserter polymerase involved with Polζ in translesion synthesis, where Rev3 specifically extends past the nucleotide inserted by Rev1. Depletion of Rev1 in GM639s resulted in increased sensitivity to MMC as indicated by an increase in breaks and radials after ICL formation. Like Rev3, Polκ is an extender of mispaired primer termini. For this reason, we were interested in whether the depletion of Polκ also resulted in increased sensitivity to ICL agents. The depletion of Polκ in HEK293 immortalized fibroblasts led to increased chromosome breakage and radial formation after treatment with MMC. Increased radial formation after ICLs is a phenotype characteristic of Fanconi Anemia (FA). For that reason, we depleted Rev3 in FA-D2 cells, to test the epistatic relationship of Rev3 to the FA pathway. Depletion of Rev3 in these cells increased the sensitivity to MMC, suggesting that Rev3 acts in a distinct ICL repair pathwaythat is, it is non-epistatic to the FA pathway. Rev1 was also tested for epistasis to the FA pathway by depletion in FA-D2. Depletion of Rev1 increased sensitivity to MMC, indicating that Rev1 is additive to the FA pathway. Our results support the conclusion that multiple pathways for ICL repair are present in higher eukaryotes and that bypass DNA polymerases act in the process outside the FA pathway.

353/T

Study of the MAGE Family in Carcinogenesis and Clinical Application of Human Colorectal Cancer. *M.J. Yang[1], J.Y. Wang[2,3], S.R. Lin[4,5].* 1) Graduate Institute of Medicine; 2) Department of Surgery, Kaohsiung Medical University Hospital; 3) Faculty of Medicine, College of Medicine; 4) Graduate Institute of Medical Genetics, College of Medicine; 5) BioMedi Innovation Incubation Center, Kaohsiung Medical University, Kaohsiung, Taiwan.

Purpose: Melanoma antigens (MAGE) are a group of tumor antigens that have tissue specific expression. In our previous study, we have found several genes of the MAGE family potentially applicable for early diagnosis, post-surgery track and prognosis evaluation of the lung cancer. In this study, we plan to analyze the mRNA expression of MAGE family and its correlation with clinicalpathological features in colorectal cancer (CRC) patients. Furthermore, we purpose to find new molecular markers for the clinical applicability in MAGE Family and explore the molecular mechanism in the carcinogenesis of CRC. Materials and Methods: We collected 97 colorectal cancer tumor tissues and their tumor-free colon tissues. Following, we used membrane array method to compare the mRNA expression profile of the MAGE family genes between colorectal cancer tissue and normal tissue. In addition, we used immunohistochemical stain to investigate the protein expression of the MAGE family and its localization. Finally, we compared the clinicalpathological features with the MAGE family genes. Results: We found 9 genes overexpressed in CRC and are shown as follows, MAGE-A2, 84.8%; -A5, 63.6%; -A8, 78.8%; -A9, 69.7%; -A12, 72.7%; -B2, 63.6%; -B3, 69.7%; -B4, 66.7%; -F1, 87.9%. After comparing the clinicopathological features with the expression of MAGE genes, we found all CRC patients express at least one of the MAGE genes except MAGE-D4. Interestingly, MAGE-D4 do not express in tumor tissue nor in normal tissue. We also found MAGE-B10 expressed in late-stage CRC(III & IV stage)(p=0.045), MAGE-C3 expressed when tumor size is below 5 cm(p=0.027). Meanwhile, MAGE-A10 had a contrary performance to MAGE-C3. Conclusion: This result suggested that MAGE-A2 and MAGE-F1 were highly-expressed and may serve as novel tumor markers in CRC. MAGE-B10 and MAGE-C3 may associate with carcinogenesis and cell proliferation but its mechanism still needs further analysis.

354/T

Genomic deletions of the *APC* gene can result both in diffuse and attenuated forms of adenomatous polyposis. *S. Baert-Desurmont[1], J. Bou[1], J. Tinat[1], J. Mauillon[1], O. Bera[2], S. Olschwang[3], T. Frebourg[1].* 1) Department of Genetics, University Hospital and Inserm U614, Faculty of Medicine, Institute for Biomedical Research, Rouen, France; 2) Department of Virology, University Hospital, Fort-de-France, Martinique; 3) Inserm U599, Institut Paoli-Calmettes, Marseille, France.

Germline mutations of the *APC* gene result into familial adenomatous polyposis (FAP) and two clinical forms of FAP can be distinguished : the typical one characterized by hundreds to thousands adenomas developing during adolescence or young adulthood, and the attenuated form called AFAP characterized by fewer than 100 adenomas at a more advanced age. While numerous studies have indicated a genotype-phenotype correlation for some *APC* point mutations, there is no clear evidence for a common phenotype associated with *APC* large deletions. Here we report the phenotypic variability of these *APC* genomic large deletions. In our French series, deletions are involved in 9% of the FAP families. To screen for *APC* genomic rearrangements, we used a QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragment) assay exploring each exon and dividing exon 15 in 5 fragments numbered 15.a to 15.e. We identified 4 distinct large deletions affecting exons 6-15, exons 11-13, exons 1-15.b, and exons 1-15.e. The three patients harbouring the partial deletions of *APC* presented typical forms of FAP with 100 to 1000 adenomas. In contrast, the complete *APC* deletion was detected in a patient presenting at 23 years of age an AFAP with 50 adenomas located in the distal colon and rectum. Although genomic rearrangements of *APC* are less frequent than point mutations, their presence should be considered in FAP families with not only with classical but also with attenuated phenotypes.

355/T

Splicing defects associated with unclassified variants of *BRCA1* and *BRCA2* detected using a novel reverse transcription-PCR design and a DNA-based *ex vivo* splicing assay. *C. Bonnet[1], S. Krieger[2], A. Rousselin[2], M. Vézain[1], I. Tournier[1], A. Martins[1], T. Frébourg[1], M. Tosi[1], A. Hardouin[2].* 1) Inserm U614, Faculty of Medicine and Department of Genetics, University Hospital, Institute for Biomedical Research, Rouen, France France, France; 2) Laboratory of Clinical and Oncological Biology, Centre François Baclesse, Caen, France.

Many unclassified variants (UV) of *BRCA1* or *BRCA2* may have an effect on pre-mRNA splicing by altering degenerate positions of splice site sequences or by inducing cryptic splice site activation. Moreover, exonic variants may be pathogenic by affecting splicing regulatory sequences such as exonic splicing enhancers (ESE). We have developed a strategy that combines the analysis of RNA extracted from peripheral blood of patients and the characterization of each variant in an *ex vivo* splicing assay. On one hand, we have optimized the conditions for RT-PCR analysis of BRCA1 and BRCA2 mRNAs. On the other hand, we have PCR-amplified from patient DNA exons and flanking sequences that carry UVs and inserted the PCR products into a splicing reporter minigene. After transfection into HeLa cells, the effects of variants on splicing were evaluated by RT-PCR. We have presently examined with both methods 20 variants: 16 were intronic, at positions different form the conserved GT or AG dinucleotides at the intron boundaries and 4 were exonic. Results obtained from RT-PCR analysis of patient RNA and from the *ex vivo* splicing assay were fully concordant. Six of the intronic variants examined induced a splicing alteration: 3 by inducing partial or total exon skipping, 2 by activating cryptic splice sites and 1 by modifying the balance of an alternative splicing towards stronger exon inclusion. Two exonic variants generated or activated new donor splice sites and one affected a novel putative exonic splicing enhancer. This work reveals an important fraction of splicing defects among unclassified variants. Moreover we show that functional analysis of splicing using a reporter minigene is reliable and is of particular value in many cases in which patient blood samples suitable for RNA extraction are not available.

356/T

Individualized risk predictions to estimate the clinical benefit of risk reduction mastectomy (RRM) and oophorectomy (RRSO) in BRCA carriers with breast cancer. *H. Burke[2], A. Hoang[2], K. Metcalfe[3], J. Culver[1], D. MacDonald[1], M. Grant[1], A. Thornton[1], M. Robson[4], S. Narod[3], J. Weitzel[1].* 1) City of Hope, Duarte, CA; 2) George Washington University, Washington, DC; 3) University of Toronto, Toronto, Ontario, Canada; 4) Memorial Sloan Kettering CA Ctr., New York, NY.

Background: Breast cancer (BC) patients with a BRCA mutation have a markedly elevated risk for new cancers. Health care providers must communicate complex information about risk-reducing surgeries. We created models that provide individualized 5-year BC survival and contralateral BC predictions and the benefit of RRM and RRSO. Method: The study population was 491 BRCA+ women treated for stage I or II BC between 1975 and 2000 (BRCA1, n = 327; BRCA2, n = 152; both BRCA1 and BRCA2, n = 12). The independent variables were age (< vs.≥50 years old), tumor size (continuous), ER status (+/-), and lymph node status (+/-). Logistic regression was used to create the model which estimate the probability of each outcome for RRM and for RRSO. Accuracy is assessed by the ROC. Results:

	RRM	RRSO
5-year BC- specific survival	Model ROC = 0.707; NS	Model ROC = 0.804; significant
5-year contralateral BC	Model ROC = 0.749;significant	Model ROC = 0.611; NS

The breast and ovary cancer-specific survival model did not differ significantly from the BC-specific survival model and is not reported here. Conclusion: In this population of BRCA women who had BC, we found that, with the exception of RRSO and risk of contralateral BC, the models were highly accurate predictors of the two outcomes. An unexpected finding was the beneficial effect of RRSO on BC survival. These are preliminary results and await validation on an independent dataset. The individualized output of the predictive models will then be incorporated into a decision support tool for use in cancer risk counseling.

357/T

Multiple rare non-synonymous variants in APC predispose to colorectal tumours. *J.P. Cheadle[1], D. Azzopardi[1], A.R. Dallosso[1], K. Eliason[2], B.C. Hendrickson[3], N. Jones[1], E. Rawstorne[1], J. Colley[1], V. Moskvina[4], C. Frye[2], J.R. Sampson[1], R. Wenstrup[2], T. Scholl[3].* 1) Institute of Medical Genetics, Cardiff University, Cardiff, UK; 2) Myriad Genetic Laboratories, Inc., 320 Wakara Way, Salt Lake City, UT; 3) Genzyme Genetics, 3400 Computer Drive, Westborough, MA; 4) Biostatistics and Bioinformatics Unit, Cardiff University, Cardiff, UK.

It has been proposed that multiple rare variants in numerous genes collectively account for a substantial proportion of multifactorial inherited predisposition to a variety of chronic diseases including colorectal cancer (CRC). We have studied this hypothesis by re-sequencing the adenomatous polyposis coli (APC) gene in 691 unrelated North American patients with colorectal tumours and 969 matched healthy controls. Rare inherited non-synonymous variants were significantly over represented in patients who did not carry conventional pathogenic mutations in the APC or MUTYH genes ('non-FAP non-MAP patients') (81/480, 16.9%) as compared to FAP/MAP patients (20/211, 9.5%; P=0.0113). Furthermore, significantly more non-FAP non-MAP patients carried rare non-synonymous variants as compared to healthy controls (P=0.0166). Seven out of sixteen non-synonymous variants were shown to alter β-catenin-regulated transcription and in silico analyses predicted that over half of the 61 different variants identified were likely to affect function. These data show that multiple rare non-synonymous variants in APC play a significant role in predisposing to colorectal tumours.

358/T

Fatigue and Sleep Disturbances in Asymptomatic BRCA1/2 Mutation Carrier Women: Preliminary Study. *E. Dagan[1,2], T. Shochat[2], R. Gershoni-Baruch[1,3].* 1) Rambam Medical Center, Haifa, Israel; 2) Nursing, Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, Israel; 3) Bruce Rappaport Faculty of Medicine, Technion-Institute of Technology, Haifa, Israel.

Aim: To investigate whether asymptomatic BRCA1/2 mutation carriers exhibit high levels of fatigue and sleep disturbances. Methods: Asymptomatic BRCA1/2 mutation carriers (n= 11) and non-carriers (n=15) from the oncogenetic clinic in Rambam Medical Center in Israel and low-risk controls (n=36) were recruited. Participants completed a battery of clinical, psychological (Brief Symptoms Inventory, BSI; Cancer Related Worry, CRW) and fatigue (Fatigue Symptoms Inventory, FSI) and sleep quality (Pittsburgh Sleep Quality Index, PSQI) questionnaires. Activity monitors (actigraphs) were worn for one week, for objective assessment of sleep quality. Results: Mean ages at interview were not statistically different between groups as well as psychological assessment by the BSI. CRW was significantly different between groups (carriers 0.83±0.49, non-carriers 0.70±0.52, controls 0.43±0.42; p=0.027); post-hoc analysis revealed significant differences between carriers and controls. Subjective sleep quality showed borderline significance between carriers (6.73±4.45) and controls (4.26±2.78); (p=0.087). Sleep duration (minutes) based on actigraphy was significantly different between groups (carriers 449±50, non-carriers 399±58, controls 434±52; p=0.047); post-hoc tests revealed borderline significance between carriers and non-carriers (p=0.071). Actigraphic measures of sleep efficiency (percentages) showed borderline significance between carriers (93±13) and controls (97±3); (p=0.085). Wake-time after sleep onset (minutes) showed borderline significance between carriers (23±28) and controls (12±10); (p=0.084). No group differences for sleep latency or specific and overall measured of fatigue were found. Conclusions: These preliminary results indicate that asymptomatic BRCA1/2 carrier women exhibit sleep disturbances compared to controls. Sleep disturbances in non-carriers were similar to carriers. Early screening for sleep disturbances may allow early management in women undergoing genetic testing.

359/T

Timing of BRCA1/BRCA2 genetic testing in women with ovarian cancer. *M.S. Daniels[1], D.L. Urbauer[2], J.L. Stanley[1], K.G. White[1], K.H. Lu[1].* 1) Gynecologic Oncology, U.T. M.D. Anderson Cancer Center, Houston, TX; 2) Quantitative Sciences, U.T. M.D. Anderson Cancer Center, Houston, TX.

Introduction: 10-15% of women with ovarian cancer have a germline BRCA1/BRCA2 mutation. Women with ovarian cancer are often diagnosed at advanced stages, when overall prognosis is poor. Results of BRCA genetic testing are both important to family members, and could impact ovarian cancer treatment. **Purpose:** To determine when, during the course of their treatment, women with ovarian cancer were seen for BRCA1/BRCA2 genetic testing, and what factors influenced timing. **Methods:** We identified 100 women who underwent treatment for ovarian cancer and had BRCA1/BRCA2 genetic testing at a single institution. Data were collected retrospectively. **Results:** 33 (33%) women were seen for genetic counseling before or during their initial treatment, 34 (34%) were seen while in first remission, and 33 (33%) were seen at or after first recurrence. Of those seen at or after recurrence, 4 (12%) died prior to disclosure of genetic test results. Women who had a history of breast cancer were seen earlier in the course of their treatment than women who never had breast cancer ($p<0.05$). 45 (45%) women tested positive for a BRCA1 or BRCA2 mutation, and women who had a history of breast cancer were more likely to test positive than women who never had breast cancer ($p<0.05$). **Conclusion:** Women with ovarian cancer are seen for genetic counseling throughout the course of their treatment, with an even distribution. Women with a prior history of breast cancer were seen earlier in the course of their treatment, implying that they are being appropriately flagged as high risk. The mutation detection rate among women with ovarian cancer seen for genetic counseling is high, and is even higher among women who had breast cancer. One third of women with ovarian cancer were not seen for genetic counseling until first recurrence or later. These women should be seen earlier in the course of their treatment, both to alleviate the difficulties of direct disclosure of results to next of kin, as well as to provide them with the opportunity to potentially benefit from therapeutics targeted to BRCA-positive patients.

360/T

Fanconi Anemia D1 Presenting as Breast Cancer caused by bi-allelic BRCA2 gene mutations. *M. Dasouki[1], K. Penning[2], A. Shwaiki[2], S. Mundt[2].* 1) Pediatrics, University of Kansas , Kansas City, KS; 2) Saint Luke's Hospital, Kansas City, MO.

Fanconi Anemia (FA) is a premalignant, autosomal recessive syndrome characterized by highly variable congenital abnormalities, progressive bone marrow failure, and susceptibility to cancer. Approximately 25-40% of individuals with Fanconi anemia have no congenital abnormalities. The Fanconi anemia phenotype is caused by mutations in 13 genes. Fanconi anemia complementation group D1 (FANC-D1) accounts for about 3.3% of all Fanconi anemia cases. Breast cancer is also a genetically heterogeneous disease. Inherited breast cancer is associated with germline mutations in ten different genes in pathways critical to genomic integrity. Mono-allelic (heterozygous) BRCA1 and BRCA2 mutations confer a high risk of breast cancer and are major causes of familial breast cancer. Recently, bi-allelic BRCA2, BRIP1 and PALB2 mutations were shown to cause FANC-D1, FANC-J and FANC-N respectively. Few kindreds had been reported with recurrent solid tumors due to recessive BRCA2 mutations. Here, we report on Hispanic kindred with breast and colon cancer associated with compound heterozygous BRCA2 germline mutations. The proband, a 22 year old, G1P1 previously healthy female presented with right breast, stage T2N1M0, Her2/nu and estrogen/progesterone receptor negative invasive ductal carcinoma. BRCA1 gene sequencing was normal, while BRCA2 analysis showed E49X (maternal) and 9927del4 (paternal) mutations. Her healthy 15 year old brother was found to carry both mutations and both siblings had abnormal chromosomal stress testing using MMC. Neither sibling had any physical features of Fanconi anemia. Identification of Fanconi anemia as the cause of breast cancer in this family necessitated a tailored chemotherapy regimen for the proband and close monitoring of affected family members for hematologic and solid tumors known to occur frequently in patients with Fanconi anemia.

361/T

Germline SDHB and SDHD mutations in a hospital-based series of patients with hereditary pheochromocytoma and paraganglioma. *E. Edelman[1], K. Zbuk[1], A. Shealy[1], R.R. Lorenz[2], C. Eng[1].* 1) Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH; 2) Head and Neck Institute, Cleveland Clinic Foundation, Cleveland, OH.

Homozygous or compound heterozygous germline mutations of autosomal genes affecting mitochondrial energy production are associated with progressive childhood or adult-onset disease, often with multisystemic involvement. More recently, heterozygous germline mutations in this pathway have been implicated in heritable neoplasia syndromes. Heterozygous germline mutations in complex II succinate dehydrogenase subunits B, C, and D (SDHB, SDHC, and SDHD) and fumarate hydratase (FH) are associated with the Hereditary Pheochromocytoma and Paraganglioma and Hereditary Leiomyomatosis and Renal Cell Cancer syndromes, respectively. Hereditary Pheochromocytoma and Paraganglioma is an autosomal dominant condition characterized by an inherited susceptibility to adrenal pheochromocytoma (PC) and extraadrenal paraganglioma (PGL). From 2006 to present, 16 patients with PC/PGL were referred for cancer genetics consultation. Of the 16, research and/or clinical SDHB, SDHC, and SDHD sequencing results are available on 9. SDHB or SDHD mutations were identified in 5/9 (56%) cases. Heterozygous SDHD mutations were identified in 3/3 familial cases (100%) and heterozygous SDHB mutations were identified in 2/6 (33%) sporadic cases. No SDHC mutations were found. The average age at presentation in mutation positive patients was 46 years (range 18 - 72 years) compared to 53 years (30 - 67 years) in mutation negative patients. A Pro81Leu SDHD mutation was identified in a patient with a personal history of multiple head and neck PGLs as well as hemangioma and chordoma, neither of which have been previously reported in SDHD carriers. These families illustrate the variability in disease presentation in individuals with SDHB and SDHD mutations. Genetic counseling issues specific to hereditary PC/PGL include highly variable age of onset, maternal imprinting in SDHD carriers, reduced penetrance with SDHB mutations, and the risks of renal cell carcinoma and papillary thyroid cancer, especially in SDHB carriers.

362/T

Recognizing A Milder Phenotype Of Tuberous Sclerosis. *H. Gaddipati, K. Nathanson.* Division of Medical Genetics, Children's Hospital of Philadelphia, Philadelphia, PA.

The tuberous sclerosis complex (TSC) is an autosomal dominant hamartomatous tumor syndrome.The severity of the TSC phenotype can range from mild skin abnormalities to life-threatening complications. The spectrum of clinical features, natural history and management recommendations for a subset of TSC patients who present with a milder phenotype has been less well characterized. We follow 50 patients in our TSC clinic at the Hospital of the University of Pennsylvania. A subset of nine patients were identified with normal development and intellect. They were diagnosed after the age of 18 because of subtle clinical stigmata, incidental imaging, positive family history or acute complications of TSC. Adolescents with no history of seizures or complications from TSC were also included in the study. Two of the patients presented with sudden hemorrhage of renal angiomyolipomas (AML) requiring embolization. Two patients were diagnosed by biopsy of facial angiofibroma and one of them was noted to have a lesion suspicious for renal cell carcinoma on routine surveillance imaging. Three patients were evaluated based on a positive family history of TSC and two of them were found to have cortical tubers. One patient was diagnosed when subependymal nodules were incidentally found on brain imaging while investigating the etiology of her headaches. She has subsequently required embolization for renal AML. Another patient presented with micronodular pneumocyte hyperplasia (MNPH). Three patients had TSC1 mutations, two patients were mutation negative. The result was inconclusive in one case and unknown in two other. After reviewing the natural history of TSC in our study population we conclude that the risk for serious and sometimes life-threatening complications remains significant and unpredictable. No specific genotype-phenotype correlations are apparent. Therefore, it is important for physicians to recognize these subtle phenotypes and facilitate timely referral of patients to specialized TSC clinics. Even for patients with seemingly benign initial presentations close surveillance and timely intervention may help prevent significant morbidity and mortality.

363/T

Determining pathogenicity of a BRCA1 missense variant - a multi-modal approach. *N. Hamel[1], M.A. Carvalho[2,3], G. Birrane[4], A. Soni[4], E.H. van Beers[5], S. Joosse[5], D. Novak[1], P.M. Nederlof[6], S. Grist[6], D. Goldgar[7], S. Tavtigian[8], A.N.A. Monteiro[2], J.A.A. Ladias[4], W.D. Foulkes[1], M. Tischkowitz[1].* 1) McGill University, Montreal, Canada; 2) H. Lee Moffitt Cancer Center & Research Institute, Tampa, USA; 3) Centro Federal de Educação Tecnológica de Química, Rio de Janeiro, Brazil; 4) Harvard Institutes of Medicine, Harvard Medical School, Boston, USA; 5) Netherlands Cancer Institute, Amsterdam, The Netherlands; 6) Flinders Medical Centre and Flinders University of South Australia, Adelaide, Australia; 7) University of Utah School of Medicine, Salt Lake City, USA; 8) IARC, WHO, Lyon, France.

New BRCA1 variants of unknown significance are regularly detected in clinical practice and create serious management problems in the families concerned. BRCA1: M1775K was identified through BRCA1/BRCA2 mutation screening by full sequencing in 2 unrelated families with a history of breast cancer. Position M1775 is invariant in a sequence alignment including 9 mammals, chicken, frog and pufferfish. Combining sequence alignment results, Grantham variation and deviation scores, SIFT scores, logistic regression results for co-segregation in both families and histopathological data from M1775K tumors, the probability that M1775K is a high-risk variant exceeds 0.99. LOH data shows partial loss of the wild-type allele in the M1775K tumor and CGH analysis of these tumors closely resembles the aberrations present in BRCA1-related tumors. The M1775K variant was evaluated for transactivation activity in the context of stringent reporters and displayed markedly reduced activity with 20% and <5% of the wild type activity in yeast and mammalian cells, respectively. M1775K resides within the C-terminal BRCT binding domain and protein crystallography showed that it specifically disrupts the phosphopeptide binding pocket, thereby inhibiting BRCA1 interaction with BRIP1. This result suggests that the integrity of the BRCA1 phosphopeptide binding pocket is required for tumor suppression. Importantly, we demonstrate that multiple lines of evidence are required to confirm the pathogenicity of very rare variants in any susceptibility gene.

364/T

The Tip60 chromatin remodeling complex functions in the Fanconi anemia pathway of DNA interstrand crosslink repair. *J. Hejna, M. Holtorf, A. Hemphill, A. Starks, P.M. Jakobs, Y. Akkari, M. Al-Dhalimy, S.B. Olson, R.E. Moses.* Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR.

Fanconi Anemia (FA) is a rare, recessive disease that results in pancytopenia, hematological malignancies, and head and neck cancer. FA cells display genomic instability, which is exacerbated by DNA interstrand crosslinking (ICL) agents. A core FA complex composed of FA proteins interacts with FANCD2 and is required for its activation by monoubiquitination in response to DNA damage. BRCA2/FANCD1, also associates with FANCD2. FANCD2 interacts with a similar protein, FANCI, which is also monoubiquitinated and required for the FA pathway. We have identified the histone acetyltransferase Tip60 as another FANCD2-interacting protein by a yeast two-hybrid screen. Interaction of Tip60 and FANCDC2 was also demonstrated by co-immunoprecipitation and co-localization. The Tip60-interacting region of FANCD2 has been mapped to roughly the same region shown to interact with BRCA2. Site-directed mutagenesis of the acetyl-CoA binding pocket of Tip60 abrogated the interaction with FANCD2. Mutation of FANCD2 lysine 561 to arginine, preventing monoubiquitination, did not alter the interaction. Tip60 has been implicated in repair of ionizing radiation damage. In view of the association of Tip60 with FANCD2, we asked whether depletion of Tip60 in immortalized human fibroblasts sensitized them to ICL agents. Cell survival of Tip60-depleted GM639 cells was significantly reduced after treatment with Mitomycin C. We then tested whether depletion of another member of the Tip60 complex, the RuvB homolog, Tip49, would also lead to increased sensitivity to MMC. Tip49 depletion in GM639 cells gave an FA-like increase in chromosomal aberrations in response to MMC. In summary, there is a direct interaction between FANCD2 and Tip60, independent of the ubiquitination state of FANCD2, but which requires the acetyltransferase domain of Tip60 and a region of FANCD2 that also binds to BRCA2. Our findings establish a role for the Tip60 chromatin remodeling complex in ICL repair.

365/T

Total leukonychia and sebaceous cysts in a novel family: are the acoustic neurinomas of the index case in relation with the disease? *C. Jeanpetit[1], G. Morin[1], C. Desenclos[2], S. Olschwang[3], N. Levy[4], M. Mathieu[1].* 1) Clinical Genetics Unit, Amiens University Hospital, Amiens, France; 2) Neurosurgery Service, Amiens University Hospital, Amiens, France; 3) Paoli Calmette Institute, Marseille, France; 4) Medical Genetics Service, Marseille, France.

This French family of five generations demonstrates total leukonychia and multiple sebaceous cysts. Six members are affected, five women and one boy. The index case, a 41-year-old woman, was initially addressed for bilateral acoustic neurinomas, resembling neurofibromatosis type II. .Her physical examination revealed leukonychia of fingers and toes and multiple sebaceous cysts of the scalp. Among the five related affected persons nobody demonstrates neurinoma. The association of total leukonychia and sebaceous cysts is a rare disease, first reported by Bauer in 1920, in a large family of nineteen affected persons with leukonychia, and seventeen with sebaceous cysts. In 1975, Gorlin and Bushkel described a family of five boys with leukonychia, four with sebaceous cysts, three with renal stones and one with acute pancreatitis. In 1986, another family with eleven individuals was reported by Friedel, inconstantly associated with trichilemnal cysts and ciliary dystrophy. In 1997, Slee published the observation of one affected mother with pancreatitis and her daughter. The etiopathogeny of this disease is completely unknown. The mode of inheritance appears autosomal dominant in all the reported families. But no analysis for localisation has been made in this apparently benign disease and no candidate gene is really evocated. However, several cases of pancreatitis and renal stones suggest the possibility of complications. To our knowledge, the acoustic neurinomas or other tumours have never been reported. This argument and the absence of this complication in the other five affected members of our family suggest a different disease. But the screening of the NF2 gene of the index case failed to find a mutation.

366/T

Response to neo-adjuvant chemotherapy in women with BRCA1-positive breast cancers. *J. Lubinski[1], T. Byrski[1], J. Gronwald[1], T. Huzarski[1], E. Grzybowska[2], M. Budryk[2], M. Stawicka[3], T. Mierzwa[4], M. Szwiec[5], R. Wisniowski[6], M. Siolek[7], S.A. Narod[8], The Polish Hereditary Breast Cancer Consortium.* 1) Dept. of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin; 2) Department of Tumor Biology, Maria Skłodowska-Curie Memorial Institute Gliwice, Poland; 3) Prophylactic and Epidemiology Center, Poznań, Poland; 4) Department of Clinical Genetics, Bydgoszcz Medical University, Poland; 5) Regional Oncology Hospital, Opole, Poland; 6) Regional Oncology Hospital, Bielsko Biała, Poland; 7) Holycross Oncology Center, Kielce, Poland; 8) Centre for Research in Women`s Health, University of Toronto, Canada.

PURPOSE: There have been no studies to date which look at the relative effectiveness of different regimens of chemotherapy in women who have breast cancer and who carry a BRCA1 germ-line mutation. We wished to compare rates of response to neo-adjuvant chemotherapy in BRCA1 mutation carriers and non-carrier controls. EXPERIMENTAL DESIGN: From a registry of 3,479 patients, we identified 44 Polish women who carried a BRCA1 founder mutation and who had been treated with neo-adjuvant chemotherapy for breast cancer, and 41 age- and hospital-matched controls. RESULTS: 35 of the 44 BRCA1 mutation carriers (80%) experienced a partial or complete response to neo-adjuvant chemotherapy, compared to 39 of the 41 (95%) non-carriers (P = 0.05). In the hereditary subgroup, response rates differed depending on whether or not a taxane (docetaxel) was given. Six of the 15 BRCA1 carrier women given docetaxel with doxorubicin responded (complete or partial), compared to 29 of 29 given other (DNA-damaging) therapies (P = 0.001). Among the non-carriers, the rates of response to the two categories of chemotherapy were similar. CONCLUSIONS: Breast cancers among BRCA1 carriers frequently do not exhibit sensitivity to docetaxel in the neo-adjuvant setting. It is likely that normal BRCA1 is required for clinical response to mitotic spindle poisons.

367/T

Two cases of malignant phyllodes tumor in patients with history of bilateral retinoblastoma - a possible novel association with RB1 germline mutations. *J. Mak.* Cancer Risk Program, University of California San Francisco, San Francisco, CA.

We are reporting two cases of patients with a history of bilateral retinoblastoma who subsequently developed malignant phyllodes tumors of the breast. Patient 1 has a large deletion encompassing the promoter through exon 23 of the RB1 gene. She had bilateral retinoblastoma diagnosed at the age of 2, followed by rhabdomyosarcoma at age 12, and malignant phyllodes tumor at age 22. Patient 2 had bilateral retinoblastoma in early childhood and, therefore, has a presumed RB1 germline mutation. She was diagnosed with malignant phyllodes tumor at age 48. Both patients were treated with mastectomy and were free of recurrence at 7 and 4 years post diagnosis.

The coincidence of these two very rare tumors - retinoblastoma and cystosarcoma phyllodes - in two separate patients suggests a possible etiological link through a germline RB1 mutation. Other sarcomas, including osteosarcoma, rhabdomyosarcoma, and leiomyosarcoma, are already known to be more frequent in patients with germline RB1 mutations. This possible novel association would be consistent with the pathological features of phyllodes tumors, which are a type of sarcoma frequently displaying alterations in the RB1 gene or pRb protein expression.

This observation could have relevance for the clinical care of patients with suspected or proven germline RB1 mutations, who may benefit from careful breast cancer surveillance starting at an early age. Of note, phyllodes tumors can be difficult to distinguish from fibroadenomas on mammogram or the results of fine needle aspiration. Surgical biopsy is often required for definitive diagnosis.

Thank you to Jerzy Klijanienko, MD of Institut Curie, Paris, France, for sharing data on Patient 2.

368/T

Swedish adenomatous polyposis families: Notably High mutation detection rate and colorectal cancer morbidity in probands. *M. Nordling[1], A. Rohlin[1], K. Fritzell[2], G. Kanter Smoler[1], J. Meuller[1], J. Björck[2].* 1) Dept Clinical Genetics, Sahlgrenska Univ Hosp, Goteborg,-Sweden; 2) Dept of Medicin, Karolinska Institute, Stockholm, Sweden.

Background and aims: Using a range of different molecular genetic techniques our purpose was to achieve as high mutation detection rate as possible. Our intention was further to uncover any not at yet described genotype-phenotype correlation. Participants and methods: Mutation screening of APC and clinical characterization of 95 unrelated FAP patients from the Swedish Polyposis Registry was performed. In addition to ordinarily used mutation screening methods analyses of splicing affecting mutations and investigations of the presence of low-frequency mutation alleles, indicating mosaics, have been performed as well as quantitative real-time PCR to detect lowered expression of APC. Results: A number of novel mutations were detected and characterised including a case with reduced APC expression The nonsense mutation, c.70 C>T in exon 1, was detected, to our knowledge the most 5' situated APC mutation reported. In total, 60 different APC mutations in 80 of the 95 families where identified in this study and 27 of those are novel. We have previously shown that 6 of the 95 patients carried biallelic MUTYH mutations. The mutation-negative cases all display an atypical FAP phenotype, indicating that the detection frequency for mutations in the APC gene in patients with classical FAP is 100%. Probands with mutations upstream from codon 1309 had a median age at diagnosis of 36 (range, 14-57) years compared to 20.5 (range, 11-34) years among those with mutations downstream of codon 1309 (P < 0.0014). The morbidity in CRC among probands, of whom more than 80 percent were diagnosed during the last three decades, was 43 and 18 percent respectively, and in total 34 percent. Conclusion: With a variety of mutation detection techniques it is today possible to achieve a 100% detection frequency in classical FAP. Despite a lower fraction of patients with dense polyposis among those with mutations upstream of codon 1309, CRC at diagnosis occurred more often.

369/T

A patient with pancreatic neuroendocrine tumors and Von Hippel-Lindau V84L mutation: A new VHL subset? *E.C. Oquendo, H.C Gaddipati, K.L. Nathanson.* Division of Medical Genetics, Children's Hospital of Philadelphia, Philadelphia, PA.

Von Hippel-Lindau (VHL) disease is an autosomal dominant tumor susceptibility syndrome caused by germline mutations of the VHL tumor suppressor gene. Patients develop hemangioblastomas of the central nervous system and retina aswell as endolymphatic sac tumors, renal cell carcinomas, cysts and neuroendocrine tumors of the adrenal gland (pheochromocytomas) and the pancreas. The VHL protein plays an important role in angiogenesis as a negative regulator of hypoxia-inducible mRNAs. We present a 43-year-old Caucasian male patient with history of benign bilateral adrenal pheochromocytomas (PCCc) status post adrenalectomy and retroperitoneal paraganglioma status post resection. At the age of 40 he developed symptoms of diarrhea, fatigue and flushing. A 3cm pancreatic mass was detected on abdominal MRI scan. The histopathology of the specimen obtained from pancreatoduodenectomy was consistent with 3 localized benign pancreatic neuroendocrine tumors (PNETs). Brain, spine MRI scans, thyroid ultrasound and retinal exams were all normal. DNA sequencing of the VHL gene detected a heterozygous missense mutation G463T changing a valine for leucine at amino acid position 84 of the protein (V84L). We previously reported the correlation between the V84L mutation and multiple early-onset PCCs in 4 unrelated families with VHL type 2C. None of these patients had PNETs. PCCs and PNETs have been found to occur concurrently in VHL patients principally in the VHL2B subtype. VHL2C is characterized by patients with isolated PCCs and low risk for renal cell cancer. Therefore this novel association between the V84L mutation, PCC and PNET may represent a new variant of VHL2B, VHL2C subtypes or possibly a new subtype VHL2D. This case illustrates the ambiguity sometimes encountered when we make genotype-phenotype correlations based on clinical classification models. Should molecular or clinical criteria predominate in classification and management? Interestingly our patient had hormonally active PNETs which is very unusual in VHL syndrome. In conclusion it is clear that VHL patients with the V84L mutation should be screened for PNETs in addition to early onset PCCs.

370/T

Multifocal pheochromocytoma in a patient with Beckwith-Wiedemann syndrome: A case report and review of the literature. *L. Palma[1], L. Feldman[2], G. Domanowski[3], E. Shoubridge[4], W.D. Foulkes[1,5].* 1) Division Medical Genetics, McGill Univ Health Ctr (MUHC), Montreal, Canada; 2) Dept Surgery, MUHC, Montreal, Canada; 3) Dept Pathology, MUHC, Montreal, Canada; 4) Depts Neurology & Neurosurgery, McGill University, Montreal, Canada; 5) Program in Cancer Genetics, Depts Oncology & Human Genetics, McGill University, Montreal, Canada.

We report the case of a 23-year-old girl with Beckwith-Wiedemann syndrome (BWS) who presented with multifocal pheochromocytoma. The patient was investigated at age 21 for hypertension and headaches. A 24-hour urine collection had high norepinephrine and normetanephrine. CT scan revealed a 2.9 cm left adrenal mass and a 2.3cm mass anterior to the aortic bifurcation. After alpha blockade, the patient underwent a laparoscopic left adrenalectomy and excision of the left para-aortic mass. Postoperative pathological findings included a 5.0 cm left adrenal pheochromocytoma and a 3 cm para-aortic mass consistent with either a completely replaced metastatic lymph node or, more likely an extra-adrenal paraganglioma arising in the organs of Zuckerkandl. At nearly two years follow-up, the patient has no further sequeale or radiologic evidence of recurrent disease. Family history was negative for pheochromocytoma/paraganglioma, von Hippel-Lindau, and multiple endocrine neoplasia type A/B. To rule out co-existing nonsyndromic pheochromocytoma due to a germline mutation in the *VHL* , *RET*, or *SDHD* genes, molecular testing of all genes was performed and no mutations were identified. To our knowledge, this is the third reported case of pheochromocytoma occurring in a patient with BWS. Two additional cases of pheochromocytoma in association with congenital hemihypertrophy have been reported. Interestingly, all five patients had hemihypertrophy; either isolated or in association with BWS. Our case provides further evidence that pheochromocytoma may be a part of the clinical spectrum of BWS, though in light of the rarity of this tumour type, the need for regular screening is at present, unclear. An awareness of the clinical sequeale of pheochromocytoma among physicians caring for patients with congenital hemihypertrophy or BWS is of utmost importance.

371/T

Intrafamilial correlation of age at breast cancer onset in *BRCA1* and *BRCA2* carriers. *S. Panchal[1, 2], M. Ennis[3], S. Canon[1], L.J. Bordeleau[1, 2].* 1) Familial Breast Cancer Clinic, Mount Sinai Hospital, Toronto, ON, Canada; 2) The University of Toronto, Toronto, ON, Canada; 3) Applied Statistician, Markham, ON, Canada.

BRCA1 and *BRCA2* mutations account for the majority of known hereditary breast cancer. For female carriers, lifetime breast cancer (BC) risks range from 56-87%. Although general data are available on cancer risk estimates by age and mutation type, personalized BC onset risk estimates are not available for individual carriers. This study attempts to determine if there is a correlation between the age at BC onset among family members in any given *BRCA* mutation positive family. Data were collected from a chart review of patients followed in the Familial Breast Cancer Clinic at Mount Sinai Hospital, Toronto, Canada. Detailed 3-generation pedigrees were constructed and analyzed and ethics approval was obtained to review and record chart information. Carrier families with 2 or more cases of BC were included. Age at diagnosis and age at onset are used interchangeably. The intraclass correlation (ICC), representing the correlation in BC diagnosis age between two randomly drawn family members from the same family, was calculated using the ANOVA method. Variability in diagnosis age was explored using multilevel (mixed model) methods. A total of 48 *BRCA1* and 42 *BRCA2* families were included in the study. The average number of family members diagnosed with BC was 2.8 (range:2-5). The average age at BC diagnosis is significantly younger (p=0.009) in *BRCA1* carriers (42.92 years) compared to *BRCA2* carriers (46.82 years). The ICC for *BRCA1* (0.08) and *BRCA2* (0.04) were not significantly different from zero. Data from our population are consistent with previous studies showing that the average age at BC diagnosis in *BRCA1* carriers is lower than in *BRCA2* carriers. ICC results suggest that there is no correlation between the age at BC diagnosis among *BRCA* carriers from the same family. This study demonstrates that the age at BC diagnosis for affected *BRCA* carriers cannot be used clinically to predict the age at which an unaffected carrier from the same family will develop BC.

372/T

Collecting Family History of Cancer in a Primary Care Setting from a Middle-Income Country. *M.F. Prearo[1], M. Floria-Santos[1], E.M.M. Santos[2], L.M. Alvarenga[1], C.M. Cenzi[1].* 1) University of São Paulo at Ribeirão Preto College of Nursing, Department of Maternal-Child Nursing and Public Health, SP, Brazil; 2) Cancer Hospital A.C. Camargo, SP, Brazil.

Introduction: This study presents the first step of a broad project which aims to show the importance of family history collection to know the risk for hereditary and familial cancers in families attended by the Family Health Program in a Middle-Income country. This program is a governmental initiative established to ensure medical attention on a community-based approach to vulnerable populations, who had been neglected under an earlier strategy that emphasized hospital care. Methods: This is a descriptive exploratory study with a quantitative approach, where approximately 5,000 familial medical records will be searched for cancer registries. Families with cancer will be visited and interviewed, using structured questionnaire and pedigree. Tumors will be classified as sporadic, familial or hereditary, and qualitative risk assessment will be conducted for family members. Results: So far, we analyzed 670 familial medical records, 15% had cancer registries of breast (19%), skin (12%), head/neck (12%), colorectal (11%), uterus (7%), and prostate (6%) among others. Discussion: This study can provide resources to planning actions for early diagnosis and cancer prevention to low-income families in urban centers. Also, can aware those people regarding family health history as a risk factor of cancer and other diseases, improving prevention and health promotion.

373/T

Assessment of the "MMRpredict" model for prediction of DNA mismatch repair gene mutations. *K.G. Rabe[1], S.K. Nigon[2], N.M. Lindor[2].* 1) Div Biostatistics, Mayo Clinic, Rochester, MN; 2) Dept of Medical Genetics, Mayo Clinic, Rochester, MN.

Background: The MMRpredict model is a web-based model developed to predict which patients diagnosed with colorectal cancer (CRC) under age 55 years have germline mutations in a DNA mismatch repair (MMR) gene [Barnetson et al., NEngJMed 2006;354:2751-63]. Stage 1 MMRpredict estimates the probability for MMR mutations from clinical parameters (age of diagnosis, proximal versus distal site, family history, multiple primary CRCs). We evaluated the performance of Stage 1 of this model in a cohort of colorectal cancer patients. **Methods:** Cases with CRC were identified through Mayo Clinic Rochester. All were consented participants in the Mayo Colon Cancer Family Registry. dHPLC followed by sequencing and MPLA were conducted on the 3 genes. **Results**: We defined 4 probability risk groups:0-10%, 11-25%, 26-50%, and 51-100%. The number of cases in each of these groups was 112, 28, 20, and 31, respectively. The corresponding percent of mutations in each group was 3.6%, 7.1%, 10.0% and 29.0%. The observed/expected ratio in the 4 groups was 1.0 (95% CI 0.27-2.56); 0.40 (95% CI 0.04-1.44) 0.29 (95% CI 0.03-1.03) and 0.39 (95% CI 0.18-0.74). Overall, 17/191 patients were found to have mutations (8.9%), compared to 20.5% predicted (95% CI 0.25-0.70%. **Conclusion:** The MMRpredict Stage 1 predicted a percentage of positive MMR gene mutations carriers was greater than what was found. In the lowest predicted risk group the model performed well, while in the higher risks groups, a trend toward over prediction was seen. Larger studies will be required to further explore the prediction properties of this model. **Acknowledgements:** This work was supported by the National Cancer Institute (NCI), National Institutes of Health (NIH) under RFA #CA-95-011 and through cooperative agreements with members of the Colon Cancer Family Registry and P.I.s. The content of this manuscript does not necessarily reflect the views or policies of NCI or any of the collaborating centers in the CFR nor does mention of trade names, commercial products or organizations imply endorsement by the US Government or the CFR.

374/T

Therapeutic implications of a differential response to rapamycin in cells from Gorlin Syndrome patients versus unaffected controls. *M.R. Rossi[1], B. Hoffman[1], J. Zhou[1], S. Westman[1], P. Beck[1], S. Mane[1], L.M. Milstone[1], J.A. Crowell[2], L. Kopelovich[2], A.G. Knudson[3], A.E. Bale[1].* 1) Yale University School of Medicine, New Haven, CT; 2) National Cancer Institute, Bethesda, MD; 3) Fox Chase Cancer Center, Philadelphia, PA.

Basal cell nevus syndrome (BCNS), also known as Gorlin Syndrome, is an autosomal dominant disease characterized by palmar pits, jaw cysts, skeletal anomalies, and multiple basal cell carcinomas (BCCs). Although the BCCs associated with BCNS are rarely life threatening, they lead to disfigurement and are a major clinical management challenge in these patients. BCNS is caused by heterozygous mutations of the PTCH1 gene, a regulator of the hedgehog (HH) signaling pathway. Hereditary and sporadic BCCs arise through a two-hit mechanism in which both copies of PTCH1 are inactivated, resulting in aberrant HH signaling mediated through the GLI family of transcription factors.

Rapamycin is an antagonist of cellular transformation by GLI, but this effect is not mediated directly by HH signaling and the mechanism is unknown. In a phase I clinical trial, global gene expression was used as an endpoint to measure the effects of rapamycin on BCNS-derived cells in vitro. Biopsies from normal-appearing skin from a total of 9 BCNS patients (5 male, 4 female) and 8 unaffected controls (4 male, 4 female) were used to generate primary keratinocyte and fibroblast cultures. These cultures were treated with 10 and 50 μM of rapamycin, and RNA was extracted and analyzed using the Affymetrix U133 Plus 2.0 expression array. The resulting data showed no statistically significant expression differences between BCNS patients and controls before treatment, but major differences in gene expression were observed following rapamycin treatment for both keratinocytes and fibroblasts. Analysis of the genes accounting for these differences revealed novel transcriptional targets of rapamycin, and also provided insight into the mechanism of action of rapamycin in blocking effects of HH pathway activation. Rapamycin or a derivative is promising as a pharmacologic agent for prevention and treatment of BCCs in vivo.

375/T

Large Germline Deletions of the Fumarate Hydratase Gene in Patients with HLRCC. *A.B. Santani[1], C. Vocke[2], L. Middleton[2], W.M. Linehan[2], C.A. Stolle[1].* 1) Dept of Pathology and Lab Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Urologic Oncology Branch, National Cancer Institute, Bethesda.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is characterized by cutaneous leiomyomata, uterine leiomyomata and renal cell carcinoma and is associated with mutations in the fumarate hydratase (FH) gene. Using DNA sequence analysis missense, nonsense, splice site and frame shift mutations may be identified in 85-90% of patients with HLRCC, while the molecular cause is unknown in the remaining 10-15%. Failure to identify mutations in all patients might be due to the inability of the current PCR-based assays to detect disease causing mutations such as large duplications and deletions. Previous reports have identified whole gene deletions in three families with HLRCC. To determine whether gene deletions could be responsible for HLRCC in some of our patients, we developed a relative quantitative (RQ) PCR assay to test probands and family members in whom HLRCC was strongly suspected but who tested negative for a point mutation in the FH gene. RQ-PCR primer/probe sets were designed for 8 exons of the FH gene and the albumin gene (internal control). The copy number of each exon was determined using the ddCt method. A complete deletion of the FH gene was identified in 17 individuals from one large family. In addition, complete FH gene deletions were identified in probands from two other unrelated families. Among our mutation positive probands, complete deletions account for about 5% of disease causing mutations. The identification of deletions by RQ-PCR, therefore, increases the detection rate for FH gene mutations to about 90-95%. Use of genetic testing for early identification of HLRCC in patients and at risk family members improves diagnostic certainty and reduces costly screening procedures for at-risk members who have not inherited a disease-causing mutation. Early recognition of clinical manifestations may also allow timely intervention and improved outcome. The RQ-PCR assay is a simple, high resolution technique for rapid detection of exon dosage, and diagnostic testing for HLRCC should include quantitative analysis of the FH gene in addition to sequence analysis.

376/T

Identifying which young women affected with breast cancer are at high risk of carrying a germline mutation in *BRCA1*. *M. Southey[1], S. Ramus[2], J. Dowty[3], G. Dite[3], G. Byrnes[3], G. Giles[4], M. McCredie[5], J. Hopper[3].* 1) Department of Pathology, University of Melbourne, Australia; 2) Translational Research Laboratory, Institute for Women's Health, University College London, UK; 3) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Australia,; 4) The Cancer Council Victoria, Australia; 5) The University of Otago, Dunedin, New Zealand.

Only a small proportion of women diagnosed with breast cancer at a young age carry germline mutations in *BRCA1*. In our population-based study of breast cancer diagnosed before the age of 40 years in Australian women we have estimated that only 3.8% (95% CI 0.3-12.6%) of cases is attributable to mutations in *BRCA1* and that family cancer history alone is not a strong predictor of carrier status. In a subset of 66 women with a strong family history (2 or more affected first- or second-degree relatives affected with breast or ovarian cancer) only 10 (15%) carry germline *BRCA1* mutations.

We sought to devise a practical strategy for identifying women at high risk of carrying a germline mutation in *BRCA1* that could be applied at the time of diagnosis, irrespective of family history, using morphological and immunohistochemical data that are routinely collected at diagnosis. Tumours arising in women participating in our population-based study were systematically reviewed and scored for morphology features and ER and PR status was collected for each cancer.

Using a simple morphology based scoring system we identified the *BRCA1* mutation carriers amongst 500 early-onset breast cancer cases and also in the subset of women with a strong family history. Sensitivity was 90% and 100% respectively and specificity was 33% and 63%. In addition we used logistic regression on the same dataset and identified a model with 3 key covariates that predicts *BRCA1* mutation carrier status with a high degree of reliability (area under the ROC curve of 0.93). These approaches offer a simple, cost-effective and practical way of identifying young women affected with breast cancer at high risk of carrying a *BRCA1* germline mutation.

377/T

Clinical testing experience for large genomic rearrangements in the *BRCA1* and *BRCA2* genes for hereditary breast and ovarian cancer. *W. Spence, T. Judkins, J. Schoenberger, S. Rajamani, C. Colvin, S. Chen, M. Frost, J. Trost, R. Wenstrup, B. Roa.* Myriad Genetic Laboratories, Inc. Salt Lake City, UT.

Testing for large genomic rearrangements throughout the *BRCA1* and *BRCA2* genes has been performed on high risk breast/ovarian cancer patients referred to our laboratory since August 2006. Our BRC*Analysis*® Rearrangement Test (BART) is a multiplexed quantitative endpoint PCR assay for deletions and duplications in the promoter and coding regions of *BRCA1* and *BRCA2*. Analytical software normalizes gene copy number and provides a statistical confidence level for mutation calls. Expert-developed criteria based on family history were used to select patients with ≥30% risk for a mutation. BART complements our panel of 5 common *BRCA1* rearrangements performed on all comprehensive BRC*Analysis*® patients. A variety of large rearrangements were identified by BART, wherein ~83% are observed in *BRCA1* and ~17% in *BRCA2*. The profile for *BRCA1* includes private as well as recurrent rearrangements. BART identified a *BRCA1* deletion of exons 9-12 in high risk patients of Latin American ancestry. Another noteworthy rearrangement is the *BRCA1* duplication of exons 18-19, which we found exclusively in unrelated patients of African descent. We also observed recurrent deletions of *BRCA1* exons 1-2, exon 17 and exons 21-24, seen predominately in patients of European descent. One very interesting *BRCA2* rearrangement consists of a triplication of exons 14-24 identified in multiple individuals, mostly traced to a large North American kindred. Recurrent *BRCA2* mutations include deletion of exons 1-2 found in patients of Western European descent, and deletion of exons 14-16. The majority of *BRCA2* rearrangements, however, appear to be private. Expanded large rearrangement testing using BART in addition to sequencing on high risk patients increased the overall *BRCA1/BRCA2* mutation detection rate from ~30% to 33%. Our clinical testing experience with BART provided key information on the *BRCA1* and *BRCA2* large rearrangement profile, and documented apparent founder mutations in specific populations. These data demonstrate the value of testing high risk individuals for large rearrangements in the *BRCA1* and *BRCA2* genes.

378/T

Rare Case of Siblings with Childhood Follicular Thyroid Neoplasia and a *PTEN* Promoter Deletion. *J. Stein[1], K. Zbuk[1], A. Stettner[2], D. Wargowski[2], C. Eng[1].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Clinical Genetics Center, University of Wisconsin, Madison, WI.

Cowden syndrome (CS) is characterized by macrocephaly, cutaneous lesions, and an increased risk for breast, thyroid and endometrial neoplasia. About 85% of those with classic CS have detectable *PTEN* mutations. Promoter mutations are detected in approximately 10% of individuals with CS without an identifiable mutation in the *PTEN* coding region. We report a family with a unique promoter deletion (-1087~ -1062 del 26) detected in three generations. A 9-year-old girl presented with follicular thyroid cancer, and subsequently her sister was diagnosed with a follicular thyroid adenoma at age 6; both carried the promoter deletion. Recent data from the largest cohort of patients with CS has shown a mean age of 31 at thyroid cancer diagnosis, 10-20 years younger than sporadic cases. Additionally, the 40-year-old father tested positive. Previously considered asymptomatic, the father was evaluated further and consequently underwent total thyroidectomy, which revealed extensive adenomatous nodules. The paternal grandfather underwent partial thyroidectomy at age 19 for multiple nodules and also tested positive. *PTEN* promoter point mutations were previously found to have a mild phenotype as defined by number of organ systems involved, but with considerable risk of breast and thyroid cancers. As no other features of CS have been described in this family, only the thyroid appears to be involved. NCCN guidelines for CS recommend annual comprehensive physical examination and consideration of baseline thyroid ultrasound starting at age 18 for most families, but additionally recommend physical examinations 5 years younger than the youngest age of diagnosis. This family illustrates the importance of providing personalized healthcare in the context of the family, as screening should begin in the first year of life in this case. Furthermore, if specific *PTEN* mutations provide evidence for childhood onset cancer, this could have important medical surveillance implications for a subset of families.

379/T

Cowden Syndrome Patients with PTEN Promoter Mutations Demonstrate Abnormal Protein Translation. *R.E. Teresi[1], K.M. Zbuk[1], M.G. Pezzolesi[1], J. Bubenik[2], D.M. Driscoll[2], K.A. Waite[1,3], C. Eng[1,3,4,5].* 1) Genomic Medicine Inst; 2) Dept of Cell Biology; 3) Taussig Cancer Center, Cleveland Clinic; 4) Dept of Genetics; 5) CASE Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH.

Cowden Syndrome (CS) is an inherited disorder characterized by hamartomas and increased risk of breast cancer, thyroid abnormalities, uterine leiomyoma, and macrocephaly. Germline mutations within PTEN (phosphatase and tensin homolog deleted on chromosome ten) account for 85% of all CS patients, where 10% are promoter mutations associated with an increase frequency of breast cancer. We studied the downstream effect of PTEN promoter variants (-861G/T, -853C/G, -834C/T, -798G/C, and -764G/A) that do not lie within known cis-acting regulatory elements from 5 unrelated CS patients. Paradoxically, protein binding to the PTEN promoter (-893 to -755) does not appear to be altered in the 5 variants, when compared to wildtype (WT). However, reporter assays indicated that 3 of the PTEN promoter variants (-861G/T, -853C/G, and -764G/A) demonstrated ~50% decrease in luciferase activity. Analysis of PTEN mRNA revealed no transcript alterations, thereby suggesting an inhibition of protein translation. MFOLD's mRNA secondary structure predictions suggest RNA structural modifications in 3/5 variants (-861G/T, -853C/G, and -764G/A) when compared to WT PTEN promoter. Additionally, PTEN protein levels were ascertained in available samples and a decrease was observed in variants that cause the largest mRNA secondary structure alterations. These data indicate that PTEN promoter variants can alter normal mRNA secondary structures and resulted in an inhibition of protein translation. Our results stress the importance of looking for PTEN promoter variants in patients that have CS features yet do not have a detectable mutation within its open reading frame, the latter of which is part of clinical routine. Additionally, nucleotide changes within the promoter region do not affect the PTEN protein sequence, therefore, a therapeutic tool that can regulate its transcription and/or translation could be highly effective in this subset of patients.

380/T

A large proportion of unclassified variants of the mismatch repair genes *MSH2* and *MLH1* are associated with splicing defects. *I. Tournier[1], M. Vezain[1], A. Martins[1], F. Charbonnier[1], S. Baert-Desurmont[1], J. Soret[2], J. Tazi[2], S. Olschwang[3], Q. Wang[4], M-P. Buisine[5], T. Frebourg[1], M. Tosi[1].* 1) Inserm U614, Faculty of Medicine and Department of Genetics, University Hospital, Institute for Biomedical Research, Rouen, France; 2) IGMM, CNRS UMR 5535, Montpellier, France; 3) Inserm UMR 599, Institut Paoli-Calmettes, Marseille, France; 4) Molecular Oncology Unit, Centre Léon Bérard, Lyon, France; 5) Laboratory of Biochemistry and Molecular Biology, University Hospital, Lille, France.

Numerous variants of unknown biological significance have been found in *MSH2* and *MLH1* involved in Lynch /HNPCC syndrome. Some of these variants may have an effect on pre-mRNA splicing by altering degenerate positions of splice site sequences. Moreover, exonic splicing enhancers (ESE) have been proposed to be frequent in *MSH2* and *MLH1*. To determine the consequences of these variants on splicing, we have developed a functional assay performed on genomic DNA. For each variant, mutant and wild-type exons to be tested, PCR amplified from genomic DNA together with ~150 bp of flanking sequences, were inserted into a splicing reporter minigene. After transfection into HeLa cells, the effects of mutations were evaluated by RT-PCR and sequencing analysis. We have examined 85 different UVs (54 missense, 10 silent, 3 deletions of a single codon and 18 intronic variants) detected in 84 HNPCC families and found that 22 (26%) affect splicing. Four exonic variants were found to affect putative splicing regulatory elements. We then analysed short stretches (~30 nt) around the latter variants or the corresponding wild type sequences by cloning them into the ESE-dependent central exon of a three exon splicing minigene and we showed that the wild-type short sequences contain functional ESEs. Moreover, using this construct, we examined short stretches around 14 additional *MSH2* or *MLH1* variants previously described as affecting putative exonic regulatory elements and we found that they contain functional ESEs. In absence of reliable bioinformatics predictions, these splicing assays represents a valuable tool for the interpretation of UVs and should contribute to the optimization fo the Lynch syndrome.

381/T

***BRCA1* c.5074+3A>G (IVS17+3A>G) is a clinically relevant splice site mutation.** *A.H. van der Hout, I.M. Mulder, M.J. Berends, R.H. Sijmons, Y.J. Vos.* Dept Genetics, University Medical Centre Groningen, Groningen, Netherlands.

During screening of the *BRCA1* and *BRCA2* genes in patients at risk for hereditary breast/ovarian cancer syndrome, numerous germline variants with unknown clinical relevance are encountered. Some of these are intronic variants, other than at the invariant GU at the 5' site of the intron or the AG at the 3' site, of which the effect on pre-mRNA splicing cannot be predicted from genomic sequence alone. We found a variant c.5074+3A>G (affecting the third nucleotide of intron 17) in *BRCA1* in index patients from three different Dutch families with several cases of breast- and ovarian cancer, that were seen in our family cancer clinic. Haplotype analysis showed that these families most likely share a common ancestor. To establish the clinical relevance of this variant we studied its effect on *BRCA1* pre-mRNA splicing. One of three on-line splice site prediction programmes predicted this mutation to abolish the splice donor of exon 17. We cultured fibroblasts from a skin biopsy from one of the carriers of the variant. Shortly before harvesting we added to half of the culture cycloheximide to inhibit Nonsense Mediated mRNA Decay. RNA was isolated and RT-PCR performed, using primers in exon 15 and exon 19. In the cycloheximide treated sample we detected an additional band, which was not present in the untreated sample or in controls. Sequence analysis showed skipping of exon 17. Analysis of a polymorphic site in exon 16 showed that one allele solely produces the wild type transcript, while the other allele solely produced the transcript without exon 17. We conclude that c.5074+3A>G disturbs the proper splicing of the *BRCA1* pre-mRNA, leading to skipping of exon 17, and therefore is a clinically relevant mutation.

382/T

Impact of the *BRCA*-genes on the burden of familial breast / ovarian cancer in South Africa. *E.J. van Rensburg[1], N.C. van der Merwe[2], M.D. Sluiter[1], C.M. Schlebusch[1].* 1) Dept Human Genetics, University of Pretoria, Pretoria, South Africa; 2) Division of Human Genetics, University of the Free State and NHLS, Bloemfontein, South Africa.

Breast cancer is the most common form of cancer to afflict women in South Africa (overall lifetime risk of 1 in 27 for all women). Between 5 and 10% of all breast cancer cases display patterns of familial inheritance. It has been estimated that 30 - 50% of familial cases are due to mutations in either of two breast cancer susceptibility genes, *BRCA1* and *BRCA2*. Germline mutations within the *BRCA*-genes are responsible for different proportions of inherited susceptibility to breast/ovarian cancer in different populations. Some mutations have frequently been reported in certain population groups, which have been shown to represent founder effects. A study was undertaken in order to determine the proportion of South African Afrikaner families who carry *BRCA* gene mutations. We fully screened 153 Afrikaner breast and/or ovarian cancer families (who had three or more affected individuals) for *BRCA1*/*BRCA2* mutations, using PTT and PCR-SSCP/Heteroduplex analysis. MLPA analysis was carried out to detect large rearrangements in the genes. In total, 103 families (67,3%) had a germ line disease-causing *BRCA*-mutation. Of these, 30 families (19,6%) carry a *BRCA1* mutation and 73 families (~47,7 %) carry a *BRCA2* mutation. Three unique founder mutations (p.E881X and c.1493delC in *BRCA1* and c.8162delG in *BRCA2*) were identified in the Afrikaner population. Collectively these three founder mutations account for 94% of mutation positive Afrikaner families with a history of ≥3 affected individuals. Genealogical studies have identified the founding couples. In conclusion: the *BRCA*-genes thus account for ~67% of South African Afrikaner breast/ovarian cancer families. The results obtained for *BRCA1* is similar to that found in populations from western Europe and America. The large contribution of *BRCA2* mutations to the burden of breast cancer susceptibility in Afrikaner families is however striking. These results now allow for better testing strategies and risk management of Afrikaner breast cancer families.

383/T

Classification of BRCA1 and BRCA2 variants using gene expression profiling of lymphoblastoid cell lines treated with ionizing radiation is affected by mutation type. *N. Waddell[1], A. Ten Haaf[1], M. Gongora[2], S. Grimmond[2], G. Chenevix-Trench[1, 3], A.B. Spurdle[1], kConFab.* 1) Queensland Inst Medical Res, Brisbane, Australia; 2) Institute for Molecular Biosciences, Brisbane, Australia; 3) Peter MacCallum Cancer Centre, Melbourne, Australia.

BRCA1 and BRCA2 mutations confer a high risk of breast cancer. Truncating mutations are usually assumed to be pathogenic, but the consequences of missense variants are difficult to predict. We used Illumina bead microarrays to expression profile 65 lymphoblastoid cell lines (LCLs) after exposure to 10Gy irradiation. The LCLs were derived from women carrying pathogenic truncating or missense mutations in BRCA1 (n=22) or BRCA2 (n=22), or from affected, non-BRCA1/2 women (BRCAX, n=21). The genes that could discriminate between BRCA1 or BRCA2 LCLs, versus all BRCAX LCLs, were determined and the data visualised using hierarchical clustering. In addition, genes specific to mutation type were elucidated because missense and truncating mutations could also be separated for both BRCA1 and BRCA2. We used Support Vector Machines with Leave One Out cross-validation to determine if the mutation status of LCLs known to carry pathogenic mutations, comparing predictions using missense-associated, truncating-associated and truncating-specific gene lists. Accuracy of prediction was improved when the gene list used for prediction was appropriate to the mutation type (truncating or missense) being tested. Pathogenic truncating mutations of BRCA1 and BRCA2 were predicted with only 53% and 84% accuracy using missense-associated gene lists, but this increased to 77% and 84% using truncating-specific gene lists. Missense mutations of BRCA1 and BRCA2 were predicted with 100% accuracy using missense-associated lists, but with only 80% and 33% using truncating-specific gene lists. This study illustrates the potential of using gene expression profiling as a predictive tool to assess the likely pathogenicity of sequence variants of BRCA1 and BRCA2 of unknown clinical significance, but highlights the importance of using classifiers that take into account mutation type.

384/T

Downregulation of NEIL1 or NEIL2 induces mutator phenotype in mammalian cells. *A.K. Maiti[1], I. Boldogh[2], S. Mitra[1], T.K. Hazra[1].* 1) Biochemistry and Molecular bio, University of Texas Medical Branch, Galveston, TX.77555; 2) Department of Microbiology and Immunology, University of Texas Medical Branch, galveston, TX, 77555.

Oxidatively induced DNA lesions have been implicated in the etiology of many diseases including cancer and in aging. Repair of oxidatively damaged bases in all organisms occurs primarily via the DNA base excision repair (BER) pathway, initiated with their excision by DNA glycosylases. Among four mammalian oxidized base-specific DNA glycosylases, the recently characterized NEIL1 and NEIL2 are unique because of their preference for excising lesions from a DNA bubble, unlike the previously characterized OGG1 and NTH1, which are active only with duplex DNA. The preference of NEILs for bubble DNA substrates raised the possibility that they function in the repair of base lesions during replication and/or transcription. A lack of phenotype in OGG1/NTH1-null mice, and efficient repair of oxidized bases from the genomes of null mouse cells, suggests a critical role for the NEILs. To investigate the role of NEIL1 and NEIL2 in preventing endogenous mutations, we examined the consequences of their deficiency on the hprt locus in chinese hamster V79 cells. Here we show that antisense-mediated downregulation of NEIL1 and NEIL2 separately induced endogenous mutation by about 4 to 6 fold. The mutation frequency was further enhanced (~25 to 30-fold) under oxidative stress. We have analyzed the mutation spectrum by amplifying and sequencing the hprt locus. NEIL1-downregulated cells accumulated mutations mostly at the AT base pairs, on the other hand NEIL2-downregulated cells accumulated mutations mostly in the CG base pairs. Thus the NEILs appear to play distinct and important roles in maintaining the functional integrity of mammalian genomes. (Research supported by USPHS grants R01 CA102271, CA91063 and P01 ES06676).

385/T

Characterization of the Finnish prostate cancer susceptibility locus HPCX. *T. Wahlfors[1], H. Mattila[1], K. Ivori[1], K. Chang Sik[2], M. Vihinen[2], H. Oja[4], T. Tammela[3], T. Ikonen[1], J. Schleutker[1].* 1) Lab of Cancer Genetics,Inst of Medical Technology,Univ of Tampere and Tampere Univ Hospital, Tampere,Finland; 2) Bioinformatics,Inst of Medical Technology,Univ of Tampere, Tampere,Finland; 3) Division of Urology,Tampere Univ Hospital and Medical School,Tampere,Finland; 4) Tampere School of Public Health,Univ of Tampere,Tampere,Finland.

Prostate cancer is the most common male cancer in the Western world but its etiology is still unclear. While most of the cancer cases are sporadic, there is evidence suggesting that 42% of the cases have a hereditary component. A locus that has been shown to be important in the Finnish population is HPCX on Xq27-28. To date, the susceptibility gene has not been found because of the complex structure of the chromosomal region. We are applying the NMD microarray technique for the analysis of HPCX. In this technique the patient sample is compared to itself after inhibition of NMD. Microarrays are then used to identify nonsense transcripts that are increased in abundance after loss of NMD. Inactivation of tumor suppressor genes is a two-step process involving mutation of the target gene and loss of the other allele. In lymphoblastoid cell lines the other normal wild type allele can mask the effect of a germline mutation. Because males have one X-chromosome, there is only one allele of the X-chromosomal genes. Therefore, tumor suppressor genes may be caught using lymphoblastoid cell lines. Cell lines for analysis were selected from six affected and six healthy persons in HPCX linked families. mRNA was isolated from cells after treatment and the altered levels of mRNA expression were studied using Agilent 44K oligoarrays. In data analysis the limma package from Bioconductor was used for differential expression analysis using the linear models. First, ten genes were selected for direct sequencing and afterwards five genes more by using different linear model. So far the following genes have been sequenced:RBMX, CSAG2, RAP2C, SOX3, MBNL3, ZNF75, MAGEC1, MAGEA1,MAGEA11, MAGEC3, RAB39B, SUHW3, RAB9A and ZBTB33. No truncating mutations were found. However, a few interesting missense mutations were detected and these genes are now under further study.

386/T

QMPSF : A novel method for detection of 1p19q deletions in gliomas. *V. Paquis-Flucklinger[1, 2], S. Monnot[2], D. Fontaine[1, 3], F. Vandenbos[1, 4], P. Paquis[1, 3], J.F. Michiels[1, 4].* 1) UMR CNRS 6543, Medicine School, NICE cedex 2; 2) Department of Medical Genetics, CHU Nice; 3) Department of Neurosurgery, CHU Nice; 4) Department of Anatomopathology, CHU Nice.

Gliomas are the most common primary cerebral malignancies. Deletions of 1p and 19q chromosomes have shown to be predictors of chemotherapeutic response and better survival in oligodendrogliomas. Different techniques are available for the detection of these alterations including LOH, FISH and CGH. Despite good concordance exists in terms of sensitivity and specificity between these methods, all have specific limitations. The aim of our study was to describe a reliable novel technique for the detection of 1p/19q deletions in gliomas. For each chromosome arm (1p and 19q), we devised a multiplex PCR assay of fluorescent fragments corresponding to exons, which belong to genes located in the minimal deleted region. A control gene, located on chromosome 1q or 19p, is simultaneously amplified in each assay. PCR products are analysed on an automated sequencer and electropherograms generated from control and tumor samples are superimposed. We have searched for 1p/19q deletions by LOH and QMPSF (Quantitative Multiplex PCR of Short Fluorescent fragments) in a series of 50 patients with a glioma. We found that QMPSF, which does not require constitutional DNA, is a simple, rapid and reliable method to detect 1p/19q deletions. There was a good concordance with LOH data in (88% for 1p deletion and in 83% for 19q deletion). Furthermore, we show that QMPSF has a higher sensitivity than LOH and allows the detection of 1p/19q duplications. In conclusion, QMPSF can be routinely used in diagnosis laboratories for the detection of 1p/19q rearrangements in glial tumors.

387/T

The Cancer Genetics Telephone Clinic Model. *K. Myhill[1], S. Shanley[1], R. Doherty[1], A. Ardern-Jones[1], S. Hall[1], C. Vince[1], S. Thomas[1], P. Aspinall[2], R. Eeles[1].* 1) Genetics, The Royal Marsden NHS Foundation Trust, London, United Kingdom; 2) Univeristy of Kent, Kent, United Kingdom.

The Royal Marsden Genetics Unit conducted a pilot project to evaluate a telephone counselling service as opposed to face to face counselling which is the standard model of care in the UK. The study commenced in March 2004 and evaluation of the clinic was conducted over 17 months from March 2005 to the end of July 2006. A total of 612 patients had telephone consultations during this time, 228 of whom were referred from primary care with a median of 30 patients counselled per month (range of 19-63, depending on staff availability with average of two staff per clinic). Waiting times were measured for GP referrals and all 228 were counselled within the national target-stipulated 13 weeks (median 6 weeks, range 1-12). An additional 132 patients who were sent appointment letters after receipt of their family history questionnaires did not attend their appointments (18% of all potential referrals) and required re-contacting by letter. After telephone counselling, 42% of patients were able to be discharged from the telephone clinic without a subsequent face to face appointment, thereby saving resources. The telephone clinic also had a short set-up time with flexibility on timing and day of administration which would be an advantage in centres where outreach clinic facilities are scarce. The telelink telephone counselling model is highly efficient in triaging high risk individuals for face-to-face counselling as per the Kenilworth model, in effecting concentration of resources and in providing a flexible individual-centred approach to cancer genetic counselling delivery.

388/T

Robotic microscopy for detection and analysis of circulating tumor cells. *F. Ntouroupi[1], A. Seppo[2], S. Wang[2], Y. Kim[2], P. Tsipouras[2], F. Tafas[2], M.W. Kilpatrick[2], W.F. Bodmer[1].* 1) Cancer Research UK, Oxford, UK; 2) Ikonisys Inc, New Haven, CT.

Identification and analysis of rare circulating tumor cells has great potential; for detection of disease recurrence or minimal residual disease following treatment, or screening for malignancies. The challenge to the utilization of rare tumor cells diagnostically is to be able to accurately detect, quantify and analyse cells present in small numbers in a large, complex cellular background. We used the Ikoniscope robotic microscopy system, developed specifically for cell identification and analysis by automated fluorescence microscopy. Slide analysis is accomplished in a completely unattended manner, allowing analysis of samples for the presence of rare cells, avoiding complex purification procedures which risk loss of the cells being sought and can create unresolvable clusters of normal and cancer cells. Blood samples were collected from 12 colorectal cancer patients, 7 prostate cancer patients and 3 healthy controls. The mononuclear cells were immunostained with Cam5.2 directed against epithelial specific Cytokeratins 7/8 and either AUA1 (directed against EpCam) or anti-PSA, then analyzed on the robotic microscope. Potential tumor cells were identified at 10x and all identified targets verified at 100x magnification. All 12 colorectal cancer patients presented at least one EpCam/cytokeratin-positive cell with the morphological characteristics of tumor cells. On average 26 ± 53 immunoreactive cells were detected per 7.5 ml blood sample. The number of cells detected was different before and after surgical resection. Five out of seven prostate cancer patients presented EpCam/cytokeratin or PSA/cytokeratin-positive cells with the morphological characteristics of tumor cells. The number of cells ranged from 1.2 to 22.5 per ml blood. No immunoreactive cells were detected in the blood samples from healthy volunteers. This data supports the potential clinical utility of circulating cancer cell detection and analysis. By maintaining the integrity of the tumor cells detected, the approach has the added advantage of allowing the further analysis of both cell morphology and phenotype.

389/T

Fine-mapping of 42 hereditary prostate cancer families narrows the interval for a susceptibility locus on chromosome 22q12.3. *B. Johanneson[1], S.K. McDonnell[2], D.M. Karyadi[1], S.J. Hebbring[3], L. Wang[3], K. Deutsch[6], L. McIntosh[4], E.M. Kwon[1], M. Suuriniemi[1], J. Stanford[4,5], D.J. Schaid[2], E.A. Ostrander[1], S.N. Thibodeau[2].* 1) Institute, National Institutes of Health, Bethesda, MD 20892; 2) Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55905; 3) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905; 4) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Box 19024, Seattle, WA 98109; 5) School of Public Health and Community Medicine, University of Washington, Seattle, WA 98115; 6) Institute for Systems Biology, Seattle, WA 98103.

Genetic studies suggest that hereditary prostate cancer is a genetically heterogeneous disease with multiple contributing loci. Studies of high-risk prostate cancer families selected for aggressive disease, analysis of large multigenerational families, and a meta-analysis from the International Consortium for Prostate Cancer Genetics (ICPCG), all highlight chromosome 22q12.3 as a susceptibility locus. Our study is the most detailed fine-mapping analysis of this region to date. Of 173 high-risk families from the Mayo Clinic and 254 from the Prostate Cancer Genetic Research Study (PROGRESS), 42 were identified as having a shared haplotype among all affected men that overlapped the 22q12.3 region. In 35 of the families, an overlapping consensus region of 8.73 Mb is defined. However, in the subset of 14 families with ≥ 5 affected men per family, a 2.53 Mb shared consensus segment is identified in 12 of the families that overlaps with previously published intervals. Combining these results with data from the other published studies, a three-recombinant consensus interval is found in 52 of 54 families which narrows down the region to 1.36 Mb between 33.72 Mb and 35.08 Mb. Overall, our results provide the most comprehensive framework achievable for candidate gene testing. Ongoing studies are aimed at evaluating genes in this region for variants associated with prostate cancer risk.

390/T

A description of the first oncogenetic clinic for BRCA1/2 mutation carriers in London: The Carrier Clinic. *E. Bancroft[1], A. Ardern-Jones[1], K. McReynolds[1], S. Shanley[1], Z. Kote-Jarai[2], R. Eeles[1,2], Carrier Clinic Collaborators.* 1) Royal Marsden NHS Foundation Trust, London; 2) Institute of Cancer Research, London.

A specialist oncogenetic clinic was established in 1996 at the Royal Marsden NHS Foundation Trust for families harbouring mutations in *BRCA1* and *BRCA2* to offer expert advice and specialist follow-up. The remit of this multidisciplinary clinic is provide individualised screening recommendations, psychological support, cascade testing, risk reduction strategies and offers an extensive research portfolio. METHODS: We have performed a retrospective analysis on uptake of prophylactic surgery, uptake of *BRCA1/2* testing and cancer incidence in 347 families identified with *BRCA1/2* mutations between January 1996 and December 2006. A total of 661 individuals have attended this clinic and 406 mutation carriers identified (239 *BRCA1*, 165 *BRCA2* and 2 *BRCA1* and *BRCA2*). RESULTS: Out of 406 gene positive individuals 85.8% choose to attend the Carrier Clinic for annual follow-up. Out of 476 individuals eligible for a predictive test 411 have proceeded to testing. The incidence of prophylactic bilateral mastectomy (PBM) contralateral mastectomy (PCM) and oophorectomy (PBO) is as follows: 70.4% mutation carriers aged 40-70 chose PBO; 32.3% unaffected women chose PBM at a mean age of 37.4 years; 22% of women chose PCM at the time of a breast cancer diagnosis; 14.3% of unaffected women chose both PBM and PBO. In unaffected women the mean time to surgery post-test was 11 months for PBM and 12.5 months for PBO. 95.6% of individuals approached to take part in research are enrolled in at least one study. The number of families identified is increasing annually both through clinical and research testing. Cancer incidence amongst these individuals matches the incidence reported in the literature. CONCLUSION: The results indicate a high demand for both prophylactic surgery and genetic testing in women from *BRCA1/2* families. This clinic model has subsequently been adopted in other centres and this will facilitate translational studies in this group. The Carrier Clinic Collaborators: Locke I, Walker L, Barwell J, Mitchell G, Dorkins H, Thomas S, Doherty R, Lynch E, Mitra A, Javhar S, Izatt L, Pichert G.

391/T

International Variation in Rates of Uptake of Preventive Options in BRCA1 and BRCA2 Mutation Carriers. *K. Metcalfe[1,2], D. Birenbaum-Carmeli[3], J. Lubinski[4], J. Gronwald[4], H. Lynch[5], P. Moller[6], P. Ghadirian[7], W. Foulkes[8], E. Friedman[9], C. Kim-Sing[10], P. Ainsworth[11], B. Rosen[12], S. Domchek[13], T. Wagner[14], N. Tung[15], S. Manoukian[16], F. Couch[17], P. Sun[2], S. Narod[2].* 1) Faculty of Nursing, Univ Toronto, Toronto,Canada; 2) Women's College Research Institute, Toronto,Canada; 3) University of Haifa, Israel; 4) Pomeranian Medical University, Szczecin,Poland; 5) Creighton University School of Medicine, Omaha,USA; 6) Rikshospitalet-Radiumhospitalet Medical Centre, Oslo,Norway; 7) Centre Hospitalier de l'Universitaire Montréal,Canada; 8) McGill University, Montréal,Canada; 9) Tel-Aviv University, Israel; 10) British Columbia Cancer Agency Vancouver,Canada; 11) London Health Sciences Centre, London,Canada; 12) Princess Margaret Hospital, Toronto,Canada; 13) University of Pennsylvania, USA; 14) Medical University of Vienna and Private Trust for Breast Health, Austria; 15) Beth Israel Deaconess Medical Centre, Boston,USA; 16) Medical Genetics Service, Istituto Nazionale Tumori, Milan, Italy; 17) Mayo Clinic, Rochester, MN.

Objective: We report on preventive practices in women with mutations from 8 countries and examine differences in uptake by country. Methods: Women with a BRCA1/2 mutation were contacted and asked about cancer preventive practices. Results: 2365 women with a BRCA1 or BRCA2 mutation from 8 countries were included. The questionnaire was completed a mean of 4.0 years (range 1.5-10.3 years) after testing. 1390 women (59%) had a bilateral prophylactic oophorectomy. Of the 1198 women without breast cancer, 228 (19%) had had a prophylactic bilateral mastectomy. Among those who did not have a prophylactic mastectomy, only 49 women (5%) took tamoxifen for breast cancer prevention. Approximately half of the women at risk for breast cancer had taken no preventive option, and relied solely on screening. There were significant differences in the uptake of the preventive options by country. Conclusion: A minority of women with a BRCA1 or BRCA2 mutation opt for prophylactic mastectomy or take tamoxifen for the prevention of hereditary breast cancer. Approximately one-half of women at risk for breast cancer rely on screening alone.

392/T

High-resolution oligonucleotide array-CGH applied to large rearrangements in BRCA1, BRCA2, MLH1 and MSH2 genes. *E. Rouleau, C. Lefol, S. Tozlu, C. Andrieu, C. Guy, F. Copigny, C. Nogues, I. Bieche, R. Lidereau.* Oncogenetic laboratory, Centre René Huguenin, St Cloud , France.

Genetic pre-disposition to breast, ovarian, colorectal cancers results mainly from alterations in BRCA1, BRCA2, and MLH1, MSH2 genes. However, the identification of large rearrangements represented a major advance in our understanding of familial forms. Indeed, 10 to 15 percent of deleterious mutations in the BRCA1, MLH1 or MSH2 genes correspond to a large rearrangements. In a less extent, some has been reported for BRCA2 gene. Nowadays, large rearrangements are starting to be included in routine genetic screening. Many techniques are available, but few give a panoramic view of the gene of interest and none explores promoting regions. We have developed a new method for detecting and characterizing large rearrangements in the BRCA1, BRCA2, MLH1 and MSH2 genes, based on high-resolution oligonucleotide array-CGH technology. We designed specific CGH arrays for the four genes and their flanking regions. Prior to use this approach in routine, we analyzed thirteen DNA samples known to contain a large deletion or a large duplication in one of the four genes, previously detected with QMPSF, MLPA or real-time quantitative PCR. All the large rearrangements from single small exon to large region were detected by the array-CGH method. Their size was estimated to within 1-2 kb. When possible, the deleted or duplicated region was sequenced to identify the break point. The characterization of the break point enabled us to develop a simple PCR screening test for other family members, and to explore the founder effect in families with the same deleted or duplicated exons. The high probe density in the microarray method described here gives the opportunity to rapidly screen a group of genes involved in a specific cancer, for instance, breast and colorectal cancer. Despite its cost, this method can assist with the development of simple PCR-based genetic test for family members. It should help to detect large rearrangements affecting long exon or promoting regions missed by other current methods. Additional studies are needed to define the role of this technique in routine genetic testing.

393/T

Digital PCR and High Resolution Melting for the Discovery of Very Low Allele Fraction Somatic Mutations in Tumor Samples. *J.T. McKinney[1], M.D. Wall[1], L.L. Cutler[1], D. Ruddy[2], B. Gorbetcheva[2], J. Monahan[2], D.H.F. Teng[1].* 1) Research and Development, Idaho Technology, Inc., Salt Lake City, UT; 2) NIBR, Oncology, Boston, MA.

Digital PCR (dPCR) is a means to identify mutations in a minor cell population fraction (i.e. primary tumor tissue). By diluting DNA samples down to a single copy, it is possible to transform the analog nature of PCR into a linear, digital signal. We sought to apply High Resolution Melting (HRM) to a modified dPCR approach for defining low fraction variants identified by an abnormal melting profile in the primary tumor sample. Digital PCR requires that a single copy of DNA is used as starting template for amplification, thus endpoint detection becomes a digital readout rather than an analog admixture of the DNA. In order to apply HRM, a more robust amplification is required for a reliable melting profile, thus we chose a target dilution of 5 copies. This dilution target is based on the limitations of downstream sequencing being approximately 20% sensitive to detect low fraction variants. HRM is sensitive to 5% mutant allele fraction; therefore even a single copy of the mutant allele would be sufficient to detect a difference in the melting profile. A total of 14 samples representing known variants or suspected low fraction somatic mutations were assayed in 6 different cancer gene targets. For samples with known variants, low fraction mixtures were created at 5% and 2.5% of the minor allele. All samples were diluted to a concentration 5 copies and amplified in replicates of 24. Replicates that did not amplify robustly were excluded from the high resolution melting analysis, leaving only reproducible melting profiles to analyze. Replicates that displayed an abnormal profile were selected for sequencing confirmation. Low fraction somatic mutations were identified in 6 of 8 tumor samples, whereas all 6 samples with known variants were successfully identified in both the 5% and 2.5% dilutions. These results indicate that HRM as a pre-screen to a modified dPCR approach can significantly reduce the downstream sequencing effort of the traditional dPCR application for identifying low fraction variants.

394/T

Evaluation of whole genome amplification in tumor genome analysis. *W. Winckler[1,2], R. Onofrio[2], N. Burtt[2], C. Guiducci[2], R. Tewhey[2], K. Ardlie[2], M. Meyerson[1,2], S. Gabriel[2].* 1) Dept of Medical Oncology, Dana Farber Cancer Institute, Boston, MA; 2) The Broad Institute of Harvard and MIT, Cambridge, MA.

Large-scale cancer genomics often requires more DNA than can be obtained from a single tumor sample. Many studies on inherited variation have dealt with similar sample limitations by using whole genome amplified (WGA) DNA. It is unclear, however, whether this amplification process performs equally well in tumors, which have added complexities like aneuploidy and stromal contamination. To evaluate WGA for tumor analysis, we compared genomic and phi29 polymerase multiple strand displacement WGA DNA by Affymetrix 250K Sty SNP array and Sanger sequencing.

Unamplified and WGA DNA from 30 tumors and 17 normals were evaluated for copy number using SNP arrays. Amplified samples had consistently poorer copy quality metrics than their unamplified equivalents. Additionally, we detected regions with reduced copy number in all WGA samples but not in unamplified ones, affecting 4% of the SNPs on the 250K Sty chip. This was particularly pronounced at the telomeres. These results indicate that phi29 WGA is inferior for copy number analysis by SNP array.

To address the effect of WGA on sequencing, we sequenced PIK3CA and TP53 in 12 breast and 12 colorectal carcinomas, including the unamplified tumor and two independent WGAs of the tumor. Each of the 8 mutations observed in the unamplified tumor was also seen in WGA DNA, indicating that there is unlikely to be a substantial false negative problem with sequencing WGA DNA in tumors. No new mutations were seen in the amplified material, suggesting that the WGA process is not introducing de novo mutations at a high rate. Also, we found that 13 of the 1,000 genes studied by the Tumor Sequencing Project, which used WGA DNA to sequence 190 lung adenocarcinomas, occur in our identified regions of low WGA copy number. 24 somatic and 34 germline mutations were discovered in these genes, further indicating that mutations can be detected using WGA material-even in the regions that performed poorly on SNP array.

395/T

Novel mutations in BHD and expansion of the spectrum of phenotypes in 50 new families with Birt-Hogg-Dubé Syndrome. *J. Toro[1], M. Weinreich[1], G.M. Glenn[1], O. Toure[1], P. Pinto[2], M. Merino[3], M. Turner[4], S.M. Steinberg[5], P. Choyke[6], L.S. Schmidt[2,7], M.H. Wei[1], W.M. Linehan[2].* 1) Genetic Epidemiology Branch, DCEG; 2) Urologic Oncology Branch, CCR; 3) Laboratory of Pathology, CCR; 4) Dermatology Branch, CCR; 5) Biostatistics and Data Management Section, CCR; 6) Department of Diagnostic Radiology, CCR, NCI, Bethesda, MD; 7) Basic Research Program, SAIC-Frederick Inc., Frederick, MD 21702.

Birt-Hogg- Dubé syndrome (BHDS) (OMIM #135150) is an autosomal dominant predisposition to the development of follicular hamartomas (fibrofolliculomas), lung cysts, spontaneous pneumothorax, and kidney neoplasms. In this study we characterize the BHD gene mutation spectrum and phenotypes of 50 new families with BHDS. Patients had a medical exam and computed tomography (CT) scans of the chest and abdomen to screen for pulmonary abnormalities and renal tumors. We performed a dermatologic evaluation to screen for cutaneous fibrofolliculomas and other skin lesions. Bidirectional DNA sequencing was used to screen for mutations in the BHD gene. Insertion and deletion mutations were confirmed by subcloning. Genotype-phenotype correlations were investigated. The mutation detection rate was 88% (51/58) in the BHDS families tested. Mutations were distributed across coding exons (4-13) except exon 8 and 10. Of the 26 different germline mutations identified, 18 were novel consisting of: nine deletions/insertions, four splice site mutations, three missense and two nonsense. Three unrelated families carried a putative splice-site mutation in the BHD gene within intron 7 (IVS7+1 G→T) and 6/8 of the family members had renal tumors. Thirty-five percent of families presenting with histologically confirmed FFs had kidney tumors. Fifty-one percent (26/51) of families had individuals with a history of pneumothorax and 88%(45/51) of families had lung cysts on CT scans. Ninety percent (46/51) of the families had individuals with histologically confirmed fibrofolliculomas. Missense BHD gene mutations are present in families BHDS expanding the spectrum of mutations associated with BHDS. BHDS is characterized by a variable expression of phenotypes among and within families.

396/T

Vascular endothelial growth factor gene polymorphisms in Taiwanese women with cervical squamous cell carcinoma. *T.Y. Chang[1], Y.C. Yang[1,2,4], Y.J. Lee[1,3,5], T.H. Su[2,4], W.F. Chen[1], H.W. Chan[1], H.F. Liu[1], C.C. Chu[1], M. Lin[1].* 1) Medical Research Department, Mackay Memorial Hospital, Taipei, Taiwan; 2) Department of Gynecology and Obstetrics, Mackay Memorial Hospital, Taipei, Taiwan; 3) Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan; 4) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 5) College of Medicine, Taipei Medical University, Taipei, Taiwan.

Human papillomavirus (HPV) is considered to be a necessary but not sufficient cause for cervical cancer. Genetic background might partake in the risk and development of cervical cancer. Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis that has been associated with many human malignancies including carcinoma of uterine cervix. The aim of this study is to evaluate the role of the functional polymorphisms of this gene as genetic markers for cervical squamous cell carcinoma (CSCC) susceptibility. The -2578 A/C, -634 G/C, and +936 C/T polymorphisms were genotyped in 141 CSCC patients and 378 age-matched healthy controls by TaqMan allelic discrimination assay. The presence and genotypes of HPV in CSCC patients were determined by PCR. We found no significant association between the polymorphisms or haplotypes and CSCC. Stratified by the positivity of HPV-16 infection also did not find marked association. Our findings provide no support for the hypothesis that VEGF polymorphisms are associated with increased risk for CSCC in the Taiwanese population.

397/T

Protein profile analysis and associated genes in laryngeal cancer treated by hypomethylation agent 5aza2dc. *Y. Guo[1], J. Li[1], Z.M Xu[2], K.L. Sun[1], W.N. Fu[1].* 1) Medical Genetics, China Medical University, Shenyang; 2) 463 Hospital of PLA, Shenyang, 110042, China.

The demethylating drug 5-aza-2'-deoxycytidine has been shown to affect many genes expression in several kinds of cancers. Proein profiles from Hep-2 cells treated with 2μm 5aza2dc for three days were obtained by two dimensional gel elctrophoresis. Several Differentially expressed protein spots were cut off and analyzed by mass spectrometry. They were S100 calcium-binding protein A4, proteasome (prosome, macropain) subunit, alpha type 5, neuropolypeptide h3, phosphoglycerate mutase 1 (brain), Chain A, 14-3-3 Protein Epsilon Complexed to Peptide, stathmin 1 and enolase 1, respectively. In conclusion, we established the protein profiles related to 5aza2dc in Hep-2 cells. The seven differentially expressed proteins identified provide us novel targets for further studying the molecular mechanisms of laryngeal carcinoma.

398/T

RNA-based mutation analysis identifies an unusual MSH6 splicing defect and circumvents PMS2 pseudogene interference. *K. Wimmer[1], J. Etzler[1], A. Zatkova[1], A. Peyrl[2], H.-U. Schildhaus[3], A. Ficek[4], C. Kratz[5], L. Messiaen[6], I. Slavc[2], C. Fonatsch[1].* 1) Department for Medical Genetics, Medical University Vienna, Vienna, Austria; 2) Department of Pediatrics and Adolescent Medicine, Medical University Vienna, Vienna, Austria; 3) Institute of Pathology, University Bonn, Bonn, Germany; 4) Department of Molecular Biology,Comenius University, Bratislava, Slovakia; 5) Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany; 6) Department of Genetics, University of Alabama at Birmingham, AL.

Recent reports provide evidence for a novel recessively inherited cancer syndrome that is characterized by early-onset malignancies and signs of neurofibromatosis type 1 (NF1). Biallelic mutations in one of the mismatch-repair- (MMR-) genes MLH1, MSH2, MSH6 and PMS2 were identified as the underlying genetic alteration in almost 30 children of 15 families with brain and/or hematological malignancies presenting also with café-au-lait spots (CLS) or other NF1 symptoms. Blood samples of two families with children suspected to suffer from this syndrome were sent to our laboratory for genetic testing. We established a RNA-based mutation detection assay for the four MMR-genes, since (i) a number of splicing defects may escape detection by the analysis of genomic DNA and (ii) DNA-based mutation detection in the PMS2 gene is severely hampered by the presence of multiple highly similar pseudogenes. Using this assay that is based on direct cDNA sequencing of RT-PCR products we identified a complex MSH6 splicing alteration in the first family and a novel PMS2 mutation in the second family. We demonstrate that RNA-based PMS2 testing effectively avoids pseudogene co-amplification as well as allelic drop-out. With this report we show that reliable and highly sensitive mutation analysis in all four MMR-genes including PMS2 is possible using a RNA-based approach. Secondly, we bring further attention to the accumulating evidence that recessive alleles in the MMR-genes, particularly PMS2, cause early-onset malignancies in children with clinical signs resembling NF1.

399/T

The psychological impact of abnormal results in high-risk breast MRI screening. *S.M. O'Neill[1,2], W.S. Rubinstein[1,2], S.F. Sener[1,2], D.K. West[1], D.B. Ekanow[1], A.W. Rademaker[2], R.R. Edelman[1,2].* 1) Evanston NW Healthcare, Evanston, IL; 2) Northwestern University Feinberg School of Medicine, Chicago, IL.

Breast MRI has been shown to be an effective screening tool for women at high risk for breast cancer. Recent guidelines published by the American Cancer Society recommend annual breast MRI screening for BRCA, PTEN, and p53 mutation carriers, their untested close female relatives, and women with a family-history-based lifetime statistical risk for breast cancer of 20-25%. However, there is widespread concern that false positive MRI results may have a persistent negative psychological effect, as has been shown repeatedly in mammography screening studies.

We measured psychological stress prior to MRI in 103 high-risk women enrolled in a longitudinal screening study, assessed change in Impact of Event Scale (IES) scores in women who had more than one MRI, (n=68) and compared women who had normal results on their previous MRI (n=32) with the group that had results which prompted recall (n=34). In a two year period 189 MRI scans were performed, of which 64 (34%) required further evaluation because of BIRADs scores ≥ 3. The recall follow-up included biopsy (n=4; 2 had breast cancer), ultrasound (n=20), mammogram (n=5), and 6-month interval MRI (n=40). When the group began MRI screening the mean IES score was 14.6 with 22.3% having clinically meaningful stress. At the time of second MRI, mean IES score was 16.3. Intrusion decreased (6.4), but mean Avoidance was significantly increased (9.9, p=0.025). Between-group comparisons revealed that the overall increase in mean Avoidance at MRI 2 was driven by the group that had recall results on MRI 1 (p=0.029), not the group with previously normal results.

Although the women with previous false positive results had an increase in cognitive avoidance symptoms, they nevertheless reported for another scan. Cognitive avoidance in this high-risk subset of women may be a developed coping strategy that does not impair compliance with screening.

400/F

Association of 1494del6 polymorphism of Thymidylate Synthase gene (TYMS) in colorectal cancer of Mexican population. *V. Peralta[1], G. Morgan[2], M.P. Gallegos[1].* 1) Medicina Molecular, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Guadalajara, Mexico; 2) Departamento de Radiodiagnostico, UMAE, CMNO, IMSS.

The polymorphism 1494del6 of Thymidylate Synthase gene (TYMS) has been associated with decreased levels of mRNA in patients with colon cancer. In this study has been determined the frequency 1494del6 polymorphism of the TYMS gene and it association with colon cancer in patients and controls of Mexican population. DNA of 317 patients and 200 controls was extracted. PCR fragment of 152pb was amplified and were digested with DraI, the products were separated on a 8% polyacrylamide gel. The genotypic frequency in patients and controls for the wild-type genotype was of 56% and 46%; heterozygotes of 27% and 43%; and homozygous was 17% and 11% respectively. When the statistical analisis were performance, not significant differences was observed ($p<0.05$). However, when was compared both groups by sex distribution, we observed that female gender showed association with genotype 1494del6, with OR 2.16(95% CI;1.07-4.53). The polymorphism 1494del6 of TYMS gene was associated with female colon cancer.

401/F

Breast cancer risk associated with genotype val/val polymorphism of the estrogen metabolizing gene CYP1A1 in Mexican population. *AM. Puebla[1,3], D. Ontiveros[2], M. Gallegos[1].* 1) Medicina Molecular, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Guadalajara, Mexico; 2) Unidad Médica de alta Especialidad, Hospital de Gineco-obstetricia, Fisiología Obstétrica CMNO, IMSS. Laboratorio de Inmunofarmacología Experimental, CUCEI, Universidad de Guadalajara. Guadalajara, Jal., México; 3) Laboratorio de Inmunofarmacología Experimental, CUCEI, Universidad de Guadalajara. Guadalajara, Jal., México.

Estrogen has been proposed to trigger breast cancer development via an initiating mechanism involving its metabolite, catechol estrogen . We conducted a case-control study to determine whether polymorphism iso/val of CYP1A1 gene that participated in metabolism of estrogen biosynthesis is associated with an elevated risk for breast cancer in Mexican population. In 243 breast cancer patients and 243 healthy controls were determine the genotypes of iso/val CYP1A1 polymorphism. The val/val genotype in breast cancer was 12% and in the control group of 6% ($p<0.05$); the risk factors of breast cancer was associated with a 2.22 fold increase in risk (95% confidence interval, 1.07-4.83). On the basis of comprehensive profiles of estrogen metabolism, this study supports the possibility that polymorphism iso/val of CYP1A1 gene in breast cancer can be play a role in metabolism of estrogen biosynthesis.

402/F

Deletions of the MEN1 gene are more common than previously thought. *P.J. Bridge[1], T.L. Gillan[1], M.E. Phillips[1], D. Gilchrist[2], A.M. Innes[1], J.S. Parboosingh[1].* 1) Molecular Diagnostic Laboratory, Alberta Children's Hospital, Calgary, AB, Canada; 2) Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada.

Multiple endocrine neoplasia type I (MEN1) is an AD inherited cancer syndrome characterized by multiple endocrine tumours in the parathyroid, pituitary and gastro-entero-pancreatic tract. Clinical diagnosis of MEN1 is made by the presence of at least two MEN1-related tumours. MEN1 is associated with germline mutations in the MEN1 tumour suppressor gene located on chromosome 11q13. To date over 400 MEN1 mutations have been described, the majority of which result in premature protein truncation due to frameshift, nonsense or splice site mutations. MEN1 mutations are detected in 80-90% of probands with a positive family history. No genotype/phenotype correlations have been observed although considerable effort has been made. Mutations in the MEN1 gene have also been identified in sporadic MEN1-associated tumours. In the Molecular Diagnostic Laboratory (MDL) we perform a comprehensive screen of the MEN1 gene using sequence analysis and the more recently implemented MLPA analysis. With this combined approach, we detect at least 95% of MEN1 germline mutations segregating in families. Eighty-seven families have been submitted for mutation screening; all patients had either a personal or family history of MEN1-associated symptoms. The first forty probands of putative MEN1 families were screened by sequencing alone while 47 probands were screened by both sequencing and MLPA analysis. Pathogenic mutations were identified in nineteen families and consisted of: 5 nonsense mutations, 3 missense mutations, 2 splice site mutations, 5 small deletions, 1 insertion and 3 large deletions. Interestingly, since the implementation of MLPA in spring 2006, the presence of three large deletions segregating in MEN1 families suggests that large deletions of the MEN1 gene may be more common than originally reported. In our sample of 19 MEN1 mutation positives, based on clinical and pedigree information provided with the requisition, they were equally likely to have a positive family history or a negative family history.

403/F

Evaluation of ODC1 genotype as a modifier of adenoma number in attenuated FAP. *M.W. Condie[1], K.M. Boucher[1, 2], R.W. Burt[1, 3], D.W. Neklason[1, 2].* 1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Department of Oncological Sciences University of Utah, Salt Lake City, UT; 3) Depatment of Medicine University of Utah, Salt Lake City, UT.

Mutations in the APC gene result in increased expression of c-Myc which activates genes involved in proliferation including Ornithine Decarboxylase (ODC1). ODC has been implicated in epithelial cancers including colon. The SNP rs2302615 (G/A) in intron 1 of ODC1 resides between two Myc/Max binding sites (E-box). c-Myc results in increased transription of the A allele ODC1 compared with the G allele. Furthermore, the competitor of c-Myc, Mad1, represses the A allele more than the G allele. Recent reports describe that individuals homozygous for the A allele have reduced prevalence of precancerous colonic adenomas and their polyps are further reduced by NSAID usage. We tested the hypothesis that this ODC polymorphism could be a genetic modifier, influencing the number of adenomatous polyps in individuals with a germline mutation in APC. Penetrance of the adenomatous polyps is highly variable in attenuated familial adenomatous polyposis (AFAP) even with the identical underlying genetic mutation. Associations between adenoma number (set as tertiles of <7, 7-43, and >43 adenomas) and age, gender or ODC genotype were evaluated using ordered multinomial regression analysis under a multivariate model in 161 individuals with the AFAP founder mutation APC c.426_427delAT. Advancing age and male gender correlated statistically with more adenomas. However, there was no statistical difference between ODC genotypes and number of adenomas. NSAID usage was also evaluated in 79 individuals, and there was no interaction between NSAID usage and ODC genotype. ODC genotype has been reported to also have a more significant effect on advanced adenomas. Based on our data, it is likely that polyp initiation is influenced by factors other than ODC in AFAP. The possibility that the ODC genotype may influence development of advanced adenomas or cancer in both AFAP and FAP remains to be evaluated.

404/F

Occurrence of germline *PALB2* mutations in ovarian cancer. *H. Erkko[1], J. Nikkilä[1], R. Bützow[2], K. Pylkäs[1], S.M. Karppinen[1], M. Reiman[1], B. Xia[3], D.M. Livingston[3], R. Wingvist[1].* 1) Dep.of Clinical Genetics, University of Oulu and Oulu University Hospital, Oulu, Finland; 2) Division of Pathology, Helsinki University Hospital, Helsinki, Finland; 3) Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA.

BRCA1 and *BRCA2* are the two major genes involved in hereditary predisposition to breast and ovarian cancer. Recently a novel BRCA2 interacting protein PALB2 (partner and localizer of BRCA2) was discovered and found crucial for the DNA damage response functions of BRCA2 (Xia et al. 2006). We have identified a relatively common heterozygous germline mutation c.1592delT in the *PALB2* gene conferring an approximately fourfold increase in the risk of developing breast cancer (Erkko et al. 2007). In the current study we wanted to investigate whether this protein truncating founder mutation is also associated with an increased risk of developing ovarian cancer, the other major phenotype of *BRCA2* mutation carriers besides breast cancer. Consequently, DNA samples of 593 unselected ovarian cancer cases were screened by conformation sensitive gel electrophoresis (CSGE) and direct sequencing for the presence of *PALB2* c.1592delT, and the results were compared to that of 2501 cancer-free controls (used previously in Erkko et al. 2007). Three mutation-positive ovarian cancer patients were identified. The frequency of carriers among ovarian cancer patients was approximately twofold higher than in the controls, but the difference was not statistically significant ($p=0.4$). Based on these results, and in contrast to the situation with *BRCA2* the relative contribution of *PALB2* mutations in ovarian cancer appears to be very limited.

405/F

BRCA1:5382insC in Individuals of Eastern European Heritage. *D. Gilchrist.* Medical Genetics, Univ Alberta, Edmonton, AB, Canada T6G 2H7.

Introduction: In 10 years of BRCA testing, our most common mutation is BRCA1:5382insC. This is commonly identified as an Ashkenazi mutation. The prevalence of the three common AJ mutations is: 0.8-1.1% for BRCA1:185delAG, 0.9-1.5% for BRCA2:6174delT and 0.13-0.3% for BRCA1:5382insC.

Method: We reviewed all charts with mutations in BRCA1:5382insC, BRCA1:185delAG and BRCA2:6174delT for geographic/ethnic heritage. We then compared our results to the total number of index cases, cases with an identified mutation, and demographic information for Edmonton.

Results: 568 index cases were clinically selected for high likelihood of HBOC. Of these, 110 had a BRCA1 or BRCA2 mutation. There were 24 BRCA1:5382insC, 3 BRCA1:185delAG, and 1 BRCA2:6174delT mutations. Of the latter four, three gave a clear history of Ashkenazi heritage. The remaining 185delAG family did not have heritage recorded on the chart. Of the 24 BRCA1:5382insC mutations (19% of the total), NONE were Ashkenazi in heritage.

The 5382insC families gave a heritage of: Ukrainian (11), German (7), Russian (2) and one each of Polish, Dutch, Croatian and Estonian. Publications from Poland, Ukraine, Russia, Latvia and Germany have reported BRCA1:5382insC to be common in their non-Jewish nationals.

The population of Edmonton is approximately one million. Over half of this population identify themselves as United Kingdom in origin. Over 30% identify themselves as being from Germany or the Ukraine. There are about 10,000 individuals of Ashkenazi heritage in the area. Referrals to our clinic roughly parallel city populations.

Conclusion: BRCA1:5382insC is not only a common Jewish mutation. Rather it is a common mutation from Eastern Europe - particularly the areas of Ukraine, Eastern Germany, Western Russia, Poland and the Baltic states. BRCA1:5382insC should be considered at high likelihood in a patient from these areas.

406/F

The importance of updating the family cancer history: Longitudinal risk assessments of 2508 breast cancer survivors. *L. Madlensky, WHEL Study Group.* Moores UCSD Cancer Center, UC San Diego, San Diego, CA.

BACKGROUND: Eligibility for cancer syndrome genetic testing is largely based on family history criteria. Over time, pedigrees change as family members are diagnosed with new cancers. We sought to quantify the change in the proportion of breast cancer patients eligible for BRCA testing over a period of time.

METHOD: Data were obtained from the WHEL study, a randomized trial of diet in breast cancer survivors. Detailed cancer family histories were taken at baseline and again at study exit (mean 7.5 yrs later, range 5-11 yrs). We classified women as "eligible" if they had at least a 10% risk of a BRCA1/2 mutation according to the Myriad prevalence tables. At study exit, we also asked women to self-report whether they ever heard of BRCA testing, and if they had ever had testing.

RESULTS: A total of 2508 women provided both baseline and exit data. At baseline, 206 (8.2%) were classified as eligible for BRCA testing. At study exit, an additional 150 women (6%) were eligible. Of those eligible at baseline, 39 (18.9%) experienced new breast cancer diagnoses (<age 50) or ovarian cancers in their families vs. 309 women (13.4%) who were not initially eligible at baseline (p=0.028). At study exit, 15% of those eligible had never heard of BRCA1/2 testing while 25% of those not eligible had not heard of testing (p<0.001). Of those who had heard of testing, 24% of those eligible vs. 7% of those not eligible reported undergoing testing (p<0.001).

CONCLUSIONS: Our data represent the largest report to date of changes in cancer family history over time. As these data were not obtained from high-risk genetics clinics, the results are relatively applicable to a community sample. However, our clinical trial sample was predominantly White and highly educated limiting broad generalization. The number of breast cancer survivors with "high risk" family histories nearly doubled over a relatively short period of time, underscoring the importance of updating the cancer family history in clinical practice.

407/F

Residual genetic effects beyond germline p53 mutations in Li-Fraumeni syndrome. *C.C. Wu[1], S. Shete[1], J. Ma[1], C.I. Amos[1], L.C. Strong[2].* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Dept Cancer Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX.

Germline p53 mutations have been identified in many families with Li-Fraumeni syndrome (LFS). Even in the presence of a stable germline p53 mutation segregating in a family, the age of cancer onset and multiplicity of tumors is highly variable, suggesting the presence of additional genetic effects. In a preceding study, we identified a sex difference in cancer risk in LFS patients with germline p53 mutations (Cancer Research, 66(16): 8287-92, 2006). Here, we investigate the residual genetic basis of risk beyond germline p53 mutations on LFS that might account for the observed genetic and phenotypic heterogeneity of LFS. We investigated 6 pedigrees with hereditary germline p53 mutations from a series of families described previously and analyzed with a single combined phenotype of invasive cancer (excluding nonmelanoma skin cancer and in situ carcinoma). In those kindreds, a total of 62 germline p53 mutation carriers have been identified. To determine the hypothetical genetic model for any unobserved non-p53 gene(s) or cancer risk modifier(s) in the presence of germline p53 mutations, we applied a complex segregation analysis based on Cox proportional hazards model and a Bayesian Monte Carlo Markov chain (MCMC) method implemented in the G.A.P. and Loki programs, respectively. The statistical approaches allowed us to associate the simultaneous effects of germline p53 mutations and unobserved gene(s) underlying LFS with cancer incidence in these families. Our findings from the segregation analysis showed that the plausible genetic models allowing for interaction between an unmeasured major gene, p53, and sex significantly improve the corresponding models with no interaction term, thus providing strong evidence for at least one modifier locus interacting with germline p53 and sex. In the MCMC analysis, we found that one or two susceptibility genes in addition to germline p53 mutations contribute to the variance in age of cancer onset in LFS.

408/F

Frequency of m1 polymorphism (CYP1A1) in adult Mexican patients with acute lymphoblastic leukemia. *C. Batista, M.P. Gallegos, G.M. Zúñiga.* Medicina Molecular, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Guadalajara, Mexico.

The etiology of most types of leukemia remains unknown. Acute lymphoblastic leukemia (ALL) is a heterogeneous disease characterized by the predominance of lymphoblasts or immature haematopoietic precursors, in which the malignant cells express diverse phenotypes with variable response to chemotherapy. Polymorphisms in genes encoding (activate procarcinogens and detoxify carcinogens) metabolism enzymes may be relevant for susceptibility to acute lymphoblastic leukaemia. We evaluated the distribution of CYP1A1 (m1) genotype in peripheral blood DNA samples from 227 healthy controls and 96 adult patients with ALL. The frequency of CYP1A1 (m1) wild type genotype was 53% (120 of 227) for controls and 29% (15 of 51) for ALL patients; heterozygote genotype was 39% (89 of 227) and 45% (23 of 51); and polymorphic genotype was 8% (18 of 227) and 26% (13 of 51) respectively with odds ratio of 4.0; 95% CI, 1.6-9.3 and p<0.05. Our observations suggest a possible interactions of CYP1A1 (m1) polymorphism to the risk of developing ALL in adults Mexican patients.

409/F

CYP1B1 variants are associated with prostate cancer in non-Hispanic and Hispanic Caucasians. *J. Beuten[1], J.J. Byrne[1], I. Balic[2], T.L. Johnson-Pais[3], I.M. Thompson[4], R.J. Leach[1,3,4].* 1) Department of Cellular & Structural Biology; 2) Department of Psychiatry; 3) Department of Pediatrics; 4) Department of Urology, UTHSCSA, San Antonio, TX.

Cytochrome P4501B1 (CYP1B1) is involved in the activation of many carcinogens and in the metabolism of steroid hormones. To test whether genetic polymorphisms within the CYP1B1 gene are associated with risk for prostate cancer (PCa) we compared allele, genotype, and haplotype frequencies of 7 single nucleotide polymorphisms (SNPs) within CYP1B1 among non-Hispanic Caucasians (482 cases, 501 controls) and Hispanic Caucasians (148 cases, 240 controls). When each of the SNPs were analyzed separately, significant differences were observed for allele frequencies between Hispanic Caucasian cases and controls for 366G/T(A119S) (p=0.026). In this group the TT genotype for 366G/T(A119S) increased the risk for PCa significantly (OR = 2.72, p = 0.004, 95%CI = 1.39-5.32). Moreover, a common G-C-C-C-G-G-A haplotype (frequency of 22.3%) for -1001C/T- -263G/A- -13C/T-142C/G(R48G)-355G/T(A119S)-4326C/G(L432V)-4390A/G(N453S) was found to occur more frequently in controls, as compared to cases in the Hispanic Caucasian samples (p = 0.04). Among non-Hispanic Caucasian men with more aggressive prostate cancer, variants -1001C/T, -263G/A, -13C/T, and 142C/G(R48G) were associated with the disease status: carrying one or both of the respective minor alleles at each variant resulted in a 1.7-3.2 fold increase among men with more aggressive PCa (p = 0.004-0.02). Furthermore, a common A-T-T-G-C-A haplotype (frequency of 27.6%) for -1001C/T- -263G/A- -13C/T-142C/G(R48G)-4326C/G(L432V)-4390A/G(N453S) was positively associated with high aggressive disease status (i.e., Gleason score greater than or equal to 7) among non-Hispanic Caucasian men (p = 0.0007). Our findings suggest that genetic polymorphisms in CYP1B1 may modify the risk for PCa and support the role of CYP1B1 as a candidate gene for prostate cancer.

410/F

The UGT2B17 gene deletion polymorphism and risk of prostate cancer: A case-control study in Caucasians. *C.J. Gallagher[1], F.F. Kadlubar[3,4], J.E. Muscat[1], C.B. Ambrosone[2], N.P. Lang[3,5], P. Lazarus[1].* 1) Health Evaluation Sciences, Pharmacology, and the Penn State Cancer Institute, Penn State College Medicine, Hershey, PA; 2) Epidemiology, Roswell Park Cancer Institute, Buffalo, NY; 3) Surgery and Epidemiology, University of Arkansas for Medical Sciences, Little Rock, AR; 4) Pharmacogenomics and Molecular Epidemiology, National Center for Toxicological Research, Jefferson, AR; 5) Central Arkansas Veterans Healthcare System, Little Rock, AR.

UDP-glucuronosyltransferase (UGT) 2B17 is a phase II metabolizing enzyme that mediates the glucuronidation of androgens and is expressed in the prostate. Variations in androgen levels have been suggested as a risk factor for prostate cancer, but results are inconsistent. Polymorphic variants in androgen metabolizing enzymes may alter androgen levels and therefore affect risk for prostate cancer. A deletion polymorphism in the UGT2B17 gene is associated with a substantial reduction in glucuronidation activity in vitro. We examined the association between the UGT2B17 deletion and the risk of incident prostate cancer in a population-based study from central Arkansas that included 411 Caucasian cases and 397 Caucasian controls. We developed a high-throughput procedure that uses real-time PCR and allelic discrimination for genotyping analysis. There was no significant difference in the prevalence of the UGT2B17 deletion genotype between prostate cancer cases (10%) and controls (12%). The odds ratio (OR), adjusted for age, smoking, and family history of prostate cancer, was not significant when comparing deletion homozygote subjects (0/0) (OR=0.89, 95% CI 0.55-1.45) or heterozygote subjects (+/0) (OR=0.99, 95% CI 0.73-1.35) to wild type subjects (+/+). There was also no association with prostate cancer risk when collapsing genotypes. These findings suggest that the UGT2B17 deletion is not associated with prostate cancer risk in Caucasians.

411/F

Common polymorphisms in the BRCA1 and BRCA2 genes are not associated with breast cancer risk. *J. Long[1], X.O. Shu[1], Q. Cai[1], Y. Gao[2], W. Zheng[1].* 1) General Internal Medicine, Vanderbilt Univ, Nashville, TN; 2) Department of Epidemiology, Shanghai Cancer Institute, Shanghai, 200032, China.

It is well established that rare mutations in the BRCA1 and BRCA2 genes increase the risk of breast cancer; however, it is not clear whether common polymorphisms in these two genes are associated with breast cancer risk. Using 1,079 cases and 1,082 controls from The Shanghai Breast Cancer Study, a population-based case-control study, we performed a comprehensive association study for these two genes. Tagging SNPs were identified through HapMap Chinese data with criteria of minor allele frequency (MAF) of ≥0.05 and r2≥0.9 for both genes plus their flanking 5kb sequences. Potential functional SNPs including those located in promoter genes or those non-synonymous SNPs with MAF≥0.05 in Asian were forced into tagging list. A total of 9 SNPs in the BRCA1 gene and 32 SNPs in the BRCA2 genes were successfully genotyped, using Affymetrix ParAllele Target genotyping system. Two SNPs, one for each gene, were dropped for association analyses because they were not polymorphic in our study population. No significant associations were observed for the other 39 SNPs through dominant, additive, or recessive model analyses. Haplotype analyses did not show any association for either the BRCA1 or BRCA2 gene. Analysis stratified by menopause status found the same null association results. CONCLUSION: It is unlikely any other common polymorphisms in these two genes are associated with increased risk of breast cancer in our study population.

412/F

Association of Tagging SNPs in *MLH1* with Prostate Cancer Susceptibility in Men of European Ancestry. *P. Pal[1], H. Xi[1], S. Guha[1], G. Sun[1], S. Indugula[1], J. Meeks[2], S. Thaxton[2], J. Mallik[1], H. Cheng[1], B.K. Suarez[3], W.J. Catalona[2], R. Deka[1].* 1) Center for Genome Information, Univ Cincinnati, Cincinnati, OH; 2) Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, IL; 3) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO.

Prostate cancer (PCa) is the most commonly diagnosed visceral malignancy and the second leading cause of cancer deaths in men in the United States. There is strong evidence that genetic factors are involved in PCa susceptibility. Mismatch repair genes play a major role in maintaining DNA integrity and are implicated in the etiology of various cancers including PCa. We have tested association of tagging SNPs (tagSNP) in *MLH1*, a known tumor suppressor and member of mismatch repair gene-family, with PCa susceptibility in men of European descent. Seven tagging SNPs were selected from the HapMap database and analyzed in a sample of 596 histologically diagnosed PCa cases and 567 ethnicity matched controls. All of the typed SNPs conform to Hardy-Weinberg expectations. We found significant allelic frequency differences between cases and controls in four of the tagSNPs (P ~ 0.046 to <0.0001). Haplotype analysis revealed the second most common haplotype encompassing all 7 tagSNPs to be significantly associated with PCa susceptibility (18% in cases vs 23% in controls; P = 0.018). Structure analyses negate the evidence of population stratification which could have confounded the association. These results indicate a primary role of *MLH1* variants in prostate carcinogenesis in men of European descent.

413/F

Polymorphism in the IL18 gene and risk of epithelial ovarian cancer in Caucasian women. *R.T. Palmieri[1], M.A. Wilson[2], E.S. Iversen[2], P.G. Moorman[3], J.R. Marks[4], A. Berchuck[5], J.M. Schildkraut[3].* 1) Epidemiology, UNC, Chapel Hill, NC; 2) Institute of Statistics & Decision Sciences, Duke University, Durham, NC; 3) Community & Family Medicine, DUMC, Durham, NC; 4) Surgery, DUMC, Durham, NC; 5) Obstetrics & Gynecology, DUMC, Durham, NC.

Inflammation may be a mechanism through which established risk factors (e.g., parity, oral contraceptive use, lifetime number of ovulatory cycles) contribute to ovarian carcinogenesis. To investigate this candidate pathway, as well as others such as DNA repair and methylation, we genotyped 1536 tagging SNPs on 170 genes using a customized Illumina OPA chip; 19 of the genes are on the inflammation pathway. Genotypes were determined for 839 incident epithelial ovarian cancer cases and 791 age-matched controls in the NC Ovarian Cancer Study, a population-based case-control study. The analysis was restricted to Caucasian women. IL18, an interleukin gene on the inflammation pathway, was identified as the most significant gene in a gene-by-gene analysis (p=0.00211, Q-value=0.24018). All 12 SNPs on IL18 were in HWE. In a separate haplotype association analysis, rs1834481 uniquely tagged a single haplotype. Compared to women with the homozygous wildtype genotype, heterozygotes were 24% more likely and homozygous rare genotypes were 64% more likely to have ovarian cancer (OR=1.24, 95% CI: 1.01, 1.53 and OR=1.64, 95% CI: 1.09, 2.46, respectively); the Armitage trend test was significant (p=0.0041). No covariates confounded the association between the SNP genotype and ovarian cancer case status; all results were age-adjusted. The effect of the variant allele was slightly stronger among women with serous invasive epithelial ovarian cancer (OR=1.30, 95% CI: 1.00, 1.68 and OR=1.92, 95% CI: 1.18, 3.11 for heterozygous and homozygous rare genotypes, respectively). Though an initial investigation into effect measure modification yielded no significant results, we will present our exploratory analyses. Overall, our analysis suggests an association between the IL18 gene, specifically rs1834481, and epithelial ovarian cancer risk. These results are currently being validated by members of the Ovarian Cancer Association Consortium.

414/F

Distribution of genetic variants associated with steroid biosynthesis and prostate cancer in Mexico. *M. Rodriguez-Dorantes, K. Carrillo-Sanchez, H. Miranda-Ortiz, C. Rangel-Escareno, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Prostate cancer is the second most common male malignancy. In Mexico, ~40% of men between 60-69 years old suffer from this disease. Androgens are essential for normal differentiation and malignant growth of the prostate. Genomic variation in genes involved in biosynthesis and metabolism of androgens have been associated with risk to prostate cancer. We screened SNPs in candidate genes to determine their geographic distribution in Mexico. DNA from 672 unrelated males were obtained from seven states of Mexico (96 each): Sonora (SON), Guanajuato (GUA), Zacatecas (ZAC), Guerrero (GUE), Veracruz (VER), Tamaulipas (TAM), and Yucatan (YUC). We genotyped *LHB* (-1184C>T, rs753307), *CYP17A1* (+27T>C, rs743572) and *SRD5A2* (-17C>G, rs523349, +10G>C, rs632148) using a TaqMan allelic discrimination system (AB). Our results show that *LHB* -1184TT in GUE and VER has a significantly lower frequency of the TT genotype (0.043 and 0.086) compared to the rest of the analyzed states (0.179+-0.063 CI 0.102-0.258; FST p<0.03). This genotype has also a significantly lower frequency in Mexican mestizos (0.14 +-0.076 CI 0.078-0.22) compared with two of the HapMap populations: CEU (0.35) and YRI (0.28), and are similar to the CHB-JPN samples. For the *CYP17A1* +27AA genotype, GUA shows a significantly higher frequency (0.22) compared to the rest of the states (0.136 +- 0.015 CI 0.071-0.212; FST p<0.001) but lower than the CEU (0.448), YRI (0.576) and CHB (0.364). *SRD5A2* +10CC showed a significantly lower frequency in GUE (0.33) vs the rest of the states (0.31 +-0.058 CI 0.248-0.482; FST p<0.005). This genotype has also a higher frequency in Mexicans (0.37+-0.059 CI 0.275-.475) compared to the CEU (0.038), CHB (0.26), JPT (0.125) and YRI (0.039). *SRD5A2* -17C>G did not show significant difference in our population, nor with those of the HapMap. The observed differences in frequencies may relate to ancestral admixture proportions in different regions of Mexico. We are currently evaluating implications of these genetic differences in steroid hormone levels and prevalence in prostate cancer throughout Mexico.

415/F

Association of the ARLTS1 Gly65Val and Cys148Arg variants with breast and prostate cancer risk. *J. Schleutker[1], S. Siltanen[1], K. Syrjakoski[1], R. Fagerholm[2], T. Ikonen[1], P. Lipman[3], K. Holli[4], T. Tammela[5,6], HJ. Jarvinen[7], JP. Mecklin[8], K. Aittomaki[9], C. Blomqvist[10], JE. Bailey-Wilson[3], H. Nevanlinna[2], LA. Aaltonen[11], P. Vahteristo[11].* 1) Laboratory of Cancer Genetics, Institute of Medical Technology, Univ of Tampere, Tampere, Finland; 2) Dept of Obstetrics and Gynaecology, Helsinki Univ Central Hospital (HUCH), Helsinki, Finland; 3) Inherited Disease Research Branch, National Human Genome Research Institute, NIH, Baltimore, Maryland; 4) Dept of Oncology, UTA and TAUH, Tampere, Finland; 5) Dept of Urology, TAUH, Tampere, Finland; 6) Medical School, UTA, Tampere, Finland; 7) Second Dept of Surgery, HUCH, Helsinki, Finland; 8) Dept of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland; 9) Dept of Clinical Genetics, HUCH, Helsinki, Finland; 10) Dept of Oncology, HUCH, Helsinki, Finland; 11) Dept of Medical Genetics, Univ of Helsinki, Helsinki, Finland.

ARLTS1 was recently found as a tumor susceptibility gene when a nonsense mutation Trp149Stop was found more frequently in familial cancer cases than in sporadic cancer patients and healthy controls. We screened the ARLTS1 gene for 1242 breast cancer, 541 prostate cancer, and 241 colorectal cancer cases as well as for 809 healthy population controls by direct sequencing. The Trp149Stop was found at frequencies 0.5-1.2% in all cancer patient subgroups, and with the highest frequency among controls. The recessive model of Cys148Arg variant was found to be more common among breast cancer cases (OR=1.48, 95% CI 1.16-1.87, p=0.001) and in prostate cancer patients (OR 1.50, 95% CI 1.13-1.99, p=0.005) when compared to controls. A novel variant that may have an effect on cancer risk is a Gly65Val alteration that was found at higher frequency among familial prostate cancer patients (8/164, 4.9%) when compared to the controls (13/809, 1.6% OR 3.14, 95% CI 1.28-7.70, p=0.016). No association was found with any of the variants and colorectal cancer risk. Our results suggest that Trp149Stop is not a predisposition allele in breast, prostate or colorectal cancer in the Finnish population, whereas the Gly65Val increase the familial prostate cancer risk and the Cys148Arg both prostate and breast cancer risk.

416/F

Familial Aggregation of Prostate and Breast Cancer in African Americans and Hispanics. *T.J. Costello[2], C. Pettaway[2], C.J. Etzel[3], S.S. Strom[3].* 1) Health Disparities Research, UT MD Anderson Cancer Center, Houston, TX; 2) Urology, UT MD Anderson Cancer Center, Houston, TX; 3) Epidemiology, UT MD Anderson Cancer Center, Houston, TX.

Although prostate cancer familial aggregation has been well studied in Caucasians and African Americans, similar information about Hispanics is rare. Family history of prostate cancer is considered one of the major risk factors, along with age and race, associated with prostate cancer risk. We investigated the role of having a positive family history of prostate cancer on cancer risk in minority populations in an ongoing case/control study. The 578 prostate cancer cases were treated at various hospitals in the Texas Medical Center in Houston, Texas between 1995 and 2004. The 669 controls that were recruited in the Houston area were matched to the cases based on ethnicity, race, residency and age (±5 years). We obtained detailed family cancer history for 5703 first-degree relatives (FDR) of the cases and 5981 first-degree relatives of the controls. We compared the reported cancer among relatives of cases to that of controls to evaluate whether there was an excess of cancer using multivariable logistic regression for Hispanics and African American probands, respectively. We also conducted stratified analyses by type of cancer (prostate and breast), relationship to the case/control and age of diagnosis of the case (≤ or > 60 years old).

Positive family history of prostate cancer increased risk for both Hispanics (OR=2.74 (1.50-5.21)) and African-Americans (OR=1.99 (1.37-2.87)). Stratified analyses also revealed a higher risk if the affected relative is a brother for both Hispanics (OR=5.07) and African Americans (OR=1.95). No increased risk of breast or colon cancer was observed in FDR of either Hispanic or African American of prostate cancer probands. Together with additional analyses that we conducted, this evidence confirms that a positive family history of prostate cancer is a risk factor for both populations and suggests that continued research is required to characterize and identify the genetic factors that influence prostate cancer susceptibility.

417/F

Genetic risk factors for asbestos-related malignant mesothelioma in a general population study. *I. Dianzani[1], M. Betti[1], M. Giordano[2], M. Bertolotti[3], D. Ferrante[3], S. Guarrera[4], D. Mirabelli[3], G. Matullo[4], C. Magnani[3].* 1) Lab.Patologia Genetica, Dept Med Sci, Univ.Piemonte Orientale, Novara; 2) Lab.Genetica, Dept Med Sci, Univ.Piemonte Orientale, Novara; 3) CPO-Piemonte and Unità di Statistica Medica ed Epidemiologia, Dept Med Sci, Univ.Piemonte Orientale, Novara; 4) Dept.Genetica, Biologia, Biochimica, Univ.Torino.

Malignant mesothelioma (MM) of the pleura is a rare, aggressive tumour associated with asbestos exposure. Only ten per cent of subjects exposed to asbestos develop MM. This behaviour and familial aggregation favour a role of genetic risk factors. Asbestos fibers can be carcinogenic as the result of mechanical effects (such as interference with segregation of chromosomes) and generation of reactive oxygen species, that lead to DNA breaks and base modification. We have performed a case-control epidemiological study to analyse SNPs in genes possibly involved in the protection against asbestos carcinogenicity (i.e. genes involved in DNA repair, in the control of red/ox status or in inflammation) in persons exposed to asbestos, who had or had not developed MM. Patients and controls had the same ethnic origin and were residents at Casale Monferrato, a town highly exposed to asbestos pollution. In a previous study we observed an association between XRCC1-399Q and MM (Dianzani et al. Mutat Res 2006). We report here: 1. analysis of the association between XRCC1-399Q and MM in a different panel of patients and controls; 2. analysis of SNPs in 10 other genes (NBS1, PCNA, APEX, ERCC1, MGMT, OPN, GPX1, SOD2, SEP15, NAT2). Unconditional multivariable logistic regression was used to estimate odds ratios (ORs) and 95 °/° confidence intervals (CIs). We confirmed the association with XRCC1-399Q (133 cases, 182 controls; increment of mutant alleles: OR=1.46; 95°/°CI= 1.01-2.11). We found an association with the NAT2 fast acetylator phenotype and thus confirmed the data reported by another Italian group (Neri et al. Mutat Res 2005); pooling the two studies: OR=1.50, 95°/°CI=1.04-2.15 (171 cases, 381 controls). Other SNPs showed increased, but not significant, ORs. Our data support the hypothesis of a multigenic predisposition to MM.

418/F

Iron-related Gene Variants Increase Childhood Leukemia Risk and Birth Weight. *M.T. Dorak[1], R. MacKay[1], C.L. Relton[1], M. Worwood[2], L. Parker[3], A.G. Hall[1].* 1) School of Clinical Medical Sciences, Newcastle University, UK; 2) Dept of Haematology, Cardiff University School of Medicine, UK; 3) Dept of Pediatrics, Dalhousie University IWK Health Centre, Halifax, Canada.

To test our hypotheses that variation in the hemochromatosis gene (HFE) and other iron-related genes modify genetic risk for childhood acute lymphoblastic leukemia (ALL) and birth weight, a population-based study of 172 incident cases and 1005 newborn controls (533 with their mothers) from the North of England was undertaken. Fifteen HFE variants, the transferrin receptor gene (TFRC) S142G variant and several other variants were examined. Using genomic control loci, cryptic population substructure was ruled out. Frequency distribution of HFE haplotypes constructed on PHASE was different between cases and controls (P = 0.01) but in males only (P = 0.02). HFE H63D (OR = 1.6) and an intervening sequence 1 (IVS1) variant (OR = 2.1) showed independent (dominant model) associations but C282Y did not. Homozygosity for TFRC S142G (recessive model) individually increased risk (OR = 1.4) and interacted with the HFE IVS1 (OR = 3.4; 95% CI = 1.5 to 7.6, P = 0.004). H63D, IVS1 and C282Y also increased birth weight statistically significantly in healthy newborns with sex effect: 79g by C282Y (240g in boys); 87g by H63D (138g in boys); 177g (188g in boys) by IVS1. These effects were greater in interaction with TFRC or when the mother was also positive for the HFE variants. IVS1 variant in combination with S142G homozygosity increased birth weight (188g) with greater effect in boys (450g). Compound heterozygosity (C282Y/H63D) increased birth weight but in boys only (434g, P < 0.01). Cord blood iron indices showed non-significant changes in 214 newborns examined. Thus, we showed that an HFE x TFRC interaction modified childhood ALL susceptibility and the same genotypes increased birth weight with maternal and sex effects. Our findings complement the previously established links between high body iron levels and increased cancer risk presumably due to iron's genotoxic effects. The influence of iron on dividing cells may, on the other hand, explain the well-known association between birth weight and childhood ALL.

419/F

Cost-effectiveness of population-based *BRCA1/2* testing and ovarian cancer prevention for Ashkenazi Jews. *W.S. Rubinstein[1], H. Jiang[2], L. Dellefave[3], A. Rademaker[2].* 1) Evanston Northwestern Healthcare, Evanston, IL; 2) Northwestern University, Chicago, IL; 3) Univ. of Chicago, Chicago, IL.

Background Identification of *BRCA1/2* carriers rests solely on family history. Yet, family history fails to identify half of carriers, as shown by breast cancer case-based studies in several populations including Ashkenazi Jews (AJs). Three prevalent founder mutations account for 40% of ovarian cancer (OC) and 10% of breast cancer in AJs, suggesting that a marked reduction in cancer burden is attainable. Population-based genetic screening may be feasible given the high prevalence of founder mutations, low cost of genetic testing and historical acceptance of Tay-Sachs disease screening among AJs. Purpose To examine the effect of population-based gene testing on life-savings and medical costs when considering ovarian cancer treatment and prevention. Methods Decision analysis using the parameters: Screening program participation rate=0.9; Mutation carrier rate=0.025; Probability that a 40 year-old carrier will have prophylactic bilateral salpingo-oophorectomy (PBSO)=0.50; OC penetrance= 0.16; PBSO effectiveness=0.96; Sporadic OC probability=0.016; Mean age at OC diagnosis 58.8 (carriers), 63 (sporadic); Commercial cost of gene testing=$460; Cost of PBSO and OC treatment as per Anderson et al.2006; OC mortality rate based on SEER 2004 data. Results Our model suggests that a population-based genetic screening program could prolong average survival by 384 days for a 40-year-old female AJ *BRCA1/2* carrier having PBSO and save non-discounted costs of ~$100 per woman screened. Conclusions Our model predicts a significant life-saving potential for a genetic screening program. The costs of breast cancer surveillance, prevention and treatment and effect on life expectancy must also be taken into consideration. While additional costs of a screening program would need to be factored in, the cost of genetic testing seems to be balanced by the savings of avoiding OC diagnosis and treatment. We think that a dialogue should begin among Jewish stakeholders, genetics professionals, and public health leaders to determine whether a population-based *BRCA1/2* genetic screening program should be pursued.

420/F

Germline BRCA2 mutations and the risk of esophageal squamous cell carcinoma. *M.R. Akbari[1,2,3], R. Malekzadeh[3], D. Nasrollahzadeh[3], D. Amanian[3], F. Islami[3], I. Zandvakili[2], R. Shakeri[3], M. Sotoudeh[3], P. Boffetta[4], S.M. Dawsey[5], P. Ghadirian[6], S.A. Narod[2].* 1) Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, Canada; 2) Women's College Research Institute, University of Toronto, Toronto, Canada; 3) Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran; 4) International Agency for Research on Cancer, Lyon, France; 5) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA; 6) Epidemiology Research Unit Research Centre, CHUM- Hôtel-Dieu, University of Montreal, Montreal, Canada.

The incidence of esophageal squamous cell carcinoma (ESCC) among the Turkmen population of the southeastern Caspian littoral is very high. Family studies suggest that there is a genetic component to the disease. Because Turkmen are ethnically homogenous, they are well-suited for studies of ESCC genetics. A previous study from China suggested that BRCA2 might play a role in the etiology of ESCC in regions of high incidence. We screened for mutations in the coding region of the BRCA2 gene in the germline DNA of 197 Turkmen patients with ESCC in northern Iran. A nonsense variant, K3326X, was identified in 9 of 197 cases (4.6%) versus 2 of 254 controls (0.8%)(OR = 6.0, 95%CI = 1.3 - 28; P = 0.01). This mutation leads to the loss of the C-terminal domain of the BRCA2 protein, a part of the region of interaction with the FANCD2 protein. We observed nine other BRCA2 variants in single cases only, including two deletions (501-513del-CCAATCTCCTGTA and 3734del-A), and seven missense mutations (Y42C, C315S, L1019V, I2490T, T2542M, K2729N and Q3227E). None of these variants detected in controls. Six of these (Y42C, C315S, I2490T, K2729N and two deletions) were judged to be pathogenic. In total, a suspicious deleterious BRCA2 variant was identified in 15 of 197 ESCC cases (7.6%). Eleven patients had mutations (K3326X, I2490T and K2729N) which have been seen in patients with Fanconi anemia. Therefore it is tempting to speculate that BRCA2 mutations increase the risk of ESCC by a mechanism related to the Fanconi anemia pathway.

421/F

A dinucleotide polymorphism in promoter of ankyrin repeat domain 9 gene associated with susceptibility to intestinal-type gastric cancer. *H. Ju, C. Kang.* Dept Biological Sci, Rm 1217, KAIST, Daejeon, Korea.

Polymorphisms in promoters can increase or decrease target gene expression, and the altered expression levels may confer susceptibility to complex diseases. We sequenced 3-kb promoter region of the ankyrin repeat domain 9 gene whose expression is down-regulated in gastric cancer tissues and found 6 single-nucleotide polymorphisms (SNPs). The SNPs were genotyped for unrelated 178 intestinal-type gastric cancer patients and 406 non-patient controls. Five successfully genotyped SNPs were significantly associated with intestinal-type gastric cancer ($P < 0.033$). Among them, two adjacent SNPs located at -112 and -111 from the transcription initiation site were in complete linkage disequilibrium ($r^2 = 1$) of each other and had the strongest association (OR = 1.6, $P = 0.00076$). Two variant sequences carrying the dinucleotide polymorphism were inserted in three tandem copies to the pGL3 luciferase vector for promoter activity assay. The risk variant had 1.8-fold reduced promoter activity versus the non-risk variant ($P = 0.0034$) in MKN45 gastric cancer cells. Although functional implications of ankyrin repeat domain 9 in gastric tumorigenesis is not elucidated yet, these results suggest that the risk variant may be functionally associated with increased susceptibility to intestinal-type gastric cancer by reducing the gene transcription level.

422/F

Gene-wide association study on ABCB1/MDR1 polymorphisms and colorectal cancer risk. *D. Campa[1,2], B. Pardini[2,3], P. Vodicka[3], S. Wilkening[1], K. Hemminki[1,4], R. Barale[2], F. Canzian[1].* 1) German Cancer Research Center(DKFZ), Heidelberg, Germany; 2) Department of Biology, University of Pisa, Pisa, Italy; 3) Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic; 4) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden.

Transporters are the gatekeepers for all cells and organelles, controlling uptake and efflux of a large variety of compounds such as sugars, amino acids, nucleotides, inorganic ions xenobiotics , including toxins, carcinogens, and drugs. Among ABC transporters, ABCB1, is the best known member of the family. Its expression in the human intestine increases from proximal to distal, resulting in the higher expression levels in the colon. ABCB1 is involved in the excretion of several carcinogens from the gut into the intestinal lumen. The aim of the present work was to study the impact of genetic variants of ABCB1 gene on risk of colorectal cancer, with particular attention to the role of putatively functional variants that have been previously shown to be associated with cancer risk, such as C3435T and S892A polymorphisms within ABCB1. In order to study exhaustively genetic variation of ABCB1 gene, we have followed a hybrid functional/tagging approach. Genotype data from the most recent release of the HapMap project have been downloaded. All polymorphisms with minor allele frequency ≥5% in HapMap Caucasians have been included. We performed a case-control study on 690 cases and 590 controls of Czech origin. We found that carriers of the T allele of ABCB1 G2677T polymorphism had an increased risk of colorectal cancer. In conclusion this findings suggest that variants impairing ABCB1 activity would lead to an increased colorectal-cancer risk due to a decreased toxin/carcinogen clearance trough the body.

423/F

Admixture and Hormone Receptor Status of Breast Cancer among African Americans. *L. Fejerman[1], E. John[2], C. Ambrosone[3], A. Whittemore[6], S. Neuhauser[7], K. Amend[4], W. Davis[5], D. Bovberg[4], S. Huntsman[1], D. Hu[1], W. Lorizio[1], E. Ziv[1].* 1) Medicine, UCSF, San Francisco, CA; 2) Northern California Cancer Center, Union City, CA; 3) Epidemiology, Roswell Cancer Center, Buffalo, NY; 4) Oncological Sciences, Mount Sinai School of Medicine, New York, NY; 5) Cancer Prevention and Population Sciences, Roswell Cancer Center, Buffalo, NY; 6) Health Services Research, Stanford University, Palo Alto, CA; 7) Epidemiology, UCIrvine, Irvine, CA.

African American women present more commonly with estrogen receptor (ER-) and progesterone receptor negative (PR-) breast cancer compared with Caucasian women. We hypothesized that this difference in incidence of PR/ER- tumors is due to the difference in the frequency of predisposing alleles between the ancestral populations (European and African). We used an admixture mapping approach to search for loci that may underlie this difference. Methods: Samples are from African American women newly diagnosed with first primary invasive breast cancer (N=333). 1,491 ancestry informative markers were successfully genotyped. Ancestry estimates were calculated with the program STRUCTURE1.2. We tested the association between locus specific ancestry and ER/PR status using logistic regression, including individual ancestry as a covariate. We used a permutation test to evaluate for genome wide significance. Results: Our results suggest an association between lack of expression of hormone receptor and African ancestry (p=0.03 for PR and p=0.059 for ER). Examining locus specific ancestry differences among PR- vs. PR+ and ER- vs. ER+, we found nine regions with a nominally significant (p<0.05) difference in ancestry. One region on chromosome 15q- was strongly associated with PR status (p=0.00006) and was significant at the genome-wide level (p= 0.017). At this locus, individuals with PR- tumors tend to have higher African ancestry, and individuals with PR+ tumors higher European ancestry. Conclusion: Breast cancer hormone receptor status risk is associated with ancestry among African American women. At least some of this difference may be determined by a locus on chromosome 15.

424/F

DNA-repair genetic polymorphisms and breast cancer risk among Cypriot women. *A. Hadjisavvas[1], M. Loizidou[1], S. Malas[3], Y. Marcou[2], K. Kyriacou[1].* 1) Department of EM/ Molecular Pathology, The Cyprus Institute of Neurology & Genetics, Nicosia, Cyprus; 2) Bank of Cyprus Oncology Centre, Nicosia, Cyprus; 3) Department of Oncology, Limassol General Hospital, Limassol, Cyprus.

Genetic factors are important in breast cancer but less than 20% are attributable to the inheritance of mutations in genes such as BRCA1 and BRCA2. The polygenic model of breast cancer suggests that there are multiple low-penetrance alleles which have a small effect on breast cancer risk. In an attempt to identify genetic variants which modify breast cancer risk we are contacting a case-control genetic epidemiology study using a cohort of 2286 Cypriot women (1109 patients and 1177 healthy controls). In the present study we genotyped 6 single nucleotide polymorphisms (SNPs) in genes which are involved in the DNA repair pathway: BRCA2 N991D, OGG1 S326C, RAD51 135G/C and 172G/C and p53 P72R. The prevalence of the 6 SNPs was compared between cases and controls. Odds ratios were generated from 2x2 tables, and statistical significance was assessed using the Pearson Chi-Square test.BRCA2 N991D was found at a significantly higher frequency in the population-based series of breast cancer patients (142/1086, 12.9%, odds ratio [OR] =1.42, 95% confidence interval [CI]= 1.09-1.85, p=0.01) than among population controls (112/1177, 9.5%). Furthermore, a marginally significant association between the p53 P72R variant and breast cancer was observed ([OR] (PP vs. PR+RR) =1.18, 95% CI (1.0-1.39), p=0.05). In addition, our results show that the effect of RAD51 135 C allele may be protective indicating that women who harbour this allele have a reduced risk of breast cancer compared with women who carry the G allele. These results suggest that a proportion of the SNPs under study are modifying breast cancer risk, but the effects of individual SNPs are likely to be small. Large numbers of samples will be needed to verify our results in other populations. We are currently expanding our analysis to include a greater number of SNPs and to evaluate potential underlying gene-gene or gene-environment interactions, in order to advance our knowledge on the effect of genetic polymorphisms on breast cancer susceptibility.

425/F

Identification of men with a genetic predisposition to prostate cncer: targeted screening in BRCA1 and BRCA2 mutation carriers and controls. The 'IMPACT' study: pilot data. *A. Mitra[1], E. Bancroft[2], R. Eeles[1,2].* 1) Cancer Genetics, Institute of Cancer Research, London, UK; 2) Cancer Genetics, The Royal Marsden Hospital NHS Foundation Trust, London, UK.

Introduction The relative risk of prostate cancer (PC) in BRCA1 and BRCA2 carriers under the age of 65 years may be as high as 1.85 and between 7.33 and 23 fold respectively. IMPACT, the largest international prospective screening study of men with a known genetic predisposition to PC, aims to assess the role of targeted PSA screening and to determine the incidence and pathology of PC in this group. Methods 500 BRCA1 carriers and 350 BRCA2 carriers aged 40-69 will be recruited over 5 years. 850 controls will be recruited from men who are predictive test negative for a known familial mutation. Annual serum PSA, free:total PSA, testosterone and sex hormone binding globulin is taken. Prostate biopsy is offered if PSA is above 3ng/ml. The pilot study is recruiting in 8 UK cancer genetics centres and 2 international centres. Results 70 men (27 BRCA2, 19 BRCA1 and 24 controls) have been recruited to the study so far. Uptake rates have varied between centres but range from 76% to 94%. 4 men have had a PSA above 3ng/ml. A BRCA1 carrier and a control group man have been diagnosed with PC (Gleason score 3+4, stage T2b, PSA 3.8ng/ml and Gleason score 3+3, stage T2b, PSA 4.3ng/ml respectively). A 69 year old BRCA2 mutation carrier, PSA 6.7, has been found to have benign prostatic hypertrophy (BPH). A 69 year old control with a PSA of 7.2ng/ml had BPH only. Conclusions One of the limiting factors of the ERSPC and PLCO studies is the low recruitment rate in the target populations. In European countries that randomise men into a screening and control group, uptake rates are 25-46%. In those countries that randomise only to a screening arm the recruitment rate is 64%. Surveys suggest that as few as 3% of eligible men participate in the PLCO study. It appears that men who are at an increased genetic risk of developing PC are more likely to enter a PC screening study. This has implications for future targeted screening as new increased risk genotypes are identified by genome wide association studies.

426/F

Replication of a Genome-Wide Mapping Case-Control Study in Esophageal Cancer. *D. Ng[1], N. Hu[1], Y. Hu[2], C. Giffen[3], Z.Z. Tang[4], X.Y. Han[4], H.H. Yang[2], M.P. Lee[2], A.M. Goldstein[1], P.R. Taylor[1].* 1) Genetic Epidemiology Branch, DCEG/NCI/NIH/DHHS, Bethesda, MD, USA; 2) Laboratory of Population Genetics, CCR/NCI/NIH/DHHS, Bethesda, MD, USA; 3) Information Management Systems, Silver Spring, MD, USA; 4) Shanxi Cancer Hospital, Taiyuan, Shanxi, PRC.

Background: Previously, we applied the Affymetrix mapping 10K SNP array in a pilot case-control study to determine differences in genotypes between esophageal squamous cell carcinoma (ESCC) cases and controls from a high-risk area in China and identified 38 SNPs in or near one of 33 genes. The present study attempted to replicate the results of these 38 gene-related SNPs in a new sample of cases and controls. Methods: A subset of 300 ESCC cases and 300 matched controls from a larger case-control study conducted in Shanxi Province, China was selected for the present study. A series of multiplex oligonucleotide ligation assays to genotype these 38 target SNPs were developed and applied to germline DNA from study subjects. Assays were validated by direct sequencing of eight SNPs in 12 pairs of cases and controls, and Hardy-Weinberg equilibrium was examined in control samples. General linear models were used to derive odds ratios (ORs) for dominant, recessive, and additive modes of transmission adjusted for baseline risk factors and one or more SNPs. Factor analysis was used to predict individual risk of ESCC. Results: Among 36 evaluable SNPs, four were significant in one or more analyses, including SNPs in EPHB1, PIK3C3, SLC9A9, and PGLYRP2. Risks were significantly increased for subjects with the T/T genotype in SNPs in EPHB1, PIK3C3, and SLC9A9, and for subjects with the A/A genotype in the PGLYRP2 SNP. The best factor analysis models accurately classified case/control status in approximately half of the subjects. Conclusions: Four of 38 previously identified gene-related SNPs remained significant in this replication study. While EPHB1, a receptor protein tyrosine kinase previously associated with colorectal cancer, merits particular consideration as a candidate tumor suppressor gene for ESCC, further exploration of all four genes in ESCC is recommended.

427/F

Increased risk of stomach and nervous system cancers in Finnish prostate cancer families. *S. Pakkanen[1], M. Matikainen[2], E. Pukkala[3], P. Koivisto[1], T. Tammela[2], J. Schleutker[1].* 1) Laboratory of Cancer Genetics, Institution of Medical Technology, University of Tampere and Tampere University Hospital, Finland; 2) Department of Urology, Tampere University Hospital and Medical School, University of Tampere, Finland; 3) Finnish Cancer Registry, Helsinki, Finland.

Clinical features of families with prostate cancer (PCa) and other malignancies associated with this disease are not well known. A family with PCa is characterized as two or more PCa cases among first degree relatives. The aim of this study was to assess weather primary tumors other than prostate carcinoma aggregate in Finnish families with PCa or weather this disease can be considered site specific. Based on the national population based Finnish Cancer Registry (FCR), we calculated standardized incidence ratios (SIR) for 5546 members of 202 Finnish families with PCa with confirmed genealogy, either using the first diagnosed PCa among brothers as a single index or multiple indexes. Information of family members were confirmed from population registry and cancer data from hospital records and Finnish Cancer Registry respectively. The total number of cancers (all sites) among males was 552 (SIR 1.79) in single index group, 234 (SIR 0.94) in multiple index group and among females 205 (SIR 0.98). The number of PCa cases was 373 (SIR 6.73) in single index group and 71 (SIR 1.21) in multiple index group. The sisters of the index person had more stomach cancer than expected (SIR 2.12, 95% confidence interval 1.02-3.90) the mothers of the indexes had increased number of central nervous cancers in the age group of 60-69 years (SIR 19.4, 2.35-70.08) when compared general population. Spouses had no increased risk to any cancer, suggesting that special environmental risk factors can be excluded. In most of the families with excess numbers of prostate cancer the disease appears to be site specific. However in a subgroup of families, a suggestive tendency towards gastric and central nervous cancers was detected. Further analysis is warranted to carry out multivariate analysis based on selected clinical and family characteristics, possibly enabling separation of families with sporadic cases to a different cohort.

428/F

Association between invasive ovarian cancer and alleles involved with breast cancer and prostate cancer susceptibility. *H. Song[1], S. Ramus[2], S.K. Kjaer[3], R.A. DiCioccio[4], L. Quaye[2], E. Hogdall[3], A.S. Whittemore[5], D.E. Easton[6], C.L. Pearce[7], G. Chenevix-Trench[8], S.A. Gayther[2], P. Pharoah[1].* 1) Department of Oncology, University of Cambridge, UK; 2) Translational Research Laboratories, University College London, London, UK; 3) Danish Cancer Society, Copenhagen, Denmark; 4) Roswell Park Cancer Institute, Buffalo, NY, USA; 5) Stanford University School of Medicine, Stanford, USA; 6) Genetic Epidemiology Unit, University of Cambridge, UK; 7) University of Southern California, Keck School of Medicine, Los Angeles, CA, USA; 8) The Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Australia.

Background: Several alleles have recently been identified by genome-wide association study in hormone related cancers (breast and prostate cancer). The aim of this study was to test these alleles for association with invasive ovarian cancer.

Methods: Eleven breast cancer associated SNPs and 2 prostate associated SNPs were genotyped in approximately 2400 invasive ovarian cancer cases and 4100 controls from 6 studies (from Australia, UK, Denmark and USA). Genotype frequencies in cases and controls were compared using a likelihood ratio test in a logistic regression model stratified by studies.

Results: Three of 13 SNPs showed a weak association with ovarian cancer: carriers of the minor allele of rs2107425 (*H19*) were at reduced risk (per-allele odds Ratio (OR)=0.92[0.85-0.99], P-trend=0.03); and the minor allele of rs7313833 (*PTHLH*) was associated with increased risk (per-allele OR=1.09[1.01-1.18], P-trend= 0.02). In analyses restricted to serous ovarian cancer, carriers of the minor allele of rs4954956 (*NXPH2*) were associated with increased risks (per allele OR=1.12[1.01-1.24], P-trend=0.03).

Conclusions: The 5 most significant SNPs from breast cancer study and 2 SNPs from prostate cancer study were not associated with ovarian cancer. However, 3 of the remaining 6 showed association with ovarian cancer which warrant confirmation in independent studies.

429/F

Investigation of mismatch repair protein expression in ovarian tumors. *J. Wey[1], D. Boulware[1], N. Valkov[2], S. Livingston[2], S. Nicosia[2], J-H. Lee[1], R. Sutphen[1], J. Schildkraut[3], S. Narod[4], T. Sellers[1], T. Pal[1].* 1) Moffitt Cancer Center, Tampa, FL; 2) Univ of S FL, Tampa, FL; 3) Duke, NC; 4) Univ of Toronto, Canada.

Background: The frequency of mismatch repair (MMR) deficiency in epithelial ovarian cancer (EOC) has ranged from 2-17%. Limited data exist regarding representative sampling from paraffin-embedded EOC tissue blocks utilized for construction of tissue microarrays (TMA) in preparation for immunohistochemistry(IHC). Methods: EOC tumor blocks from 59 cases were investigated by IHC for expression of hMLH1, hMSH2, and hMSH6. TMAs were created using three replicate 1 mm cores sampled from the center of a donor tissue block. Loss of expression of at least one protein was observed in an unexpectedly high number of cases, prompting creation of full sections of the donor blocks, which revealed lack of expression in the central portion, but positive expression in the periphery. Follow-up analyses for cases initially lacking expression were performed by obtaining cores from the periphery of up to 5 additional donor tissue blocks (triplicate cores per block). A linear mixed model for each protein was used to investigate differences between results from the initial donor block and follow-up blocks. Results: Loss of expression of at least one protein was revealed for 17 of the 59 (29%) cases. Follow-up analyses of the 17 cases that initially showed loss of protein expression revealed loss of expression in only 6 cases (10%). For each protein, statistically significant differences ($p<0.05$) were detected between the initial donor block and the majority of the follow-up blocks. Conclusions: When performing IHC analyses for loss of MMR protein expression in ovarian carcinomas, it is important to preferentially sample from the periphery of tumor blocks where exposure to tissue fixatives is optimal. This may reduce the likelihood of tissue fixation as the cause of the lack of protein expression.

430/F

Are VEGF and Interleukin Haplotypes risk factors in prostate cancer etiology among African Americans? *K. Yanamandra[1], M. Ankem[2], D. Napper[1], P.B. Boggs[3], H. Chen[1], S.A. Ursin[1], G. Mills[4], J.A. Bocchini Jr.[1], R. Dhanireddy[5].* 1) Dept Pediatrics, LSU Health Sciences Ctr, Shreveport, LA; 2) VA Medical Center, Shreveport, LA; 3) Allergy Clinic, Shreveport, LA; 4) Feist-Weiller Cancer Center, Shreveport, LA; 5) Dept Pediatrics, UT Health Sciences Center, Memphis, TN.

Angiogenesis is a major feature and is an essential process in the development, growth and metastasis of malignant tumors. A host of genetic factors also influence the tumorigenesis and cancer etiology, especially the combination of low-penetrance gene polymorphisms. We have been studying the influence of genetic single nucleotide polymorphisms (SNP) for the past five years including the interleukins (IL), vascular endothelial growth factor (VEGF), prothrombin gene, Methylenetetrahydrofolatereductase (MTHFR), etc.in the tumorigenesis and the etiology of various cancers including multiple myeloma, breast, lung, head and neck cancers. In the present investigation we have studied the influence of IL-1, IL-8, IL-10, and VEGF SNPs in the etiology of prostate cancer in seventeen prostate cancer patients and one hundred and seventy four controls among African Americans,using microplate PCR RFLP and allele specific PCR genotyping methods. Among the genetic markers studied, mutant allele in the promoter region of VEGF gene showed a significant difference (OR 2.8, p value 0.03 for -460T). Among the haplotypes studied, the IL-1 beta -511C/IL-8 -251T/IL-10 -1082G/VEGF-460T/VEGF-1154G haplotype frequency was found to be significantly higher among the Prostate cancer patients compared to the controls (OR 1.4, p value 0.04). Based on our experimental data we conclude that the IL-1 beta -511C/IL-8 -251T/IL-10 -1082G/VEGF -460T/VEGF-1154G haplotype could be a significant risk factor in the etiology of prostate tumorigenesis among African American population. Data and statistics on different markers will be presented.

431/F

Finding Genetic Risk Factors for Breast Cancer by Pedigree-Free Identity-By-Descent Mapping. *B.L. Merriman, Z. Chen, S.F. Nelson.* Human Genetics, UCLA, Los Angeles, CA.

The most well known genetic risk factors for familial breast cancer are the BRCA1 and BRCA2 genes. However, mutations in these genes explain less than half of all familial breast cancer, and the rest remains largely unexplained, despite extensive searches using standard linkage and association methods. In this study, we apply a new method we have been developing, Pedigree-Free Identity by Descent Mapping, which has novel power to identify risk alleles if there are strong founder effects in the population. This method uses high-density SNP genotyping data to directly infer shared DNA fragments between affected individuals, without relying on any pedigree information. In order to enrich for the necessary founder effects, we carry out the strategy in the Ashkenazi-Jewish population. As proof of principle, we first demonstrate that within this population, as few as 5 carriers of each of the known mutations suffice to localize the BRCA1 and BRCA2 genes. We then go on to apply the approach with 100 cases free from known BRCA mutations, and show that this highlights several regions and specific risk haplotypes, as well as identifying novel BRCA-related risk haplotypes not previously identified in this population. This demonstrates that Pedigree-Free IBB mapping is a powerful technique for identifying risk loci, with strengths that are complementary to those of standard methods for linkage and association analysis.

432/F

The MSH2 sequence variant p.Gly322Asp (c.965G>A) is a benign polymorphism. *T.B. Bradley[1], N. Williams[1], D. Reinhardt[1], N. Andrew[2], D.U. Baty[2], H.R. Davidson[3], Z. Miedzybrodzka[4], M.G. Dunlop[5], M.E. Porteous[1], J.P. Warner[1].* 1) Molecular Genetics Service, Western General Hospital, Edinburgh, UK; 2) Molecular Genetics Laboratory, Ninewells Hospital, Dundee, UK; 3) Duncan Guthrie Institute of Medical Genetics, Yorkhill Hospital, Glasgow, UK; 4) Department of Medical Genetics, University of Aberdeen, UK; 5) MRC Human Genetics Unit, WGH, Edinburgh, UK.

Variations in the mismatch repair genes contribute to the pathogenesis of hereditary non-polyposis colorectal cancer (HNPCC). In Scotland, MLH1, MSH2 and MSH6 gene analysis, using bidirectional sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA), is carried out for Amsterdam positive families. If tumor specimens are available, microsatellite instability studies (MSI) and immunohistochemical (IHC) staining are performed. Medium risk families are pre-screened and only patients with tumors showing loss of staining or MSI are selected for gene analysis. The confirmation that sequence variants identified by gene analysis are pathogenic and causative is critically important as misleading predictive test results could lead to inappropriate withdrawal of routine surveillance. The MSH2 missense variant p.Gly322-Asp (c.965G>A) has been reported both as a pathogenic and a benign variant. DNA from 630 affected patients from high and medium risk HNPCC families was tested and this variant was present in 14 (2%). In a control cohort of 400 DNAs from individuals with no known family history of colorectal cancer it was seen in 16 (4%). Typical p.Gly322Asp pedigrees from our colorectal patient panel are illustrated. A family with an individual homozygous for p.Gly322Asp, who developed colorectal cancer aged 60 and a second family with p.Gly322Asp in combination with an MSH6 exon 5-6 deletion are shown. All tumors analysed, where the variant p.Gly322Asp was seen were MSI low. SIFT analysis (http://blocks.fhcrc.org/sift/SIFT.html) using a panel of representative proteins shows that this position is unlikely to be critical to MSH2 protein function. These data provide conclusive evidence that the variant p.Gly322Asp is a benign polymorphism.

433/F

Frequency of C677T Polymorphism of MTHFR Gene in Mexican Adult with Acute Lymphoblastic Leukemia. *D. Carbajal[1], AM. Puebla[2], LE. Figuera[3], M. Gallegos[1].* 1) Medicina Molecular, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Guadalajara, Mexico; 2) Laboratorio de Inmunofarmacología Experimental, CUCEI, Universidad de Guadalajara; 3) Division de Genetica, CIBO, IMSS.

The factors governing susceptibility to acute lymphoblastic leukemia (ALL) have not yet been identified. The MTHFR enzyme is involved in carcinogen metabolism and have been shown to influence the risk a variety of solid tumors in adults. Folate availability is critical for DNA integrity, required for the transfer of methyl groups in the biosynthesis of thymidilate. Reduction of 5,10-methylenetetrahydrofolate, a donor for methylating dUMP to dTMP in DNA synthesis, to 5-methyltetrahydrofolate, the primary methyl donor for methionine synthesis, is catalyzed by 5,10-methylenetetrahydrofolate reductase (MTHFR). The MTHFR polymorphisms C677T have been shown in some studies to alter the risk of a range of different malignancies. We evaluated the role of the C677T polymorphism on acute lymphoblastic leukemia (ALL) risk by genotyping 107 patients and 170 healthy controls. The odds ratio of ALL associated with 677TT and 677CT genotypes were 0.49 (95% CI; 0.21-1.07) and 1.1 (95% CI; 0.66-1.84) respectively. This data indicate that the MTHFR polymorphism C677T do not significantly contribute to an inherited genetic susceptibility to ALL in adult Mexican population.

434/F

Variation in Global Cancer Incidence Rates is Associated with Population-Level Genetic Background. *N.V. Campbell[1], C.M. Nievergelt[1], E.O. Lillie[1], N.J. Schork[1,2,3].* 1) Center for Human Genetics and Genomics, University of California San Diego, La Jolla, CA; 2) Scripps Genomic Medicine, The Scripps Research Institute, La Jolla, CA; 3) Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA.

Cancer incidence rates vary widely across different human populations. The reasons for this are complex and reflect the interplay between variation in genetic susceptibility, environmental exposures, medical and public health practices, and social factors. Although the influence of genetic background on cancer incidence has been probed by many model organism and anecdotal or small-scale studies on humans, few, if any, studies have actually tested the hypothesis that genetic background is associated with human cancer incidence rates on a global scale using relevant incidence and population genetic data. We have examined the relationship between variation in global human cancer incidence rates and genetic background using a wide variety of resources, including statistics collected by the WHO and other international organizations as well as data available on the Human Genetic Diversity Panel (HGDP). We attempted to control for environmental and social factors using available population data and social indicators. We ultimately find very strong associations between genetic background and global human cancer incidence rates. We do, however, acknowledge the limitations of our studies as well as areas for further research.

435/F

Lack of association between HFE gene mutations and breast cancer in Azorean patients. *P.R. Pacheco[1,2], H. Polena[1], C. Ballart[1], T. Eloi[3], C.C. Branco[1,2], R. Cabral[1,2], V. Santos[3], V. Carneiro[4], L. Mota-Vieira[1,2].* 1) Mol Genetics Pathol Unit, HDES; 2) IGC, Oeiras; 3) Cirurgic Dept, HDES; 4) Anatomic Pathology Dept, HDES, Azores, Portugal.

Iron overload, caused by HFE mutations, may be carcinogenic because it can catalyse the formation of free radicals, suppress the immune system and increase tumour cells growth. Hence, HFE mutations have been evaluated as risk factors for cancer. The purpose of this study was to assess if HFE mutations are related to the risk of breast cancer (BC). To that end, we compared frequencies of C282Y and H63D mutations in 86 Azorean BC women and in 183 healthy controls. Samples were obtained after written informed consent. The C282Y allele frequency in the BC group was 4.07%, higher than in control group, 3.28%; with an OR=1.25 (95% CI, 0.48 - 3.24). Regarding H63D mutation, the allele frequency in the BC group was 21.51%, very similar to the frequency found in the control group, 21.04%; OR= 1.03 (95% CI, 0.66 - 1.6). The mean age at diagnosis for BC patients was 60.4 yr (range, 33-87) and 54.8 yr (range, 31-84) in the healthy control group. As age is known to influence breast cancer risk and thus could be a confounding factor, we stratified the BC and the healthy control groups into three age stratum, according to the menopausal status (<48, 49-58 and >59 yr). Odds ratio for breast cancer risk associated with H63D mutation was 1.29 (95% CI, 0.54 - 3.13) in women bellow 48 yr; 0.69 (95% CI, 0.24 - 2.01) in the range of 49-58 yr, and 0.97 (95% CI, 0.53 - 1.78) in women above 59 yr. On the other hand, odds ratio for breast cancer risk associated with C282Y mutation increased with age, from 1.14 (95% CI, 0.29 - 4.52) in women bellow 48 yr to 2.03 (95% CI, 0.44 - 9.27) in women above 59 yr. The risk for breast cancer was higher in older women bearing the C282Y mutation than in healthy controls, although not statistically significant. In conclusion, the results suggest that HFE gene mutations are not associated with an increased risk for breast cancer and do not significantly contribute to the community prevalence of breast cancer in the Azorean population (paularpacheco@hdes.pt). Azorean Government founded.

436/F

Interaction between Estrogen Receptor Alpha Genotypes and Human Herpesvirus 8 Infection Resulting in an Increased Risk of Prostate Cancer. *P.R. Shea[1], C.H. Bunker[2], P.V. Benos[1], D.L. Corcoran[1], A.L. Patrick[3], F.J. Jenkins[4], R.E. Ferrell[1].* 1) Dept of Human Genetics, University Pittsburgh, Pittsburgh, PA; 2) Dept of Epidemiology, University of Pittsburgh, Pittsburgh, PA; 3) Tobago Regional Health Authority, Scarborough, Trinidad and Tobago; 4) Dept of Pathology, University of Pittsburgh, Pittsburgh, PA.

The role of interaction between viral infection and host genetic susceptibility has increasing become recognized as an important factor in the etiology of human cancer. Several epidemiologic studies have suggested that prostate cancer is a complex disease involving host genetic factors and environmental exposures that modify risk. Here we report a novel interaction between infection with human herpesvirus 8 (HHV8) and the human estrogen receptor alpha XbaI polymorphism which is associated with an increased risk of prostate cancer (p=0.032; OR=3.11 95%CI (1.42-6.77)) in an Afrocaribbean population from Tobago. Further, we have identified estrogen response elements in the promoter regions of genes in the HHV8 genome, suggesting a direct form of interaction. Using gel shift and luciferase reporter assays we have shown these sequences are capable of binding human estrogen receptor proteins and are responsive to estrogen induction. Our results suggest that direct interaction between the estrogen receptor and HHV8 gene transcription may play a role in the etiology of prostate cancer and that common polymorphisms in the estrogen receptor alpha gene may increase risk.

437/W

A molecular signature for identification of platinum resistant ovarian cancer. *M. Bonin[1], J. Hoffmann[1], T. Fehm[2], M. Walter[1], K. Sotlar[3], D. Wallwiener[2], O. Riess[1], E. Solomayer[2], H. Neubauer[2].* 1) Medical Genetics Dept, Inst. of Human Genetics, The Microarray Facility, Tuebingen, BW , Germany; 2) Gynecological Hospital, Eberhard-Karls-University, Tuebingen, Germany; 3) Inst. of Pathology, Eberhard-Karls-University, Tuebingen, Germany.

Ovarian cancer is one of the most common malignant tumors in women. So far, there are no histopathological parameters which indicate platinum resistance. Therefore, nearly all patients are treated with platinum-based chemotherapy postoperatively. Patients who suffer a relapse within 6 months are termed as platinum resistant. So far, molecular profiling of platinum resistant ovarian tumors plays no role in the selection of adjuvant therapy. The goal of this study was to identify a set of genes, which can predict resistance to platinum-based chemotherapy in ovarian cancer. 12 platinum resistant and 12 platinum sensitive ovarian carcinomas were selected by a pathologist who defined the subtype and the proportion of carcinoma. Only samples containing at least 50 % malignant tiussue were used to isolate RNA from frozen tissue sections and were analyzed on Illumina Human-6v2 BeadChips to determine differentially expressed genes. Data analysis with Genespring was followed by class prediction with Support Vector Machines (SVM) that was undertaken with a predictive set of 55 genes. It offered a correct classification into platinum resistant and platinum sensitive patients in all samples. Analysis of our findings with Ingenuity Software showed a functional relevance to regulation of transcription, apoptosis and cell cycle. For validation of the predictive gene set, we used qRT-PCR to measure the mRNA expression level of 13 selected genes in 20 samples. Again, we used SVM for analysis and found out that 18 samples were predicted correctly. The mean expression value in 10 of 13 genes was consistent with the trend observed in the microarray data. We can conclude that we found a predictive set of 55 genes that is able to classify ovarian carcinomas according to their sensitivity for platinum-based chemotherapy. The predictive power of the 55-gene set needs to be further validated in an independent set of ovarian cancer specimen.

438/W

Cervical intraepithelial neoplasia and factors in chromatin remodelling: A comprehensive serial analysis of gene expression. *J.Y. Kennett, A. Shadeo, R. Chari, C. MacAulay, W.L. Lam.* Cancer Genetics, BC Cancer Research Centre, Vancouver, B.C., Canada.

Cervical cancer affects approximately 500,000 women worldwide each year with highest rates found in developing countries. Cervical intraepithelial neoplasia (CIN) is a precursor lesion to cervical cancer and can be further described as CIN I, CIN II and CIN III (mild, moderate and severe dysplasia, respectively). Most CIN I lesions spontaneously regress to normal cervical epithelia however CIN III lesions are much more likely to progress to cervical cancer if left untreated. CIN II may be a critical junction in disease development. Social, clinical and genetic factors indicate that frontline monitoring will continue to play an important role in cervical cancer prevention and improved methods for detection and biological markers are needed. A thorough understanding of genetic events in precancerous cervical intraepithelial neoplasia is required to delineate important causal events in cervical cancer. In this study we have analyzed the transcriptome across sixteen cases (CIN I, CIN II, CIN III and normal cervical epithelium) using an unbiased long serial analysis of gene expression (L-SAGE) method. In total, sixteen L-SAGE libraries were sequenced to 2,481,387 tags, establishing the largest SAGE data collection for cervical tissue worldwide. We identified 108 tags increased in frequency in CIN III and 138 tags decreased and overall observed 246 tags differentially expressed between normal cervical tissue and CIN III. Biological functions most influenced by these genes include cell death, cell growth/proliferation and cellular movement. In evaluation of expression differences between normal and CIN lesions, we identified two gene networks targeted. Several of these genes directly or indirectly involve chromatin remodelling or the SWI/SNF ATPase chromatin remodelling complex. Further, these disruptions may be targeted to the critical stage of moderate dysplasia (CIN II) and provide candidate markers for screening at this junction.

439/W

Common human cancer genes discovered by integrated gene-expression analysis. *Y. Lu, Y.J. Yi, P.Y. Liu, W.D. Wen, M. James, D.L. Wang.* Department of Surgery and the Alvin J. Siteman Cancer Center, Washington University, St Louis, St Louis, MO.

Background: Discovery of genes commonly regulated in cancer may have an important implication in understanding the common molecular mechanism of cancer. Here, we described an integrated gene-expression analysis of 2,074 samples from 36 different studies to identify and validate a cancer type-independent gene signature that can identify cancer patients for a wide variety of human malignancies. **Methods:** The commonness of gene expression in 20 types of common cancer was assessed by permutation analysis in training datasets with 1,139 samples from 20 datasets. The discriminative power of a signature defined by these common cancer genes was evaluated by hierarchical clustering in the other 16 independent datasets with 935 samples including novel cancer types. QRT-PCR and tissue microarray were used to validate commonly regulated genes in multiple cancer types and identify a refined list of significantly and consistently regulated genes associated with malignancy. **Results:** The integrated analysis identified 187 genes dysregulated in nearly all cancerous tissue samples, regardless of their tissue of origin. The 187-gene signature can robustly predict cancer versus normal status for a wide variety of human malignancies with an overall accuracy of 85%. We further refined our signature to 28 genes that were confirmed by QRT-PCR analysis. The refined signature still achieved about 80% accuracy of classifying samples from mixed cancer types. This signature performs well in the prediction of novel cancer types that were not represented in training datasets. We also identified three biological pathways including glycolysis, cell cycle checkpoint II and plk3 pathways in which most genes are systematically up-regulated in many types of cancer. **Conclusions:** The identified signature is cancer type-independent and has captured essential transcriptional features of neoplastic transformation and progression in general. These findings will help to elucidate the common molecular mechanism of cancer, and provide new insights into cancer diagnostics, prognostics and therapy.

440/W

Microindels due to highly error-prone processes. *W. Scaringe[1], K. Li[1,2], D. Gu[1], K. Gonzalez[1], K. Hill[1,3], S. Sommer[1].* 1) Dept Molecular Genetics, City Hope Natl Medical Ctr, Duarte, CA; 2) SNP Institute, Nanhua University, Hengyang, Hunan, China; 3) Dept of Biology, The University of Western Ontario, London, ON, Canada.

Little is known about the nature of microindels. We present the first analysis of somatic microindels in i) an endogenous and universally transcribed mammalian gene, and ii) in human cancer. Analyses of reported TP53 microindels in cancer reveal that they occur at a frequency of about 0.3% without obvious tissue or age specificity, and have a "molecular anatomy" consistent with an endogenous etiology. TP53 microindels in cancer are remarkably similar to spontaneous microindels in the non-transcribed lacI transgene in normal Big Blue mouse tissues suggesting that the selective pressures associated with oncogenesis as well as any mutagens associated with cancers have minor effects relative to endogenous mechanisms. The molecular anatomy of microindels as a class is different from that of pure microdeletions, pure microinsertions, and tandem-base mutations, suggesting unique mechanisms. Three pairs of similar but not identical recurrences were observed showing the identical deletion with a nearly identical insertion ("recurroids"). Microindel sequence contexts suggest diverse mechanisms including error-prone mechanisms. In contrast to microinsertions which duplicate the adjacent sequence in the overwhelming majority of cases, the inserted sequences in microindels appear to derive predominantly from nearby but not adjacent sense or antisense sequences. The data suggest that indels arise from the bypass of blocking lesions by a mechanism that utilizes nearby sense or antisense sequences to help bridge the blocked lesion. The process is highly error prone with an estimated rate of 12% per bp, about two orders of magnitude greater than that measured for Y family polymerases, although those measurements were made without a bulky blocking adduct. In conclusion, the data herein describe the molecular anatomy of somatic microindels in cancer, constrain hypotheses of their nature and origin, and are consistent with these microindels generally deriving from spontaneous highly error-prone endogenous processes.

441/W

Expression profiles of ependymal tumors correlate with clinical characteristics and help to identify involved oncological pathways. *T. Palm[1], F. Scaravilli[2], I. Salmon[3], J. Mikol[4], D. Figarella-Branger[5], C. Lacroix[6], F. Chapon[7], D. Ellison[8], M. Vikkula[1], C. Godfraind[9].* 1) de Duve Institute, UcLouvain, Belgium; 2) UCLondon, UK; 3) Erasme University Hospital, Belgium; 4) Lariboisière Hospital, France; 5) Hôpital de La Timone, France; 6) Hôpital de Bicêtre, France; 7) CHU de Caen, France; 8) Oregon Health & Science University, USA; 9) Laboratory of Neuropathology, UcLouvain, Belgium.

Ependymal tumors are primary tumors of the central nervous system. The tumorigenesis of the ependymal tumors is not well understood and current histoprognostic markers only imperfectly predict the clinical evolution of the WHO grade II and III tumors. For several cancers, novel specific treatments have been developed, but for ependymal tumors we have not even characterized and understood their tumorigenesis pathways. In this study, we used a series of 38 ependymal tumors to perform an array-based expression study with the aim to identify tumor sub-type specific gene expression profiles and to characterise pathways leading to tumor development. By a non-supervised bio-informatic analysis, the ependymal tumors first clustered depending on tumor grade and secondly depending on tumor localisation. By a supervised analysis, opposing the expression profiles of grade II and grade III tumors, a series of differently expressed genes was isolated. The expression level of these genes could become molecular markers to be used to help to determine tumor grade and to improve quality of diagnostics. Furthermore, by analysing specific pathways known to be important in cancer, we identified alterations in expression levels of specific genes which confer one, or another, tumor-inducing characteristic to the ependymal cells. Although, these characteristics were prominent, they were not exclusive to one single tumor group. In conclusion, we show that each ependymal tumor has a specific expression profile, depending firstly on tumor grade and secondly on tumor localisation. However, expression profiles also differ regarding suggested tumor-inducing pathways, demonstrating different profiles even for tumors of the same grade and localisation. (Catherine.Godfraind@anpg.ucl.ac.be).

442/W

Multi-ethnic Comparisons of Genome-wide Alterations in Breast Cancer Using Paraffin Embedded Samples. *L. Baumbach[1], M.E. Ahearn[1], M. Jorda[1], C. Gomez[1], T.A. Halsey[2], K. Ellison[2], S.M. Farragher[2], G.L. Jellema[2], S. Gluck[1].* 1) Miller School of Medicine, University of Miami, Miami, FL; 2) Almac Diagnostics, Durham, North Carolina.

Approximately 178,000 US women will be identified with invasive breast cancer (BC) this year; about 41,000 will die from the disease. It is recognized that ethnic-specific disparities in stage of presentation/survival rates exist in BC patients. **These disparities remain an enigma**. To investigate a possible genetic basis, we are extending our study of genomic changes in BC samples from African-American (AA) women to a multi-ethnic cohort consisting of 20 each AA, Hispanic white and non-Hispanic white (Caucasian) women matched for age of diagnosis, cancer stage, and hormone receptor status. Tissue samples are evaluated for gene expression differences, as well as DNA copy number (CNV)/chromosome alterations by CGH arrays. We completed a feasibility study using paraffin embedded tissue samples. Gene expression differences in tumor vs. normal breast tissue were analyzed in sections from three AA and three Caucasian BC pathology specimens matched for age and receptor status (ER+/PR+/Her2-). Slides were macro-dissected for tumor vs. normal tissue. RNA was isolated, labeled cDNA generated, and hybridization of tumor and normal cDNA performed using Almac Diagnostics proprietary Breast Cancer DSA Research Tool. Approximately 18,000 transcripts were analyzed for expression differences in normal vs. tumor tissue. Distribution analysis, hierarchical clustering and principal component analysis were used to analyze the data within and across ethnic groups. There were 1735 unique differentially expressed genes in the AA tumor samples, 787 unique differentially expressed genes in the Caucasian tumor samples, and only 194 differentially expressed genes in common. We now are extending the study to additional samples, as well as assessing CNV by CGH arrays. It is likely that the completed study will result in an increased understanding of the biological basis of ethnic-specific BC disparities, which may ultimately lead to individualized, ethnic-specific diagnostic and therapeutic approaches.

443/W

Association of the -308G>A polymorphism of TNF alfa gene in Mexicans patients with breast cancer. *A. Escoto[1], D. Ontiveros[2], A.M. Puebla[1,3].* 1) Medicina Molecular, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Guadalajara, Mexico; 2) Unidad Médica de alta Especialidad, Hospital de Gineco-obstetricia, Fisiología Obstétrica CMNO, IMSS; 3) Laboratorio de Inmunofarmacología Experimental, CUCEI, Universidad de Guadalajara. Guadalajara, Jal., México.

Purpose Genetic polymorphisms in the promoter region of the tumour necrosis factor (TNF) gene can regulate gene expression and have been associated with inflammatory and malignant conditions. We have investigated the polymorphism in the promoter of the TNF gene (-308 G>A) in breast cancer susceptibility in Mexican population. Methods Using a Unmatched case-control design, breast cancer patients (n = 188) and woman controls (n = 122) were genotyped for these TNF polymorphism. Results Allele frequencies for patients and controls were different ($p<0.05$) with OR 7.71(IC95% 4.39-14.28). Conclusions We demonstrated association between the -308G>A polymorphism in the promoter region of TNF and susceptibility to breast cancer, in a sample of Mexican population.

444/W

An investigation into why the T877A androgen receptor mutant found in prostate cancer grows in the absence of androgens. *B. Gottlieb[1,3,5], J. Southwell[1], S. Chowdhury[2], L.K. Beitel[1,3,4], R. Lombroso[1], E. Purisima[2], M. Trifiro[1,3,4].* 1) Dept Cell Genetics, Lady Davis Inst Medical Res, Montreal, PQ, Canada; 2) Biotechnology Research Institute, National Research Council of Canada,Montreal, PQ, Canada; 3) Dept of Genetics, McGill University, Montreal, Canada; 4) Dept of Medicine, McGill University, Montreal, Canada; 5) Dept of Biology, John Abbott College, Montreal, PQ, Canada.

Prostate cancer (PCa) may progress by circumventing ablation therapy due to mutations in the androgen receptor (AR) gene. The most intensively studied is the mutation T877A in the ligand binding domain (LBD) which causes the AR to become promiscuous, i.e., respond to a number of different ligands. The T877A mutation alters; the inverse relationship between the N-terminal CAG repeat length, and transactivation; increases N/C terminal interactions; and decreases binding of TIF2, a coactivator that plays a critical role in N/C terminal interactions. We have used a molecular dynamic (MD) modeling approach involving the AR and TIF2 to investigate how altered N/C terminal interactions might affect the binding of different ligands. We compared the MD structures of the wild type (wt) and T877A AR bound to dihydrotestosterone. In T877A this revealed an increase in flexibility of amino acid residues in the ligand-binding domain (LBD), as well as a larger solvent accessible surface in the LBD compared to the wt. Thus, the improved induced fit of the N-terminal domain FXXLF containing peptide into the LBD, that would explain its promiscuity, could be due to the increased flexibility and solvent accessibility of the residues present in the C-terminal peptide-binding pocket of the mutant LBD. These new observations clearly help explain T877A's promiscuity and further our understanding of hormone-refractory PCa.lp.

445/W

The leukemia associated gene *RUNX1T1* (*MTG8/ETO*) is involved in brain and heart development. *L.A. Larsen[1], L. Zhang[1], G. Barbi[2], K. Møllgård[3], E. Bendsen[4], R. Møller[1], R. Ullmann[5], Z. Tümer[1], N. Tommerup[1].* 1) Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark; 2) Department of Human Genetics, University of Ulm, Ulm, Germany; 3) Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark; 4) Department of Obstetrics and Gynaecology, University Hospital of Odense, Odense, Denmark; 5) Max-Planck-Institute for Molecular Genetics, Berlin, Germany.

The chromosome breakpoints of the t(8;21) translocation associated with acute myeloid leukemia disrupt the *RUNX1* (*AML1*) gene and the *RUNX1T1* (*MTG8/ETO*) gene and generate a fusion protein. Involvement of RUNX1 in hematopoitec development is well documented but the normal function of RUNX1T1 is poorly understood. We report a 28 year old male patient with a constitutional balanced t(5;8)(q33;q22) translocation, where the chromosome 8 breakpoint disrupts the *RUNX1T1* gene. The patient has moderate mental retardation with autoaggressive behaviour, congenital heart defect (VSD) and minor craniofacial dysmorphism. Using FISH the chromosome breakpoints were mapped to a 27 Kb region at 8q21.3 (within intron 1 of *RUNX1T1*) and a 102 Kb region at 5q31.3 (no gene in breakpoint region). Immunohistochemistry analysis using human and rat embryonic tissues showed that RUNX1T1 is expressed in several tissues including the central nervous system, eye, heart, pancreas, colon and kidney during embryonic development. In the developing neurons of human embryonic brain RUNX1T1 expression was high in the cortical plate and moderate in the intermediate and subventricular zone, while expression was not observed in the ventricular zone. In the human embryonic heart RUNX1T1 expression was moderate. Real-time quantitative RT-PCR analysis confirmed a high expression of RUNX1T1 in the developing brain and revealed a gradually decreased expression of the gene in the heart during embryonic day 40-65. The phenotype of our patient combined with the expression during embryonic development suggests that RUNX1T1 is involved in the development of human brain and heart.

446/W

Midkine siRNA as anti-tumor molecules against osteosarcoma. *H. Maehara[1], T. Kaname[2,3], K. Yanagi[2], H. Hanzawa[1], I. Owan[1], K. Naritomi[2,3], F. Kanaya[1].* 1) Dept Orthopedics, Univ Ryukyus, Okinawa, Japan; 2) Dept Medical Genetics, Univ Ryukyus, Okinawa, Japan; 3) SORST, JST, Kawaguchi, Japan.

It is important to find a suitable molecular target for tumor therapy to make improvements in osteosarcoma. On the previous meeting, we reported a heparin-binding growth factor, midkine, is overexpressed in osteosarcoma, the level of midkine expression correlates with the prognosis of patients with osteosarcoma. In addition, we also presented that functional antibodies or a small interfering RNA (siRNA) against midkine effectively inhibit growth of osteosarcoma cells in vitro and the growth inhibition by midkine siRNA is participated in apoptosis. To apply such anti-tumor molecules for the therapy, in vivo study should be needed. Thus, we investigated whether the midkine siRNA has a potential to prevent the growth of osteosarcoma in vivo. Saos-2 osteosarcoma cells (2×10^6 cells with Matrigel) were injected and transplanted into the subcutaneous region of each nude mouse (BALB/c). After seven weeks from injection, the mice in which the tumor was transplanted and increased appropriate size were separated into two groups, a treatment group and a non-treatment group. The midkine siRNA solution or the control solution was injected into around the tumor region in each mouse for the treatment group or non-treatment group, respectively. The injection was performed every two weeks for eight-week-therapy. The body weight, tumor volume, serum alkaline phosphatase (ALP) value, and serum midkine value were measured in each mouse every two weeks. After eight weeks of therapy started, the mice were killed and the tumors were dissected. Then, the tumor weight was measured and histological examination was preformed. In mice of non-treatment group, tumor volume significantly increased more than 30 fold of the initial volume and the value of serum ALP was also increased. In contrast, the tumor volume significantly reduced in mice of treatment group. In mice had the most obvious effectiveness by the siRNA, the tumor was almost disappeared and the value of serum ALP was not changed. Our results may suggest that the midkine siRNA is effective for tumor therapy in osteosarcoma.

447/W

Genomic Heritage of Axillary Lymph Node Metastases in Breast Cancer Patients. *H.L. Patney[1], T.E. Becker[1,3], B. Deyarmin[1], R.M. Jordan[1], J.A. Hooke[3], R.E. Ellsworth[1,2], C.D. Shriver[3], D.L. Ellsworth[1].* 1) Clinical Breast Care Project, Windber Research Inst, Windber, PA; 2) Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD; 3) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC.

Metastatic breast cancer is an aggressive disease associated with recurrence and decreased survival. To better understand how genomic alterations in metastases may affect outcome in patients with metastatic disease, we used allelic imbalance to determine the molecular heritage of primary breast tumors and corresponding metastases to the axillary lymph nodes. Paraffin-embedded samples from primary breast tumors and matched metastases (n=146) were collected from 26 patients with node-positive breast cancer involving multiple axillary nodes. Hierarchical clustering was used to assess overall differences in patterns of allelic imbalance and phylogenetic analysis inferred the molecular heritage of axillary lymph node metastases. Genetically divergent lineages of metastatic tumors were present in the axillary lymph nodes, suggesting that multiple molecular mechanisms may govern the process of metastasis in individual patients. Progenitor cells for some metastases appeared to acquire metastatic potential early in the disease process and progressed with few genomic alterations, while other metastases may have developed later and harbored many chromosomal alterations present in the primary tumor. Genomic heterogeneity among axillary lymph node metastases may be associated with response to adjuvant therapy, recurrence, and survival, and thus may be important to improving clinical management of breast cancer patients.

448/W

Development and Validation of New Molecular Diagnostic Assays for the Jak2 V617F Screening and Quantification. *O. Biglia[1], J.P. LeCouedic[2], S. Hermouet[3], F. Hermitte[1], N. Maroc[1].* 1) Ipsogen, Marseille, France; 2) INSERM U790-Institut Gustave Roussy, Villejuif, France; 3) Laboratoire d'Hématologie & INSERM U601, CHU Nantes, France.

JAK2 V617F is an acquired mutation found in > 95% of patients with polycythemia vera (PV), and in > 50% of patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). The discovery of this mutation has profoundly modified the diagnosis of Ph- (BCR-ABL negative) Myeloproliferative Disorders (MPD). Using different technologies, often multi step and time consuming, variable incidences of this mutation in each pathology subtypes have been reported. Those variations are mainly due to a great heterogeneity in the technologies used and sometimes to their poor sensitivity, highlighting the needs for a simple, standardized, accurate and sensitive assay. We developed two assays: Jak2 MutaScreen assay, based on TaqMan® allelic discrimination, is dedicated to Jak2 mutation screening on DNA samples at the time of diagnosis, while Jak2 MutaQuant assay is an allele specific RQ-PCR and is dedicated to mutation load quantification on follow-up samples. We performed a multi centre performance evaluation of our Jak2 MutaScreen genotyping assay. Multi centre study in 14 labs on 7 different apparatus (ABI-prism®, LightCycler®, iCycler®) on 296 MPD samples demonstrated 98.65% correlation with technologies used at diagnosis. Parallel internal validation on 142 samples allowed identifying 13% more mutated cases than direct sequencing method. We will also present analytical validation of both assays, inter and intra-laboratory reproducibility, and technology performance comparison (sequencing versus Jak2 MutaScreen, Jak2 MutaScreen + Reference Scale versus Jak2 MutaQuant). Sensitivity, dynamic range and inter platform capability of Jak2 MutaScreen assay are compatible with a wide use for highly accurate and sensitive detection of JAK2 V617F mutation at diagnosis. Jak2 MutaQuant provides a tool for the monitoring of minimal residual disease in clinical research studies; clinical utility of this test will have to be addressed in multicentric prospective clinical trials.

449/W

Autosomal recessive malignant paraganglioma associated with mutations in the succinate dehydrogenase B gene. *C.A. Friedrich[1], S. Majumdar[2], C.A. Koch[3], G.W. Moll[4].* 1) Dept Prev Med, Medical Gen, Univ Mississippi Medical Ctr, Jackson, MS; 2) Div Hem/Onc; 3) Div Endocrin; 4) Div Ped Endocrine, UMMC, Jackson, MS.

Paragangliomas are rare tumors and seldom cause endocrine hypertension. Germline mutations in the succinate dehydrogenase subunit B gene (SDHB) have been associated with autosomal dominant malignant paragangliomas. We here report a family with non-classic congenital adrenal hyperplasia (CAH), in whom one member suffered from metastatic paraganglioma. A 13 yo boy presented with delayed puberty, nocturnal enuresis, and headaches. Thyroid and adrenal tests were within normal limits. MRI of the brain showed a lesion in the diploic space in the frontal region. Head CT revealed a lytic bone lesion resembling a histiocytic eosinophilic granuloma. A bone scan showed another lytic lesion in the right proximal femur. Bone biopsy of the skull lesion identified a paraganglioma. Further imaging showed an 8x3x3 cm mass close to the vena cava and aorta. The patient was normotensive; 24 h urinary norepinephrine was slightly elevated. After surgical tumor removal, postoperative MIBG showed persistent uptake in the abdomen and right femur. His sister had been diagnosed with 3-beta-dehydrogenase deficiency; his maternal grandmother had a pituitary adenoma. Germline mutation analysis of the SDHB gene, located at 1p36.13, revealed a known missense mutation (C.418G>T) and a previously unreported splice donor region DNA sequence variation in intron 2 (C.200+7A>G). His sister and father were heterozygous for the splice donor variation, whereas his mother was heterozygous for the known missense mutation. Whole body imaging of the sister who suffered from nonclassical CAH was within normal limits. His parents had no known clinical manifestations to suggest a pheochromocytoma or paraganglioma. Autosomal dominant paragangliomas due to SDHB mutations have been reported, associated with loss of heterozygosity in tumor specimens. The combination of sequence variations in the SDHB gene in the same patient may have been necessary to trigger tumor growth of selected chromaffin cells. Autosomal recessive etiology of paraganglioma has not been reported previously.

450/W

A germline SDHB start codon mutation in a patient with abdominal paraganglioma and two renal cancers. *K. Heimdal[1], A. Silye[2], S. Ariansen[2], S. Raicevic[3], T. Schreiner[3], H. Scott[4], O.J. Nilsen[5], A. Schultz[5], P.A. Andresen[2].* 1) Dept Medical Genetics; 2) Lab Molecular Patology; 3) Section of Endocrinology, Dept Internal Medicine; 4) Dept Pathology; 5) Section of Urology, Dept Surgery, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway.

Germline mutations in the SDHB gene are associated with paragangliomas, phaeochromocytomas, renal cancers of varying histology and thyroid cancer. We report on a family with a start codon mutation SDHB c.3G>A (p.Met1Ile) heterozygote. Start codon mutations have not been reported in this gene previously.

The proband was a 25 yr old male who presented with an abdominal paraganglioma (extra-adrenal phaeochromocytoma) and two tumors in one kidney (one carcinoma not classifiable, one mixed oncocytoma/chromophobe carcinoma). Investigations revealed that the mutation was inherited from the paternal side. LOH-analyses with markers from 1p32.1-1p36.32 (including the SDHB-locus) showed that all three tumors had lost the maternal allele, retaining the mutated paternal allele. Attempts to perform mutation analyses in tumor DNA have been unsuccessful so far due to inability to amplify a PCR-product. LOH-analyses with markers on 3p (covering the VHL locus) showed LOH in one out of two blocks from the renal carcinoma NOS and retention of heterozygosity in the other two tumors.

A renal tumour removed recently from a younger brother (age 23) is undergoing molecular investigations.

Another brother and the father have increased/borderline catecholamines but no detectable paragangliomas at ages 28/52.

Conclusions: Our data support the notion that the start codon mutation is pathogenic and that loss of SDHB gene function is important in the tumorogenesis in hereditary paraganglioma type IV. Somatic loss of VHL does not seem to be critical to the development of these tumors. Predisposition to renal cancer may be strong in some SDHB-families.

451/W

KLHDC8B, a novel candidate Hodgkin's lymphoma susceptibility gene, is targeted by Epstein-Barr virus microRNAs. *M.E. Mealiffe[1], T. Kirchhoff[2], P.H. Wiernik[3], H.T. Lynch[4], M. Daibata[5], A.-M. Gerdes[6], W.H. Raskind[1], K. Offit[2], L.R. Goldin[7], M.S. Horwitz[1].* 1) Medical Genetics, U. Washington, Seattle, WA; 2) Medicine, Memorial Sloan-Kettering Cancer Ctr., New York, NY; 3) Our Lady of Mercy Cancer Ctr., New York Medical College, New York, NY; 4) Preventive Medicine, Creighton U., Omaha, NE; 5) Hematology and Respiratory Medicine, Kochi Medical School, Kochi, Japan; 6) Biochemistry, Pharmacology and Genetics, Odense U. Hospital, Odense, Denmark; 7) Cancer Epidemiology and Genetics, NCI, Bethesda, MD.

Epstein-Barr virus (EBV) exposure and heritable factors both contribute to Hodgkin's lymphoma (HL) risk. We ascertained a family in which multiple individuals carrying a constitutional translocation (t(2;3)(q11.2;p21.31)) developed HL. Notably, a predisposition locus for another EBV-associated malignancy, nasopharyngeal carcinoma, maps to 3p21. Molecular cloning of both translocation breakpoints shows that the 2q breakpoint is intergenic, but the 3p breakpoint disrupts intron 1 of an uncharacterized gene, KLHDC8B (Kelch domain-containing 8B). To assess KLHDC8B's significance in familial HL, we sequenced its coding region in affected probands from 52 families with two or more cases of HL, but detected no coding region variants. However, we found a variant (+42C>T), in a conserved region of the 5'-UTR, present in 3 of 52 familial HL probands (5.8%) compared to 4 of 307 controls (1.3%; Odds Ratio [95% C.I.] = 4.6 [1.0-21.4]), prompting consideration of the possibility of post-transcriptional dysregulation of KLHDC8B. We asked if recently described EBV miRNAs target the KLHDC8B 3'UTR and utilized the Rna22 algorithm, shown to be highly predictive of bona fide target sites. Remarkably, Rna22 predicts that 20 of 32 of the known EBV microRNAs target KLHDC8B with 1-4 target sites each. The EBV-related rhesus macaque herpesvirus, rLCV, similarly contains miRNAs predicted to target rhesus Klhdc8b, implicating the association as likely important in virus-host interaction. We have experimentally validated targeting of the KLHDC8B 3'UTR by a subset of the EBV miRs and will present ongoing experiments exploring its biological importance.

452/W

MicroRNAs: Negative Regulators of PTEN and Modifiers of Cowden Syndrome. *M.G. Pezzolesi[1,2], K.A. Waite[1,2,3], C. Eng[1,2,3].* 1) Genomic Medicine Inst,; 2) Lerner Research Inst,; 3) Tausig Cancer Center, Cleveland Clinic Foundation, Cleveland, OH.

Germline mutations in PTEN are associated with a number of clinically distinct heritable cancer syndromes, including both Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS). While the majority of patients with CS (85%) and BRRS (60%) harbor PTEN mutations, for a subset of patients without identifiable mutations, the etiology of their disease remains unknown. Furthermore, among patients with PTEN mutations, an imprecise genotype-phenotype correlation exists, suggesting that additional genetic factors may act as phenotypic modifiers. MicroRNAs (miRs), represent one such class of potential modifier. In order to investigate their role in regulating PTEN in CS/BRRS, we chose to assess the expression of miR-21, a miR previously shown to target PTEN, in lymphoblastoid cell lines obtained from 30 CS/CS-like patients without detectable PTEN mutations and 20 healthy controls. In total, 4/30 patients had increased mir-21 expression and all 4 with over-expressed miR-21 were classic CS patients (P = 0.038). We subsequently searched for further putative miR's that are computationally predicted to target and regulate PTEN. To determine real functionality, we found that miR-519e negatively regulates luciferase expression through its interaction with PTEN's 3'UTR while, conversely, miR-26a, miR-20a, and miR22 do not. Additionally, we show that this inhibitory effect is disrupted upon deletion of miR-519e's putative seed site, while its over-expression in MCF-7 cells reduces endogenous PTEN expression. Transfection with an antisense miR-519e inhibitor restored PTEN expression to its basal level. Taken together, these data provide compelling evidence of miR-519e's involvement in the regulation of PTEN. Our results suggest that miR-21 and miR-519e act as modifiers of PTEN expression and that modulation of mir-21, and possibly miR-519e, may contribute to CS in patients lacking PTEN mutations. Furthermore, these data suggest that miRs may play a role in modulating phenotype and penetrance in patients with CS/BRRS, even those with identical PTEN mutations.

453/W

Molecular Characterization of BRCA1 and BRCA2 genes in breast cancer mexican mestizo patients. *S. Vidal, L. Taja-Chayeb, V. Rosas, O. Gutierrez, A. Dueñas.* Dept Basic Research, Inst Nal de Cancerologia, Mexico City.

Breast and ovarian cancer are the most frequent causes of death in women, generating an important public health problem. A small proportion of these tumors results from alterations in cancer susceptibility genes. Two of these genes are BRCA1 and BRCA2, which are described as hereditary breast and ovarian cancer genes. To date, over 500 sequence variants have been reported for BRCA genes. Specific mutations to particular ethnic groups have been described. There are no known mutations for the Mexican mestizo population. The purposes of this work were to determine the mutation frequency of BRCA1 and BRCA2 genes in breast cancer patients under 40 years old or patients with familiar breast and/or ovarian cancer history, through DHPLC analysis and, to establish genotype-phenotype correlations. Those families with mutations will be follow up for early detection, and will receive genetic counseling. 40 breast and/or ovarian cancer patients were included. DNA was obtained from peripheral leukocytes, and was amplified for the 24 exons of BRCA1 using 31 pairs of oligonucleotides, and for the 26 exons of BRCA2 with 39 pairs of primers. The primers were designed to include each exon flanked by a small portion of the corresponding introns. The amplifications were analyzed by DHPLC (Transgenomics). The alterations found were corroborated by direct sequencing. We have analyzed the entire BRCA1 and BRCA2 genes for 40 patients, we found in BRCA1 gene 1 polymorphism at exon 13 in 5 patients, 1 intronic deletion in exon 7 in 5 patients, the same deletion plus 3 base-changes in 9 patients, 1 missence mutation without clinical significance in 7 patients at exon 11 and 2 small deletions in exon 11 in 2 patients. In BRCA2 we found 1 polymorphism at exon 4 in 4 patients, 4 different polymorphism at exon 11 in 24 patients, 1 patient with a missence mutation in exon 11 and 1 patient with a deleterius mutation in exon 11. We have found an elevated percentage of patients with polymorphisms (up to 30%). However, until now we have found only 2 mutations with clinical significance in BRCA1 and 1 for BRCA2. It seems that mutations in BRCA genes are not frequent in our population.

454/W

DNA Methylation as an Epigenetic Modifier in Li-Fraumeni Syndrome. *C.D. Wilson[1,2], B. Zhang[1], L.L. Bachinski[1], L.C. Strong[1,2], R. Krahe[1,2].* 1) Cancer Genetics; 2) Human Molecular Genetics Grad Prog, Univ Texas MD Anderson Cancer Ctr, Houston, TX.

Li-Fraumeni syndrome (LFS) is a genetically heterogeneous cancer syndrome. Most cases (~70%) are associated with dominant germline mutations in the tumor suppressor TP53 (p53). We have shown genetic heterogeneity in LFS kindreds at loci other than p53. LFS is characterized by early tumor onset, multiple tumors in individuals and multiple affected family members. There is evidence for significant heterogeneity in p53 and non-p53 LFS, suggesting additional risk modifiers. Tumorigenesis is a multistep process, in which germline mutations alone are not sufficient for tumor development. Epigenetics has been recognized as important in sporadic cancers. While gene-specific hypermethylation is an important mechanism for silencing tumor suppressor genes, global hypomethylation has been identified as an important epigenetic factor in the remodeling of chromatin structure. To test whether DNA methylation plays a role in an inherited cancer syndrome such as LFS, we studied genome-wide and gene-specific hypo- and hyper-methylation using Pyrosequencing Methylation Analysis (PMA) in 10 LFS tumors. We determined the methylation status of 12 tumor suppressor genes hypermethylated in sporadic cancers that are part of the LFS tumor spectrum. Six genes showed significant hypermethylation (BRCA1, ESR1, HIN1, RASSF1, TCF21, TP73). In 10-50% of LFS tumors, BRCA1, ESR1, HIN1 and TP73 were hypermethylated compared to normal PBL samples. RASSF1, which is hypermethylated in sporadic soft tissue sarcomas (STS), was hypermethylated in all STS but no other LFS tumors. TCF21, which encodes a transcription factor involved in epithelial-mesenchymal transition, was hypermethylated in 100% of LFS tumors. Using PMA of SINE and LINE elements as surrogate markers, we determined genome-wide levels of hypomethylation to be intermediate between those of control tumor cell lines and normal PBL samples. The identification of tumor hyper- and hypo-methylation in LFS indicates that, similar to sporadic tumors, epigenetic alterations also play an important role in the genesis of inherited tumors.

455/W

Single nucleotide polymorphisms of follicle-stimulating hormone receptor gene are associated with testicular cancer susceptibility. *C. Foresta, M. Pengo, R. Selice, A. Garolla, A. Ferlin.* University of Padova, Department of Histology, Microbiology and Medical Biotechnologies, Centre for Male Gamete Cryopreservation, Padova, Italy.

Testicular germ cell tumour (TGCT) is the most common cancer in young adult men. Epidemiological and clinical features suggest that TGCT development is under endocrine control but definitive proofs are lacking. FSH levels are increased in numerous conditions associated with increased risk of TGCT and some single nucleotide polymorphisms (SNPs) in the FSH receptor (FSHR) gene influence the sensitivity of the receptor to FSH. However, a possible effect of FSH on testicular carcinogenesis has never been explored. Here we studied the association of FSHR SNPs with TGCT. Analysis of 12 potential SNPs of the FSHR gene in 188 TGTC cases and 152 controls, revealed 4 informative SNPs, represented by two polymorphisms in exon 10 (Ala307Thr and Ser680Asn), and two polymorphisms in the promoter region (-114 T/C and -29 G/A). Specific haplotypes and genotypes determined by the association between two or more of these SNPs were associated with TGCT. In particular, the Ala307/Ser680 allele lowers the risk of TGCT, especially in combination with the -29 G allele and the -114 T allele (P = 0.009, relative risk 0.73; 95% confidence interval 0.57-0.92). The genotype homozygous for the Thr307-Asn680 allele increases the risk of TGCT, especially in combination with the -29 A/G alleles and the -114 T allele (P = 0.018, relative risk 2.20; 95% confidence interval 1.14-4.29). The associations were stronger for non seminoma than seminoma. These data provide evidence that FSHR gene polymorphisms modulates susceptibility to TGCT. The variants associated with higher risk are those with higher activity of the FSHR, suggesting a role for FSH in the carcinogenesis process of this tumour.

456/W

***APITD1*, a tumor suppressor candidate gene with transcriptional inactivation and growth suppressive properties in the neuroblastoma deletion region on 1p36.2.** *C. Krona, H. Kryh, K. Ejeskär, L. Olsson, H. Carén, R-M. Sjöberg, T. Martinsson.* Dept Clinical Genetics, Gothenburg University, Gothenburg, Sweden.

High-risk neuroblastoma tumors are often characterized by amplification of the *MYCN* oncogene and deletion of a distal part of chromosome 1p, indicating the presence of one or more tumor suppressor genes which are inactivated on the remaining chromosomal allele. Recently, a novel gene denoted *APITD1* was identified in the region of common deletion on 1p36.22. Although ubiquitous expression levels of this gene was observed in samples derived from normal fetal and adult tissues, a significant down-regulation of *APITD1* was found in high-risk tumors when compared to low-risk tumors. Cell growth was reduced in neuroblastoma cells transfected by different amounts of in vitro transcribed *APITD1* mRNA compared to control cells transfected by the same amounts of *EGFP* mRNA and a large proportion of the *APITD1* transfected cells entered apoptosis. Western blot using antibodies towards peptides from the encoded *APITD1* sequence against nuclear and cytoplasmic cell fractions show that the protein is preferably located in the nucleus. Studies of interaction partners of the APITD1 protein performed both by a yeast two-hybrid screening and immunoprecipitation followed by MALDI-TOF mass spectrometry indicate that *APITD1* encodes a chromatin binding protein involved in cell cycle regulation or maintenance of cellular integrity. Based on its cytogenetic location, 1p36.2, and its biological features in neuroblastoma tumors, *APITD1* therefore presents as a candidate tumor suppressor gene. Further functional studies of *APITD1* in vitro and in vivo are ongoing.

457/W

The molecular mechanism of radiation effect on human colorectal cancer cell lines by using oligonucleotide membrane arrays. *C.W. Kuo[1], M.Y. Huang[2], S.R. Lin[1,3], J.Y Wang[4].* 1) Graduate Institute of Medical , Kaohsiung Medical University, Kaohsiung , Taiwan; 2) Departments of Radiation Oncology ,Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) BioMedi Innovation Incubation Center, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Department of Surgery, Kaohsiung Medical University Hospital, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background and purpose: Hypoxia, glycolysis and DNA damage response were recently suggested highly correlated with resistance to radiotherapy, but the molecular mechanism is still unclear. The purpose of this study is to identify radiation related molecules which can evaluate the effect of radiation for colorectal cancer cell lines Method: Colorectal cancer cell lines were purchased from ATCC, and irradiation with high energy photo beam. Total dose is 16 Gy. Radiation sensitivity was determined by the survival cells using the ATPlite assay. RNA was extracted from nonirradiated and radiated cells, respectively. Gene expression analysis was performed by membrane array containing 60 target oligonucleotides. Result: Analysis of radiation survival curve showed that cell lines SW620, HCT116 and Colo205 were sensitive to radiation; however, SW480, SW403 and T84 were resistant to radiation. The result of membrane array shows that 8 genes significantly overexpress in resistant colorectal cancer cell lines, such as HIF-1α, VEGF, and LDH. Conclusions: In the present study, we successfully identify several molecular markers for using to construct a radiation chip that can predict effects of radiotherapy. Comprehensive gene expression of radiation sensitive and resistant cell line might provide a new insight into the mechanisms of resistance or sensitivity to radiotherapy.

458/W

A Homozygous Splicing Mutation in PMS2 Causes Early Onset Tumors in an Inuit Family. *L. Li[1], B. Balachandra[2], K. Baker[2,4], J. Jarry[3], L. Kasprzak[4], J. Zhu[5], G. Chong[3], J.R. Jass[2], W.D. Foulkes[1,4].* 1) Dept Cancer Genetics, Lady Davis Inst, Montreal, PQ, Canada; 2) Department of Pathology, McGill University; 3) Dept Pathology, SMBD-Jewish General Hospital; 4) Div Med. Genet, McGill Univ Health Centre; 5) Institute for Genome Sciences & Policy, Duke University, Durham, North Carolina.

Early onset tumors (duodenal, stomach and colorectal cancer as well as an astrocytoma, all diagnosed before the age of 30) have occurred in 4 siblings from an Inuit family living in northern Quebec. No evidence of malignancies was found in the parental generation. Immunohistochemical staining for MLH1, MSH2 and MSH6 showed positive normal staining of tumor and adjacent non-neoplastic tissue with adequate internal controls. There was absence of staining with PMS2 in all neoplastic as well as in normal tissue from all affected siblings, indicating PMS2 is the cancer-causing gene in this family. The presence of highly homologous PMS2 pseudo-genes on chromosome 7 made conventional exon-by-exon sequencing difficult. Long range PCR was employed to specifically amplify the genuine PMS2 gene from blood DNA, followed by exon amplification and sequencing. A homozygous mutation (C.2002A>G, NM_000535.3) was found in individuals affected by cancer and both parents were found to be heterozygotes. The mutation creates a cryptic 5' splice site causing a 5 bp deletion in exon 11, consequently introducing a premature stop codon. "Polony" (polymerase colony) assay showed the 5 bp deletion is exclusively located in transcripts derived from the genuine PMS2 locus, but not those derived from the pseudo-locus resembling the C-terminus of PMS2. The predicted truncated form of PMS2 lacks the carboxyl terminus (amino acid residues 668-862), which is required for nuclear localization and endonucleolytic activity. Western blot utilizing an antibody recognizing PMS2 carboxyl terminus showed no expression of the wild-type PMS2 in transformed lymphocytes from a homozygous mutation carrier. Further work is undergoing to elaborate the pathogenic mechanism of this mutation.

459/W

High frequency of SDHB mutations in a series of head and neck paraganglioma from Belgium. *A. Persu[1], V. Grégoire[2], P. Garin[3], H. Reychler[4], G. Mortier[5], J.-F. De Plaen[1], M. Hamoir[6], M. Vikkula[7].* 1) Nephrology Dept; 2) Radiotherapy Dept, Clin univ St-Luc, UCL, Brussels, Belgium; 3) Otolaryngology Dept, Clin univ Mont-Godinne, UCL, Yvoir, Belgium; 4) Oral and Maxillofacial Surgery, Clin univ St-Luc, UCL, Brussels, Belgium; 5) Center for Medical Genetics, Gent University Hospital, Gent, Belgium; 6) Otolaryngology Dept, Clin univ St-Luc, UCL, Brussels, Belgium; 7) Laboratory of Human Molecular Genetics, de Duve Institute, UCL, Brussels, Belgium.

Mutations of SDH genes encoding subunits of complex II of the mitochondrial respiratory chain are involved in the pathogenesis of paraganglioma (PG) and pheochromocytoma. While SDHD is more frequently involved in the pathogenesis of head and neck PG, SDHB mutations are mainly associated with malignant and/or extra-adrenal pheochromocytoma. To look for the nature and frequency of SDH mutations as well as for possible genotype-phenotype correlations in head and neck PG from Belgium, screening of the coding parts of SDHD and SDHB was performed in 31 patients without familial history of PG and 6 families including 18 subjects with known PG. The screening done by SSCP, heteroduplex analysis and/or DHPLC, was followed by sequencing whenever a shift was observed. Eight different SDHD mutations (3 deletions, 2 splice site mutations and 3 substitutions) were found in 10 different patients including the 6 familial cases and 4 apparently sporadic cases. Furthermore, 6 different SDHB mutations (1 deletion, 1 splice site mutation and 4 substitutions) were found in 8 unrelated patients with apparently sporadic PG. One of them, found in 3 of the 8 subjects, had been already described in a family with malignant pheochromocytoma. (Young et al. 2002). Surprisingly, in this Belgian series, SDHB mutations were almost twice as frequent as SDHD mutations (26 vs. 13) in sporadic head and neck PG without evidence of dissemination, partly due to a single mutation previously associated with familial metastatic pheochromocytoma. (alexandre.persu@card.ucl.ac.be).

460/W

FUNCTIONAL ANALYSIS OF HNPCC RELATED MISSENSE MUTATIONS IN MSH6. *J. ou[1], R. Niessen[1], K. Kooi[1], J.H. Kleibeuker[2], R.H. Sijmons[1], R.M.W. Hofstra[1].* 1) Dept. of Genetics, University Medical Center of Groningen, groningen, groningen, Netherlands; 2) Gastroenterology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

Inherited pathogenic mutations in the mismatch repair (MMR) genes MLH1, MSH2 and MSH6 predispose to HNPCC. A major challenge in HNPCC diagnostics are the DNA variants with an unclear pathogenic nature (unclassified variants, UVs) such as single amino acid substitutions and small or large in-frame deletions. In particular MSH6 UVs account for a substantial proportion of these UVs. This study was to evaluate the pathogenicity of 5 of such inherited MSH6 UVs found in patients suspected of HNPCC. The mutated MSH6 proteins (all single amino acid substitutions) were tested for expression and stability in a MSH2/MSH6 deficient cells (LOVO cells), MSH2/(mutant)MSH6 interaction by yeast two-hybrid and for the sub cellular localization of the mutatnt proteins. Protein expression of 4 of the 5 MSH6 mutants (S144I, A1021D, A326V and T1219I) was significantly decreased after transfection when compared with WT. Possibly this is due to mutant protein instability or to lower mRNA levels. No effects was seen on protein-protein interaction (with MSH2) In our yeats two hybrid screen and the subcellular localization was normal for all 5. Conclusion Our data shows that 4 of the 5 tested MSH6 UVs influence protein abundancy. These UVs might therefore be pathogenic. In vitro assays are currently being performed to further evaluate the pathogenicity of thes UVs. Our data does however suggest that missense variants in MSH6 do play a role in HNPCC development.

461/W

Dosage-sensitive genome instability: A comprehensive genetic screen in *Saccharomyces cerevisiae* to identify heterozygous mutations that impact chromosome stability. *S.E. Plon[1], E.D. Strome[2], X. Wu[3], M. Kimmel[3].* 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Molecular and Cellular Biology Graduate Program, Baylor Col Medicine, Houston, TX; 3) Dept Statistics, Rice University, Houston, TX.

Aneuploidy is a common feature of human cancers and many of the genes whose disruption results in aneuploidy were first identified in budding yeast based on the phenotypes of haploid strains containing null mutations. In contrast, increased cancer susceptibility is often seen in heterozygous mutation carriers. To directly identify dosage sensitive genes that mediate genomic stability, we performed a comprehensive genome-wide screen in *Saccharomyces cerevisiae* for heterozygous mutations which increase chromosome instability in a checkpoint-deficient background. We used two assays for spontaneous events sensitive enough to detect the impact of heterozygous mutations: (1) increased sectoring of colonies based on loss of a chromosome fragment (CF) and (2) quantitative assessment via fluctuation analysis of loss or recombination of an endogenous chromosome. Of the 30,000 heterozygous strains screened, 170 demonstrated CF loss. Of this group, 45% also conferred modest but statistically significant instability of endogenous chromosomes. Further analysis of heterozygous deletions of a subset of genes demonstrated that the majority increased chromosome instability in both checkpoint deficient and wild-type backgrounds. Strains heterozygous for the genes encoding the conserved COMA kinetochore complex were particularly unstable. Over 50% of the genes identified in this screen have human homologs and several have already been associated with cancer susceptibility including *CHEK2* and *TOPBP1*. Given their potential impact on spontaneous chromosome instability, the homologous gene list should be included in epidemiologic studies of cancer susceptibility and genetic modifiers of other known cancer loci. These results also support further analyses of heterozygous phenotypes in yeast as models of human disease resulting from haploinsufficiency.

462/W

Frequent loss of heterozygosity at the IRF-1 gene locus in breast cancer. *L.R. Cavalli, R.B. Riggins, R. Clarke, B.R. Haddad.* Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC.

Several key observations indicate that the interferon regulatory factor-1 gene (IRF-1) localized on 5q31.1 may be a potentially important breast cancer tumor suppressor gene. IRF-1 is mutated or rearranged in several cancers, including some hematopoietic and gastric cancers. IRF-1 can reverse the oncogenic transformation of cells induced by the overexpression of oncogenes including both RAS and MYC in mouse models. Since functional roles for RAS and MYC are established in human breast cancer, a loss of IRF-1 function might also be important in this disease. IRF-1 can induce apoptosis through both p53-dependent and p53-independent signaling. Loss of 5q12-31 was reported in 11% of sporadic breast cancers and 5q deletion was detected in 86% of BRCA1 positive tumors. More recently, high-resolution array CGH studies have shown loss at 5q31.1, in 50% of BRCA1 positive breast cancers. Taken together, these observations offer a rationale to investigate the hypothesis that the IRF-1 gene plays an important role in breast cancer and to explore the usefulness of evaluating its loss of function in breast cancer. For this reason, we initiated a study in order to determine the frequency of IRF-1 allelic loss in breast tumors. Towards this end, we designed and optimized an approach to determine loss of heterozygosity (LOH) at the IRF-1 locus using an intrageneic dinucleotide polymorphic marker. Using this approach, we analyzed breast tumor specimens from 42 women with invasive breast cancer. 30 cases were informative and LOH at the IRF-1 locus was detected in 9/30 cases (30%). Our findings are consistent with a tumor suppressor role of the IRF-1 gene in breast cancer and justify further studies to confirm our initial LOH findings in a larger cohort of patients and to demonstrate functional inactivation of the IRF-1 gene in breast cancer (e.g. via gene mutations and/or an epigenetic process).

463/W

Expression profiling of primary prostate tumor free-relapse and primary prostate tumor with relapse using 44K whole human genome microarray. *S. Jacolot[1], A. Valeri[2], G. Fournier[2], L. Doucet[2], A. Volant[2], G. Fromont[3], O. Cussenot[4], J. Leger[5], C. Ferec[1,2].* 1) INSERM U613, Brest, France; 2) CHU Brest, France; 3) CHU Poitiers, France; 4) Hopital Tenon, Paris, France; 5) Genopole Grand Ouest, Nantes, France.

Prostate Cancer is the most frequently diagnosed cancer in Caucasian men. Screening for prostatic antigen (PSA) has led to an earlier detection of prostate cancer as well as an increasing number of men diagnosed with organ-confined, which are potentially curable. Early diagnosis favors curative surgery. However, up to 30% of men undergoing radical prostatectomy will relapse. DNA microarray technology offers the capacity to screen the expression of thousands of gene at the same time. Gene chips have been widely used for the identification of genes associated with the progression of PCa by assessing expression profiles of clinical samples and cell lines. All the studies show that some primary tumors are pre-configured to metastasize and this propensity can be detected at initial diagnosis, then the clinical outcome of individuals suffering from cancer can be predicted by using the gene-expression profiles of primary tumors at diagnosis. Unlike other studies which compare normal tissue versus primary tumor or primary tumor versus metastasis, we chose to examine gene expression in 60 tumor samples derived from two groups of primary tumors: the first group includes 30 samples from relapse-free patients after at least 3 years and the second group includes 30 patients with relapse. We analysed total RNA of these two groups using 44K whole human genome microarray (Agilent Technology). We used the Significance Analysis of Microarrays (SAM) software and we performed currently hierarchical clustering analysis using Cluster-TreeView software. We have been able to point out a differential expression of 515 probes (out of the 44000 present on the pangenomic chips) between two groups of patients: one representative of an early relapse (before 6 months) and the other relapse-free group beyond 4 years. 308 probes were upregulated and 207 were downregulated. Further studies are necessary to validate this molecular profiling.

464/W

Personalized Monitoring For Breast Cancer Recurrence. *Q. Liu, Z. Chen, C. Mroske, C. Yang, J. Yan, J. Feng, G. Somlo, M. Palomares, S. Sommer.* Dept Molecular Genetics and Molecular Diagnosis, City of Hope Medical Ctr, Duarte, CA.

Mortality from breast cancer may be reduced substantially if recurrence is detected earlier than is possible by conventional means. To effectively monitor early recurrence, we analyzed plasma to detect the cancer mutation signature of DNA and RNA fragments released from apoptotic or necrotic cancer cells. Specifically, cancer candidate genes were sequenced from breast cancer tissue samples to identify a personalized cancer signature of somatic mutations. Following sequencing, pyrophosphorolysis activated polymerization (PAP) (www.cityofhope.org/PAP), a method for detecting ultra-rare mutations, was performed to detect the cancer-specific signature in DNA/RNA isolated from the plasma of patients. In addition, the cancer mutation signature of circulating intact epithelial cells (CEC) was determined. Our preliminary data demonstrate the identification of cancer-specific somatic mutations in breast cancer cells, and the rapid development of PAP assays to detect even a single copy of the cancer-specific somatic mutations in patient circulation. Both somatic mutation levels and rates of increase within circulation will be measured at multiple intervals in a multi-year follow up. Our ultimate goal is to achieve effective monitoring of adjuvant and neo-adjuvant chemotherapy so that recurrence can be identified months to years earlier than is possible through conventional detection.

465/W

Breast cancer risk and C677T thylenetetrahydrofolate reductase polymorphism in Mexican population. *M. Gallegos[1], D. Ontiveros[2], J. Alfaro[1,3], AM. Puebla[3].* 1) Dept Med Molec, Guadalajara, CIBO, IMSS, Jalisco, Mexico; 2) Unidad Médica de alta Especialidad, Hospital de Gineco-obstetricia, Fisiología Obstétrica CMNO, IMSS; 3) Laboratorio de Inmunofarmacología de productos Naturales, CIBO, IMSS, Universidad de Guadalajara.

Methylenetetrahydrofolate reductase (MTHFR), a polymorphic enzyme involved in folate metabolism, plays a role in DNA biosynthesis, methylation, and repair in actively dividing cells. The polymorphism C677T of MTHFR gene, lead to decreased enzyme activity and affect chemosensitivity of tumor cells. We investigated whether this MTHFR polymorphism could be a risk factor for breast cancer in Mexican patients. Methods: In this case - control study, we genotyped 280 patients with breast cancer and 170 women controls, was assessed for the presence of the C677T mutation by PCR amplification. The allele frequencies of the MTHFR 677T were 50% in the breast cancer cases and 42% in the controls. Frequencies of MTHFR 677TT, 677TC and 677CC were 31, 39 and 30% in the breast cancer patients and 19, 53 and 34% in the controls, respectively. The results of a 2 analysis indicated that the MTHFR 677T allele was significantly distributed (2 = 4.89; p = 0.027). Likewise, the MTHFR T677T genotype showed a 1.91 fold increased risk for breast cancer. In conclusion, our data suggest that the MTHFR 677TT genotype is genetic risk factors for women with sporadic breast cancer.

466/W

Genome-wide Linkage Analysis of Utah high-risk Melanoma Pedigrees using a high-density SNP genotyping set. *L.A. Cannon-Albright[1], J.M. Farnham[1], S.A. Leachman[1,2].* 1) Univ Utah Sch Medicine, Salt Lake City, UT; 2) Huntsman Cancer Institute, Salt Lake City, UT.

We ascertained and sampled 21 high-risk melanoma pedigrees from the Utah Population Data Base resource, which is linked to Utah Cancer Registry records. Each pedigree had 3 or more melanoma cases (one of whom, aged 50 years or less, screened negatively for p16 CDK4, and ARF) and each had a significant excess of melanoma among the descendants of the pedigree founder. Melanoma cases were genotyped with the Illumina 550k SNP set. We performed genome-wide (GW) linkage analysis of different sets of markers, attempting to reduce bias due to LD between SNPs. We selected markers based on genetic distance (0.2 - 0.4cM) to reduce the possibility of LD; we also selected a subset of markers with no evidence for LD using published HAPMAP data. We excluded SNPs in LD based on pairwise r2, and selected maximally informative SNPs at a density determined to extract full information for linkage analysis. The SNP sets analyzed varied from n = 5,000 to n=27,000 markers. The set of SNPs selected for no evidence of LD had minimum heterozygosity = 0.30, with maximum r2 = 0.16. MCLINK, a Monte Carlo, Markov Chain linkage analysis tool, was used to perform multipoint linkage analysis using the TLOD statistic, and using an affecteds only model for melanoma with disease allele frequency = 0.003 and a low sporadic rate. GW analysis of the SNPs selected for average distance between markers = 0.2 showed significant evidence of linkage (het TLOD > 3.0) for chromosomes 8 and 22; analysis at greater average distances suggests these results are biased due to the presence of some LD. GW analysis of the SNPs selected to have no LD showed no regions with significant evidence for linkage, but identified 2 suggestive regions on chromosome arms 6p and 21q (heterogeneity TLODs 2.05 and 2.15, respectively). Several "linked" pedigrees were identified for 6p and for 21q that had at least nominal linkage evidence (Lod>0.59). For chromosome 6p there were 8 linked pedigrees with a range of TLOD from 0.78 - 2.00; for chromosome 21q there were 5 linked pedigrees with a range of TLOD from 0.71 - 2.05).

467/W

Fine Mapping of The 15q Glioma Susceptibility Locus And Candidate Gene Analysis. *H. Jiao[1], N. Paunu[2], I. Fransson[1], H. Haapasalo[2], J. Kere[1].* 1) Karolinska Institute, Sweden; 2) University of Tampere, Finland.

Gliomas are the most common brain tumors. The incidence and mortality of gliomas increase in many developing countries. Gliomas occasionally occur as familial with a likely genetic component, but no single gene has been identified as a causative factor. We mapped a low-penetrance locus for familial glioma on chromosome 15q23 (Paunu et al. 2002; OMIM 607248). Current work is focused on the fine-mapping and candidate gene studies on the 15q region. Four possibly functionally relevant candidate genes, NTRK3 (OMIM191316), SIAT8B (OMIM602546), NR2F2 (OMIM107773), and ADAMTS17 (OMIM607511), have been sequenced and studied. We did not detect putative mutations in exons or exon-intron boundaries in any of these genes, but identified a set of previously unknown variants. To pinpoint new candidate gene we have performed more intensive genome-wide scan using high-density Affymetrix GeneChip Human Mapping 500K Array. Fifteen individuals, 4 trios and one affected sibling with one parent were selected for genotyping. Based on linkage analysis and TDT, a new functional candidate gene has been found, and sequencing of the gene is on the way. All variants found by sequencing analysis will be evaluated in case-control samples for association to understand their roles in the development of brain tumors, in particular gliomas.

468/W

Reduced nuclear β-catenin may suppress intestinal tumorigenesis in Dnmt1 hypomorphic ApcMin/+ mice. *M. Luo[1], M.A. Renelt[1], J. Chen[1], Y. Chen[1], B. Zheng[1], L. Wang[1], X. Hao[2], P.W. Laird[3], C. Shao[1], J.A. Tischfield[1].* 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ; 3) Department of Surgery and Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.

Intestinal tumorigenesis is substantially suppressed in Dnmt1-hypomorphic ApcMin/+ mice, but the mechanism by which DNA hypomethylation contributes to tumor suppression remains to be determined. Apc is known to be a negative regulator of the Wnt signal transduction cascade. Mutation or loss of Apc is found in a vast majority of human colorectal carcinomas and in the adenomas of ApcMin/+ mice. Dysregulation of the Wnt pathway caused by Apc inactivation allows β-catenin to translocate to and accumulate in the nucleus, where it functions as a co-activator of transcription. In the current study, we examined intestinal tumorigenesis and β-catenin distribution in the Dnmt1 hypomorphic ApcMin/+ mice model. We find that while tumor formation can still be initiated, manifested as microadenomas, in those mice, tumor progression is significantly suppressed. Macroscopic tumors were rarely observed, even in relatively old mice. While the absence of Apc is correlated to an intensive accumulation of β-catenin in macroscopic tumors of Dnmt1 wild-type mice, the macroscopic adenomas, but not microadenomas, formed in Dnmt1 hypomorphic mice showed little accumulation of β-catenin protein in their nuclei even though Apc is absent. Meanwhile, intensive nuclear β-catenin staining was observed in Paneth cells from the same mice. There was no significant difference in the β-catenin mRNA level between mice with different Dnmt1 status, though the β-catenin protein level was lower in Dnmt1 hypomorphic mice. Our results suggest that the reduced nuclear accumulation of β-catenin, which can be a consequence of reduced expression, accelerated degradation and/or impaired transport, may be responsible for the suppression of tumor progression in ApcMin/+ mice with DNA hypomethylation.

469/W

Introduction of prkar1a -/- Mouse Embryonic Fibroblasts in Nude Mice Leads to Tumor Formation: Expression profiling reveals consistent alterations in Cell Cycle Regulation Genes. *S.A. Boikos, C. Giatzakis, A. Robinson-White, K. Tsang, H.P. Hsiao, F. Wen, Y. Patronas, M. Nesterova, C. Stratakis.* SEGEN, DEB, NICHD, NIH, Bethesda, MD, USA, 20892-1103.

PRKAR1A-inactivating mutations cause primary pigmented nodular adrenocortical disease, Carney complex, a multiple neoplasia syndrome, and sporadic endocrine tumors. R1a, is the main regulator of cAMP-dependent PKA a pathway that when activated leads to inhibition of growth and/or proliferation in several cell lines. Enhanced expression of R1a has also been shown in several human cancer tissues and cell lines. In order to investigate the mechanisms, regulation of Prkar1a gene and the interraction with other genes, we established a model using Mouse Embryonic Fibroblasts (derived from the heterozygote knockout prkar1a +/- mouse) grown in athymic nude mice. In order to identify genes regulated by Prkar1a, gene expression was assessed by oligonucleotide microoarrays in 4 groups: Prkar1a+/-, Prkar1a-/- not selected in soft agar, Prkar1a-/- selected in soft agar and RNA pool from tumors grown in the nude mice. We used the Illumina s Sentrix HumanRef-8 Expression BeadChip. Z-transformation for normalization was performed on each Illumina sample/array. We used the Ct model for the statistical analysis of real time. Using a cut-off of >/=2 times, we identified a total of 156 genes that had altered expression in any of the above 4 groups. Confirmation of the microarray data was done for selected genes by real-time PCR; the latter confirmed significant up-regulation of Cdkn2c, Cdkn2a, Cdkn1b and Cdkn2b, while Cyclin D2 was significantly down-regulated. We conclude that cell cycle regulation genes appear to have a crucial role in prkar1a-related tumorigenesis in mice, consistent with similar studies in human tissues. Ongoing protein and proliferation assays using siRNA on genes controlling the cell cycle aim at further investigating the interaction of these genes with the PKA signaling and related pathways.

470/W

Absence of EGFR mutations in Papillary Renal Cell Carcinoma. *S.J. Bender[1], B. Wondergem[1], K. Buzzitta[1], M. Avallone[1], P. Condit[1], M. Condit[1], A. Massie[1], S.K. Khoo[2], B.T. Teh[1].* 1) Cancer Genetics, Van Andel Research Institute, Grand Rapids, MI; 2) Germline Modification, Van Andel Research Institute, Grand Rapids, MI.

Purpose: EGFR is a transmembrane receptor tyrosine kinase that when activated engages in mitogenic signaling and other tumor promoting activities. Activation of this receptor can happen several ways, including receptor overexpression, gene amplification, overexpression of receptor ligands and activating mutations. Reports of high levels of EGFR and mutations on various exons in a variety of tumor types have led to treatment strategies to be developed to block EGFR and therefore inhibit growth of cancer cells. Anecdotal observations have been reported in papillary renal cell carcinoma which is the second most common type of renal cell carcinoma. The purpose is to examine if papillary RCC harbors any EGFR mutations that may suggest response to EGFR inhibitors. Methods and Results: DNA from 22 cases of papillary RCC and 6 cases of matched normal kidney were extracted and bidirectional sequencing of all coding exons and splice junctions of the EGFR gene were carried out on the tumor DNA. If a variant was detected, DNA was re-amplified and re-sequenced on those papillary cases and the matching normal. Known polymorphisms were detected but no DNA mutation is detected. Conclusion: EGFR mutation does not play an important role in papillary RCC. Other activating mechanisms such as protein expression, gene copy number may be worth exploring in papillary renal cell carcinoma.

471/W

Absence of Ras and Raf mutations in Clear Cell Renal Cell Carcinoma. *S.K. Khoo[1], K. Buzzitta[2], B. Wondergem[2], M. Avallone[2], P. Condit[2], M. Condit[2], A. Massie[2], S.J. Bender[2], B.T. Teh[2].* 1) Germline Modification, Van Andel Research Institute, Grand Rapids, MI; 2) Cancer Genetics, Van Andel Research Institute, Grand Rapids, MI.

Purpose: The Ras/Raf/MEK/ERK kinase pathway is a major intracellular mediator of mitogenic signals. This important pathway contributes to cell differentiation, proliferation and survival. Mutations of Ras and Raf are common in human cancer. Davies et al reported that a B-raf mutation leading to constitutive activation is common in many human cancers. More recently, an orally active small molecule inhibitor of B-raf and C-raf kinases, BAY 43-9006, has been approved for treating renal cell carcinoma, especially the clear cell type. The purpose is to examine if clear cell RCC harbors any mutations in the Ras and Raf-related genes that may suggest response to this drug. Methods and Results: DNA from 40 cases of clear cell RCC and matched normal kidney was extracted and bidirectional sequencing of all coding exons and splice junctions of the B-raf, H-ras, N-ras, K-ras and C-raf genes were carried out on the tumor DNA. If a variant was detected, the matched normal kidney DNA was sequenced to determine its somatic nature followed by re-amplication and re-sequencing of both DNA. Only known polymorphisms were detected but no DNA mutation is detected. Conclusion: We did not detect any mutation in Raf and Ras genes in clear cell RCC. It will be worthwhile to examine other activating mechanisms of the Ras/Raf/MEK/ERK kinase pathway such as expression pattern and the phosphorylation status of these genes in this group of tumors.

472/W

Somatic *TP53* mutations associated with microenvironmental genomic alterations and clinicopathological features in sporadic but not *BRCA1/2*-related breast carcinomas. *P. Platzer[1], A. Patocs[1], L. Zhang[2], Y. Xu[2], F. Weber[1], T. Caldes[3], G. Mutter[4], C. Eng[1].* 1) Genomic Medicine Institute; 2) Sect of Statistical Genetics; Cleveland Clinic Foundation, Cleveland, OH; 3) Laboratory of Molecular Oncology, San Carlos University Hospital; 4) Department of Pathology, Brigham and Women's Hospital, Harvard Medical School.

TP53 mutations, occurring in sporadic and *BRCA1/2*-related (HBOC) breast carcinomas, have variably been associated with clinical outcome. The tumor microenvironment (eg tumor stroma) of sporadic and hereditary breast cancers exhibit differences in the frequency and location of genomic alterations, suggesting different pathways for progression. No studies concurrently assess the *TP53* mutation status and its associations with genomic alterations and clinicopathological variables at the tumor microenvironment level, which is known to play an important role in the initiation and progression of sporadic breast cancers. We performed *TP53* mutation analysis and whole-genome loss-of heterozygosity (LOH) analysis on epithelial and stromal DNA from 175 sporadic and 43 archived HBOC-related cancers. We analyzed compartment-specific patterns of LOH and *TP53* mutations and computed associations between *TP53* status, LOH and patient clinicopathological characteristics. We found *TP53* mutation is associated with increased LOH in both HBOC and sporadic breast cancers. The stroma of sporadic breast carcinomas had 67 loci associated with mutated *TP53*, in comparison to only 1 locus (2p25.1) in the HBOC stroma samples. Somatic *TP53* mutations in the stroma, but not epithelium, of sporadic breast cancers were also associated with nodal metastases (pN). No such associations were observed for HBOC-related cancers. Our data indicate that stroma-specifc LOH is associated with somatic *TP53* mutation and an increased likelihood of regional metastases in sporadic breast carcinoma, but not HBOC-related breast cancers. A subset of 5 loci associated with increased LOH in the stroma of sporadic cases also contribute to the association between *TP53* mutation and pN, suggesting they may harbor modifiers which increase stromal p53's effect on sporadic breast cancer progression.

473/W

Association of non-synonymous coding SNPs with risk of colon cancer. *M.S. Cicek[1], S.L. Slager[2], T.C. Smyrk[1], K.C. Halling[1], K.G. Rabe[2], S.J. Achenbach[2], L.A. Boardman[3], D.J. Sargent[4], G.M. Petersen[5], J.R. Cerhan[5], S.N. Thibodeau[1], P.J. Limburg[3].* 1) Departments of Laboratory Medicine and Pathology; 2) Biostatistics; 3) Gastroenterology; 4) Cancer Center Statistics; 5) Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN.

Rationale: Despite its morbidity, much remains unknown about the etiology and progression of colon cancer. It is the third leading cause of cancer-related mortality affecting both gender in the US. Colon cancer is a complex disease and believed to be heterogeneous, with the presence of several low penetrant susceptibility genes. Alterations that are present on these genes are likely to play a crucial role in colon tumorigenesis. **Methods:** We have taken a two-stage approach. In stage 1, we screened 10,000+ non-syn cSNPs using the Affymetrix GeneChip® Human 10K cSNP Panel I in 195 cases and 130 controls. Cases were ascertained between the years 1995-1999 and age- and gender-matched controls were ascertained after 2000 at Mayo Clinic Rochester. In stage 2, we genotyped the top significant cSNPs (MAF≥0.05 and p<0.05) from stage I in 991 cases and 1009 matched controls using the Illumina Golden Gate Assay. The DNA MMR status, as determined by testing tumor for MSI and the absence of protein expression by IHC for hMLH1, hMSH2, hMSH6, was available for each case in stage I (65 MSI-H and 130 MSS) and has been collected on the majority of new cases in stage II (130 MSI-H and 752 MSS). Associations were assessed by single SNP logistic regression analyses. **Results:** In stage I, we found 281 cSNPs and 917 cSNPs were significant at a p-value of 0.01 and 0.05, respectively. Of the 917 cSNPs, we selected 587 cSNPs that had MAF>0.05 for genotyping in stage II. We found that 58 of these cSNPs remained significant in stage II with a p-value < 0.05. Among these, 10 cSNPs were significant with a p-value of 0.01 and one cSNP was significant at each of the p-values; p<0.0001 and p<0.001. **Conclusion:** These data provide new target genes for further research in colon tumorigenesis. Additionally, since outcome information is available on a subset of the cases, we will also test whether these cSNPs are associated with overall survival.

474/W

HUMAN PBX1 INTERACTING PROTEIN (HPIP) PROMOTES PRIMITIVE HEMATOPOIETIC CELL GROWTH. *P. Kaur[1], N. Arseni[1], F. Ahmed[1], C. Abramovich[2], W. Hiddemann[1], K.R. Humpheries[2], C. Buske[1], M. Feuring-Buske[1].* 1) Klinische Kooperationsgruppe am Hämatologikum der GSF, Medizinische Klinik III, Klinikum der LMU München-Großhadern, München; 2) The Terry Fox Laboratory, Vancouver, Canada.

Hematopoietic PBX interacting protein (HPIP) is a 731 amino acid protein recently discovered as a novel partner of the key Hox transcription factor co-factor PBX, in hematopoietic cells (Abramovich C. et al 2000, 2002; Oncogene).HPIP has been implicated as a nuclear-cytoplasmic shuttle molecule and shown to have the capacity to bind to the cytoskeleton. Intriguingly, HPIP was found to be highly expressed in human CD34+ progenitor cells, but silenced in differentiated CD34- cells.To gain further insights into the possible functional role of HPIP and its domains HPIP cDNA was cloned in pMSCV-IRES-YFP cassette. Umblical cord blood CD34+ enriched population of stem cells was obtained to perform in vitro and in vivo experiments. HPIP induced a significant increase in erythroid colony formation (p=0.002, n=6) in colony forming unit(CFC) assay. In order to test the impact of HPIP in vivo, infected cells were transplanted into NOD/SCID mice and engraftment of HPIP expressing cells was traced back by YFP expression. HPIP induced a significant increase in the proportion of engrafted myeloid cells compared to the control (p<0.005) and decrease in lymphoid cells (p=0.03) Intriguingly, there was no significant increase observed in Competitive Repopulation Unit frequency as compared to control in transplanted (6 weeks post) NOD/SCID mice. However, a significant increase in CD16/56 (p=0.02) positive and CD10 (p=0.04) positive cells and a disequilibrium in Lymphoid/Myeloid cell ratio was observed.Our data point to HPIP as a previously unrecognized potent regulator of primitive haematopoietic cell growth and lineage commitment. These findings together with the potential of HPIP to intersect with both the Hox regulatory network through its interaction with PBX and with key signalling pathways as an adapter molecule (recently described by Manavathi B. et al PNAS 103: 15981, 2006) add impetus to further studies to further elucidate HPIP's function in both normal and leukaemia hematopoiesis.

475/W

Integrated genetic and epigenetic studies of breast cancer progression and metastasis using Affymetrix SNP chips. *M. Lee, N. Diaz-Meyer, M. Kadota, H. Yang, W. Lin.* Laboratory of Population Genetics, National Cancer Institute, Bethesda, MD 20892 USA.

Breast cancer is one of the most common human cancers. The prognosis for primary breast cancer varies considerably from patient to patient. Lymph node metastases and tumor stage appear to be the most reliable prognostic indicator for primary breast cancer. Breast cancer progression and metastasis depends on the interactions among genetic background, environmental exposures, and somatic genetic/epigenetic alterations. To identify genetic/epigenetic mechanisms that determine cancer progression, we perform genotyping, copy number alteration, and allele-specific DNA methylation analyses using Affymetrix SNP chips (10K and 500K) in 21 pairs of breast cancers that were from ductal carcinoma in situ (DCIS), invasive breast cancer (IBC), and lymph node positive cancers and their matched normal samples. In addition, we performed genomic and epigenomic studies on four MCF10A series of breast cancer cell lines that can give rise to benign tumor, differentiated carcinoma, and poorly differentiated metastatic carcinoma in xenograft. Our studies identified ten genes including SPOCK, GRM7, AQP9, RPS6KA5, AGPS, CRY1, DSCAM, PKHD1L1, PBX1, and LSAMP that showed significant methylation changes between normal and tumor. We also identified 8 genes including GPC6, TIMP2, FBN1, SYTL3, DGKH, KCTD10, SH3GL3, and ARID1B that showed significant methylation difference between node positive and negative tumors. Interestingly, Kaplan-Meier survival analysis showed significant difference when samples are split by the gene expression for five of the 8 genes (GPC6, TIMP2, FBN1, SYTL3, SH3GL3). We also identified a 10-Mb region on 2q14 showing contiguous hypomethylation in tumor samples. Intriguingly, hypermethylation on 2q14 was recently identified as a common global epigenetic marker in colon cancer. We are currently validating our results in a panel of 200 pairs of breast cancer samples. Our goal is to identify genetic/epigenetic signature that can predict breast cancer progression and metastases.

476/W

Frequent inactivation of NDRG2 by promoter hypermethylation in human colon cancers. *A. Piepoli[1], R. Cotugno[1], G. Merla[2], A. Gentile[1], B. Augello[2], M. Quitadamo[1], A. Merla[1], M. Carella[2], R. Maglietta[3], N. Ancona[3], A. Andriulli[1], F. Perri[1].* 1) Unit and Research Laboratory of Gastroenterology, "Casa Sollievo della Sofferenza", Hospital, IRCCS, San Giovanni Rotondo, Italy; 2) Medical Genetics Service, "Casa Sollievo della Sofferenza", Hospital, IRCCS, San Giovanni Rotondo, Italy; 3) Istituto di Studi sui Sistemi Intelligenti per l'Automazione - CNR, Bari, Italy.

BACKGROUND & AIM: Epigenetic aberrations have been shown to play an important role in the pathogenesis of most human cancers. In the present study, promoter hypermethylation status in colorectal tumor were investigated to identify and validate novel target genes. METHODS: Using qRT-PCR assay, the gene expression profiles of colon cancer cell lines before and after treatment with the demethylating agent 5-aza-2'-deoxycytidine were evaluated and compared. The expression levels of seven responding genes were compared with the microarray expression data obtained on primary colorectal carcinomas. These down-regulated genes were subjected to bi-sulfite sequencing and methylation-specific polymerase chain reaction (MSP) using colon cancer cell line (n= 3), tumor and normal tissue (n= 30) of patients with colorectal cancer (CRC). RESULTS: In colon cancer cell lines, hypermethylation was subsequently identified in four of seven genes analyzed, HPDG (67%), PRDX6 (34%), STX12 (34%) and NDRG2 (100%). For the latter three genes, absence or reduced gene expression was not associated with promoter hypermethylation. The methylation status of NDRG2 was moreover investigated in primary colon tumor and in normal colon tissue of 30 CRC patients using both bi-sulfite sequencing and MSP. Twenty-two of 30 (73%) carcinomas were hypermethylated for this gene. Finally, analysis of normal colorectal mucosa demonstrated that the observed promoter hypermethylation was cancer-specific. CONCLUSION: These findings highlight the utility of combining microarray, expression, and epigenetic data to identify clinically significant tumor biomarkers, and suggest that NDRG2 expression will be a useful and functionally relevant biomarkers to predict patients with colorectal cancer.

477/W

Analysis of FFPE Specimen for Somatic Mutations and Epigenetic Alteration. *J.A. Durocher[1], K.B. Walters[2], S. Mahurkar[1], J.D. Karkera[1], M.L. Nickerson[1].* 1) Genome Research Division, Transgenomic Inc, Gaithersburg, MD; 2) Department of Biological Sciences, The George Washington Univesity, Washington, DC.

Formalin-fixed, paraffin-embedded (FFPE) tissue has traditionally been used to archive pathological samples from patients. The FFPE procedure preserves tissues for extended periods of time while retaining histologic information. This method of archiving samples is commonly used to preserve sections of tumors from cancer patients. Although they are a benefit for histological examination, use of FFPE samples for molecular genetic analysis has been a challenge due to degradation, crosslinked nucleic acids, and other chemical modifications which vary depending on where and how the specimen was fixed. Genomic DNA isolation from FFPE samples is difficult and previous methods resulted in insufficient quality and quantities of nucleic acids for subsequent analyses. As the field of oncology transitions to personalized medicine, researchers and physicians require additional genetic information from cancerous cells to provide a better understanding of the disease at the molecular level and to aid in determining the optimal course of treatment. Here we describe methods for isolation and analysis of genetic material from FFPE samples. Ample quantities of nucleic acids have been obtained using optimized DNA isolation protocols and we have found that quality is most accurately accessed by quantitative PCR. Genetic regions associated with disease phenotypes, including G-C rich regions, can be amplified from FFPE isolated using improved and highly developed PCR protocols. We have successfully detected inherited and low-level somatic mutations using endonuclease scanning combined with double stranded sequencing. An approach using bisulfite treatment and DNA sequencing has been used to locate methylation sites in CpG islands in gene promoters associated with disease status and drug response. All results obtained from our assays are confirmed through repeated analysis of independent PCR products. This comprehensive approach allows detailed, accurate and robust genetic data to be generated from FFPE samples to facilitate an insight to the molecular mechanisms of carcinogenesis.

478/W

Improved identification of von Hippel-Lindau gene alterations in DNA from clear cell renal tumors reveals a large percentage of mutations that appear to reside in a subpopulation of total tumor cells. *M.L. Nickerson[1], J.A. Durocher[1], S. Mahurkar[1], K.B. Walters[1,2], J.D. Karkera[1].* 1) Genome Research Division, Transgenomic, Gaithersburg, MD; 2) Department of Biological Sciences, The George Washington University, Washington, DC.

Considerable progress has been made in understanding the genetic basis of kidney cancer. Molecular studies examining tumor DNA from sporadic clear cell renal cell carcinoma (ccRCC) have revealed that von Hippel-Lindau (VHL) alterations are a common, early event in the carcinogenic process and may be associated with prognosis and response to therapy. DNA from 205 patient tumors was analyzed for alterations in the VHL protein coding region, splice junctions, and promoter. Endonuclease scanning and Sanger sequencing were applied in parallel to screen for VHL somatic mutations. Using this approach, mutations were identified in 82.4% (169/205) of the cases. Seven tumors (3%) possessed two mutations. Detailed analysis of fluorescent sequencing chromatograms revealed that almost half of the mutations exhibited very low signal compared to wild type. Potentially these findings have implications for genetic progression during tumor formation or metastasis if only a small number of total tumor cells possess the VHL mutations that were identified. Detailed analysis of 11 CpG sites in the VHL promoter identified an additional 8.3% of tumors that were potentially silenced through hypermethylation. Interestingly, hypermethylation was found exclusively in tumors that were VHL mutation negative. Together, a total of 91% of RCCs exhibited apparent alteration of VHL. High throughput, sensitive methods for genetic analysis of tumors will be essential to stratify patients for individualized treatment using targeted therapeutics. Examination of VHL in ccRCC provides validation for combinatorial application of endonuclease scanning and Sanger sequencing and provides a practical, robust means of identifying somatic mutations in other types of cancer. Genetic analysis of VHL is particularly relevant to treatment of RCC given the recent success of several related targeted therapeutics.

479/W

Overlap between *WTX* and *WT1* mutations in Wilms tumors. *E.C. Ruteshouser, N. Alam, S.M. Robinson, V. Huff.* Department of Cancer Genetics, The University of Texas M. D. Anderson Cancer Center, Houston, TX.

Wilms tumor is genetically heterogeneous, and until recently only one Wilms tumor gene was known, *WT1* at 11p13. However, *WT1* is altered in only ~20% of Wilms tumors. A second Wilms tumor gene, *CTNNB1* encoding β-catenin, is altered in ~15% of Wilms tumors, but *CTNNB1* mutations are rarely observed in the absence of *WT1* mutations. Recently a new Wilms tumor gene, *WTX* at Xq11.1, was reported (Rivera et al., *Science* 2007;315:642-5). This study identified mutations in *WTX* in 30% of a group of 51 Wilms tumors and reported no overlap between tumors with mutations in *WTX* and *WT1*.

To assess the frequency of *WTX* mutations and their relationship to *WT1* mutations in a second, larger panel of Wilms tumors (n=124), we conducted a complete mutational analysis of *WTX* that included sequencing of the entire coding region as well as quantitative PCR to identify deletions of the *WTX* gene. Twenty-six (21%) tumors carried a total of 27 *WTX* mutations; 7 of these were point mutations (5.6%) and 20 were deletions (16.1%). Surprisingly, given the results of the previous study, we observed an equal frequency of *WTX* mutations in tumors with *WT1* mutations (22.2%) and tumors with no *WT1* mutation (20.3%). However, *WTX* mutations were rare in tumors carrying known protein-stabilizing mutations of *CTNNB1*. *WTX* has been shown to negatively regulate WNT/β-catenin signaling (Major et al., *Science* 2007;316:1043-6), and our data are consistent with this observation and suggest that *WTX* and *CTNNB1* mutations may be functionally redundant.

We assessed expression of *WTX* through real-time quantitative RT-PCR analysis and observed an apparently random distribution of *WTX* deletions between the active and inactive X chromosomes in Wilms tumors from females, with the deletions in 5/9 tumors occurring on the inactive X. Taking these data into account, we have found *WTX* mutations on the active X chromosome in 21 of 124, or 16.9%, of Wilms tumors overall. Our findings suggest that the process of Wilms tumorigenesis requires inactivation of more than one cellular pathway, one involving *WT1* and the other involving *CTNNB1* and *WTX*.

480/W

High-resolution SNP array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications. *I.G. Campbell[1], E.R. Thompson[1], A. Sridhar[1], W. Qiu[1], S. Jacobs[2], D.Y.H. Choong[1], K.L. Gorringe[1].* 1) Research Division, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; 2) Affymetrix, Inc, Santa Clara, California.

Genetic changes in sporadic ovarian cancer are relatively poorly characterized compared with other tumor types. We have evaluated 31 primary ovarian cancers and matched normal DNA for loss of heterozygosity and copy number alterations using 500K SNP arrays. In addition to identifying the expected large-scale genomic copy number changes, over 380 small regions of copy number gain or loss (<500kb) were identified among the 31 tumors including 33 regions of high level gain (>5 copies) and 26 homozygous deletions. The existence of such a high frequency of small regions exhibiting copy number alterations had not been previously suspected since earlier genomic array platforms lacked comparable resolution. Interestingly, many of these regions harbor known cancer genes. For example, one tumor harbored a 350 kb high-level amplification centered on FGFR1 and three tumors showed regions of homozygous loss 109 kb - 216 kb in size involving the RB1 tumor suppressor gene only. These data suggest that novel cancer genes may be located within the other identified small regions of copy number alteration. Analysis of the number of copy number breakpoints and the distribution of the small regions of copy number change indicate high levels of structural chromosomal genetic instability in ovarian cancer.

481/W

Selection underlies most doublet somatic EGFR mutations in lung cancer: about 1/3 occur at five amino acid pairs. *Z. Chen, j. feng, j. saldivar, D. Gu, A. Bolkholt, S. Sommer.* Molec Gen & Molec Diagnosis, City of Hope Natl Med Ctr, Duarte, CA.

Doublet mutations are generally not well characterized. We find that doublet mutations were present at high frequency and on one allele of EGFR tyrosine kinase (TK) domain in lung cancers. Sequencing of 470kb elsewhere in the EGFR gene did not demonstrate any additional somatic mutations, the doublets were not obiously associated with tumor hypermutability. When doublets from the literature were added, a total of 94 doublets became available for analysis. The frequency of doublets overall is 5.6%, which is seven-fold greater than that observed in normal somatic tissue in mouse. All characterized doublet mutations are in cis. About half of all doublet mutations contain one or two of 12 distinct missense mutations at five amino acids: E709, G719, S768, T790, and L861. These 12 missense mutations are uncommonly or never been reported in singlets. Moreover, when the common L858 target is included, more than one third of EGFR doublet mutations occur at one of five pairs of missense mutations: G719/E709, G719/S768, G719/L861, L858/E709, and L858/T790. While analysis of the frequency of silent mutations in doublets is consistent with a random and "hitchhiker" mutation in spontaneous somatic mouse doublets in normal tissue and for p53 doublets in human lung cancer, that is not the case for EGFR mutations. Curiously, the frequency of doublets is decreased in smokers despite high mutagen exposure from cigarette. We conclude that most EGFR doublet mutations arise by sequential functional selection.

482/W

Apoptosis induced in human melanoma cells with a *PAX3* antisense oligonucleotide is associated with down-regulation of BCL2 and CDK2. *M.R. Eccles[1], S. He[1], J. Ineson[1], H.-S. Yoon[1], C.G. Li[1], A. Jeffs[1], E. Marshall[2], B. Baguley[2].* 1) Dept Pathology, University of Otago, Dunedin, Otago, New Zealand; 2) Auckland Cancer Society Research Centre, University of Auckland, Auckland, New Zealand.

Melanoma is an aggressive skin neoplasm involving melanocytes, and frequently metastasised by the time it is detected. The treatment of disseminated melanoma is difficult, as it is often associated with lack of response due to resistance to therapy. This can be largely attributed to the enhanced ability of melanoma cells to survive. *PAX3*, a member of the paired box family of genes, plays a critical role during the development of the neural crest and its derivatives, including melanocyte progenitors, and recently *PAX3* has been demonstrated to play a role in melanoma cell survival. Here we demonstrate that PAX3 protein is not only expressed in melanoma tissues and cell lines, but also in normal skin melanocytes and nevi, and in epidermal melanocytes adjacent to melanoma tissues. In addition, we have used antisense-mediated gene knockdown as a strategy to explore the mechanisms of cell survival in metastatic melanoma cell lines. The treatment of melanoma cell lines with *PAX3* antisense oligodeoxynucleotides specifically decreased the level of PAX3 protein, and induced more apoptosis than in control treatments. The cell death induced by *PAX3* antisense could be prevented efficiently if the cells were immediately pre-treated with Z-VAD-FMK, a caspase-specific inhibitor. Furthermore, treatment with *PAX3* antisense was associated with the down-regulation of pro-survival factors BCL2 and cyclin-dependent kinase, CDK2. Taken together these data suggest that PAX3 transcriptionally regulates an assembly of downstream survival factors, and that coordinated expression of PAX3, BCL2 and/or CDK2 may represent important mechanisms for the survival of melanoma cells in vitro.

483/W

Disruption of clock genes confers a breast cancer phenotype. *J. Esposito, S. Rossetti, F. Corlazzoli, N. Sacchi.* Cancer Biology, Roswell Park Cancer Institute, Buffalo, NY.

Exposure to artificial light correlates with higher incidence of breast cancer. Shift workers, whose day/night rhythms are altered by their odd hours, appear more prone to develop breast cancer. In response to natural light, a master clock in our brain regulates molecular clocks in cells of the peripheral tissues, triggering clock-regulated genes that govern fundamental cellular functions. Critical clock genes - the period genes PER1, PER2, and PER3 - were found to be deregulated in breast cancer. It is currently unknown whether disruption of the peripheral clock in human breast epithelial cells leads to transformation. By using a modified serum shock protocol, we entrained human untransformed breast epithelial cells in vitro and found that a few key clock genes, including the PER genes are indeed transcribed in a rhythmic fashion in untransformed but not in transformed breast epithelial cells. For this reason we tested whether disruption of one of the key clock genes, PER2, can induce breast epithelial transformation in vitro. Stable knock down of PER2 in human untransformed breast epithelial cells by RNA interference leads to three-dimensional (3D) morphological phenotypes recapitulating the changes observed in early breast tumorigenesis. These findings support our hypothesis that disruption of peripheral circadian rhythm genes initiates breast tumorigenesis. A US Army DOD Concept Award (BC052954) to NS supported this work; SR is supported by A Susan Komen Postdoctoral Fellowship; FC is supported by a DOD Predoctoral fellowship.

484/W

Differential Expression of TGIF1 Homeobox Gene Transcripts Variants in Oral Squamous Cell Carcinoma: A Preliminary Study. *T.N Liborio[1], M.G Silva-Valenzuela[2], L.F Matizonkas-Antonio[1], J. Câmara[3], M.R Tavares[4], F.D Nunes[1].* 1) Molecular Pathology Laboratory, School of Dentistry, University of São Paulo, Brazil; 2) Biochemist Department, School of Chemistry, University of São Paulo, Brazil; 3) Pathology Department, School of Dentistry, Federal University of Amazonas, Brazil; 4) Hospital das Clínicas, School of Medicine, University of São Paulo, Brazil.

The study of developmental genes, especially in the homeobox family, can provide insights into processes that differ between normal and neoplastic cells. Interestingly some of these genes may do a process called alternative splicing, in which different variants of mRNA are generated from the same gene. Different transcript variants may be associated with distinctive behaviors in the same cancer. TGIF1 homeobox gene transcripts were already found in oral squamous cell carcinoma, a type of cancer that accounts for at least 95% of all types of oral cancer worldwide, although the participation of the different transcripts variants of TGIF1 in this cancer is currently unknown. The aim of this study was to analyze the expression of TGIF1 variants 2, 4, 5, 7 and 8 in oral squamous cell carcinomas (OSCC) and compare to the adjacent non-tumoral margin (NT). Were analyzed 25 samples of OSCC and 16 of NT. Total RNA of each sample was extracted using TRIzol solution. A generic pair of primers first amplified each sample and then those showing amplicons were submitted to primers specific for each variant. The generic primer amplified 92% of OSCC samples, and variant 7 (var7) was in 91,3% of those followed by var5 and 8 (52,2%), var4 (39,1%) and var2 (30,4%). For the NT, the generic primer amplified 87,5% of cases, in which, 57,1% presented equally var7 and 8, followed by var2 and 5 (35,7%) and var4 (28,6%). These results shows that all studied TGIF1 variants are expressed in OSCC. However, var7 was significantly more expressed in OSCC when comparing with NT samples. The increase of some variants expression and the loss of others, suggest that different TGIF1 transcripts have diverse roles in oral carcinogenesis.

485/W

Nucleolar Localization of p19Arf Is Important for Tumor Suppressor Function During Transformation by the Abl Oncogene. *R. Stackpole, N. Rosenberg.* Graduate Program in Genetics, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, Massachusetts.

The *Ink4a/Arf* locus is the second most common site of mutation in cancer. Our lab has shown that p19Arf, one of two tumor suppressors encoded by this locus, plays an important role in transformation of lymphoid cells by *abl* oncogenes. Like most oncogenic events, *abl*-mediated transformation is a multi-step process involving an initial proliferative phase, followed by a crisis phase characterized by erratic growth and high levels of apoptosis. During crisis, selection for fully malignant cells occurs. Both the p19Arf and p53 tumor suppressors are required for crisis, and fully malignant transformants usually contain alterations that allow the cells to bypass tumor suppressor responses mediated by these proteins.

To determine if expression of p19Arf is the cellular trigger of the crisis response, we explored the way in which Abl expression affects p19Arf. Consistent with the ability of Abl to stimulate Myc, a transcription factor known to induce p19Arf expression, analyses of normal bone marrow cells soon after stimulation with Abl reveals that both *myc* and *arf* are induced in the cells prior to the onset of crisis. Immunofluroescence analyses reveal that many Abl-positive cells express p19Arf but do not show characteristic signs of apoptosis. Thus, expression of p19Arf is not sufficient to induce crisis. Further examination of the images reveals that localization of p19Arf changes as transformation proceeds. Early after Abl expression, p19Arf is predominantly found in the nucleoplasm; as crisis begins, the protein becomes nucleolar. In addition, the intensity of nucleolar staining increases. These data together suggest that the localization and expression levels of p19Arf modulate the effects of the protein during oncogenesis and that simple expression of the molecule is not sufficient for its anti-tumorigenic effects.

486/W

siRNA-mediated suppression of wildtype and low molecular weight forms of cyclin E protein in NIH-OVCAR-3 ovarian cancer cells. *M.C. Todd, K. Meerbrey.* Biology Department, Southwestern University, Georgetown, TX.

The mutually exclusive loss of the G1 cell cycle regulatory proteins, RB or p16, appears to play a role in the development of the majority of human cancers. In contrast, most ovarian cancers coexpress RB and p16 proteins. Although the latter finding suggests the absence of 'Rb pathway' defects in these cells, we have shown that the cell cycle distribution profiles of RB/p16-coexpressing ovarian cancer cell lines are not affected by the adenoviral-mediated overexpression of functional p16, which indicates the existence of a defect(s) downstream of p16 in these cells. In the current study, we have shown overexpression of the wildtype and low molecular weight (LMW) forms of the cyclin E protein in the p16-insensitive (RB/p16-coexpressing) ovarian cancer cell line, NIH-OVCAR-3. Further, we determined that the high levels of cyclin E were not due to a reduction in the rate of degradation of the cyclin E protein. Following transient transfection of small interference RNA specific for cyclin E into NIH-OVCAR-3 cells we were able to inhibit wildtype expression by approximately 70 percent; and completely eliminate the expression of the LMW forms of cyclin E protein. Associated with the down-regulation of cyclin E expression, the cells underwent a marked shift in RB protein expression to the active, hypophosphorylated state. This contrasts with cells transfected with the non-targeting siRNA that showed no change in the phosphorylation status of the RB protein. These data provide evidence that cyclin E over-expression plays a major role in the loss of G1/S cell cycle control in NIH-OVCAR-3 ovarian cancer cells (and might well be implicated in the development of the subpopulations of other types of cancer that coexpress RB and p16) and that the suppression of cyclin E protein expression may prove effective in restoring regulation to this critical cell cycle restriction point.

487/W

The DNA damage signalling kinase ATM is aberrantly reduced or lost in BRCA1/BRCA2-deficient and ER/PR/HER2-triple-negative breast cancer. *J. Tommiska[1], J. Bartkova[2], M. Heinonen[3], L. Hautala[1], O. Kilpivaara[1], H. Eerola[1,5], K. Aittomaki[4], J. Lukas[2], C. Blomqvist[5], A. Ristimaki[3], P. Heikkila[3], J. Bartek[2], H. Nevanlinna[1].* 1) Dept Obst & Gyn, Helsinki University Central Hospital (HUCH), Finland; 2) Inst Cancer Biology and Centre for Genotoxic Stress Research, Danish Cancer Society, Copenhagen, Denmark; 3) Dept Pathology, HUCH, Finland; 4) Dept Clinical Genetics, HUCH, Finland; 5) Dept Oncology, HUCH, Finland.

The ATM (Ataxia Telangiectasia-Mutated) kinase is a key transducer of DNA damage signals within the genome integrity network, and a tumor suppressor whose germline mutations predispose to familial breast cancer. Recently, the ATM-regulated signalling cascade was found constitutively activated in early stages of diverse types of human malignancies and cell culture models in response to oncogene-induced DNA damage, and proposed to provide a barrier against tumor progression. As BRCA1 and BRCA2 are also components of the genome maintenance network and their mutations predispose to breast cancer, we have examined the ATM expression in series of human breast carcinomas of BRCA1/2 mutation carriers, sporadic cases and familial nonBRCA1/2 patients. Our immunohistochemical results show that ATM protein expression is aberrantly reduced or lost more frequently among BRCA1 and BRCA2 tumors (in 33.3% and 30.0%, respectively) than in nonBRCA1/2 tumors (10.7%) (p= 0.0003 and p=0.0009, respectively). Furthermore, the nonBRCA1/2 tumors with reduced ATM expression were more often estrogen receptor (ER) negative (p=0.0002), progesterone receptor (PR) negative (p=0.004), and of higher grade (p=0.0004). In our series of 1013 nonBRCA1/2 cases, ATM was more commonly deficient among the difficult-to-treat ER/PR/HER2-triple-negative subset of tumors compared with cases which expressed at least one of these markers (p=0.0006). Overall, our results support the participation of ATM, BRCA1 and BRCA2 in a DNA damage-induced anti-cancer barrier and suggest higher demand for the tumor suppressor function of ATM (and consequently higher rate of its inactivation) during development of the more genetically unstable BRCA1/2 and triple-negative breast carcinomas.

488/W

Interleukin-10 Promoter Polymorphisms and Breast Cancer Risk in Iranian Women. *R. Asadollahi[1], N. Hakakian[1], E. Kamali-Sarvestani[1], A. Talei[2].* 1) Immunology department, Shiraz Medical School, Shiraz, Fars, Iran; 2) Department of surgery of Shiraz Medical School,shiraz, Fars, Iran.

Background: IL-10 is an anti-inflammatory cytokine which is involved in tumorigenesis. Over production of IL-10 and elevated number of IL-10 generating mononuclear cells in breast tumor tissue has already been shown. Objective: To determine the association of IL-10 promoter polymorphisms with increased risk of breast cancer and its association with breast cancer prognostic factors. Methods: Peripheral blood samples from 275 female breast cancer patients and 320 cancer free controls were used to detect three single nucleotide polymorphisms in IL-10 promoter region (-1082, -819, -592) by PCR method. Results: The frequency of genotypes and alleles of three mentioned regions of IL-10 promoter and their haplotypes (GCC, ATA, and ACC) showed no statistically significant difference between patients and controls. In the case of prognostic factors, progesterone receptor (PR) status exhibited significant relation with -1082 genotypes (P= 0.03) and haplotypes (P=0.02). -1082 AA genotype was associated with negative PR expression whereas AG and GG genotypes of this site were positively associated with PR expression. Similarly GCC haplotype correlated with positive PR expression and ATA and ACC with negative PR expression. Conclusion: The data of this study showed that IL-10 promoter gene polymorphisms may not be considered as one of the risk factors for breast cancer in Iranian patients.

489/W

Identification of cSNPs in environmental response genes contributing to breast cancer etiology. *R. Ellsworth[1], J. Weyandt[1], H. Patney[1], K. Anthony[1], C. Shriver[2].* 1) Clinical Breast Care Project, Windber Research Inst, Windber, PA; 2) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC.

Sporadic cancer is likely caused by a number of DNA variants, each making a small contribution to overall cancer risk. The Environmental Genome Project has identified 57 potentially deleterious cSNPs in 40 genes involved in metabolism of toxins, drug clearance, and DNA repair with population frequencies ranging from 0.01 - 0.38. To determine whether these cSNPs contribute to breast cancer etiology, we examined the prevalence of these variants in women with invasive breast cancer (n=212) and age- and ethnicity-matched (n= 212) female controls enrolled in the Clinical Breast Care Project (CBCP). The patient population was comprised of 21% African American and 79% Caucasian women; other minority populations were excluded due to small sample size. Genotypes were determined by RFLP assays or by direct sequencing. Minor allele frequencies (MAFs) in controls were in agreement with published values; most cSNPs had MAFs <0.01, while SNPs in GSTZ1 (rs3177427), GCKR (rs1260326), MTHFR (rs1801133 and rs1801131) and ERCC5 (rs17655) had relatively high minor allele frequencies (>0.20). Overall allele frequencies differed significantly (P<0.05) between cases and controls for SNPs rs1799950 (BRCA1), rs1799853 (CYP2C9), and rs3218778 (POLI). Examination of the data by ethnic group revealed that a number of the cSNPs differed between cases and controls within a single population: the Q356R BRCA1, A232V RFC2 and R71G POLI variants in Caucasians, and the W452C ERBB2 variant in African Americans. Altered expression of ERBB2 has been associated with poor prognosis in women with breast cancer and African American women frequently have aggressive tumors with poor clinical outcomes. CYP2C9 is involved in estrogen metabolism, and BRCA1, POLI, and RFC2 are largely involved in proper DNA synthesis and thus deleterious SNPs may contribute to increased risk of DNA damage, genomic instability and disease pathogenesis. Functional variants in environmental response genes may, therefore, impair the ability to respond to exogenous exposures and increase the risk of developing breast cancer.

490/W

Dichloroacetate Induces Apoptosis via Metabolic Targeting in Endometrial Cancer Cells which Exhibit Aerobic Glycolysis. *J.Y.Y. Wong[1], M. Debidda[2], I. De Vivo[1,3].* 1) Medicine, Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) The Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston Massachusetts; 3) Harvard School of Public Health, Boston Massachusetts.

Aerobic glycolysis due hyper-polarization of the mitochondrial membrane is a unique hallmark of numerous cancers. It is characterized by reliance on glycolysis for ATP production despite a readily available oxygen source. This may confer apoptotic resistance in cancer cells since the electron transport system is required for reactive oxygen species and Cytochrome C release for mitochondrial-mediated activation of Caspases. A landmark study showed that treatment of several cancer cell types with Dichloroacetate (DCA) inhibited mitochondrial pyruvate dehydrogenase kinase (PDK), and inhibitor of pyruvate dehydrogenase (PDH), which shifted metabolism from glycolysis to glucose oxidation. This was accompanied by a decrease in mitochondrial membrane potential (MMP) and promoted apoptosis in cancer but not normal cells (Bonnet et al. 2007). The objective of our study is to assess the efficacy of DCA to induce apoptosis specifically in endometrial cancer cells. Seven endometrial cancer cell lines were treated with DCA and analyzed for apoptosis using flow cytometry. Five endometrial cancer cell lines responded to treatment while 2 did not respond. Those which responded to DCA treatment showed a 2 to 5-fold increase in early and late apoptotic cells, a decrease in intracellular calcium levels, a decrease in MMP, and decreased Survivin expression. Endometrial cancer cells which did not respond to DCA showed no difference in the percentage of apoptotic cells, an increase in Survivin expression and no significant decrease in MMP with treatment. The transcript abundance of PDH was found to be greater in a non-responding cell line compared to a responding cell line which may indicate that those unaffected by treatment may be utilizing glucose oxidation. Our results suggest that DCA is a promising cancer therapeutic agent and is effective in those endometrial cancer cell-types which exhibit reliance on anaerobic respiration.

491/W

Mutation showers over the DNA landscape. *S. Sommer[1], J. Wang[1], K. Gonzalez[1], W. Scaringe[1], K. Tsai[1], N. Liu[1], D. Gu[1], W. Li[1], V. Buettner[1], K. Hill[2].* 1) Dept Molecular Genetics, City of Hope, Becker Res Inst, Duarte, CA; 2) Dept of Biology, University of Western Ontario, London ON, Canada.

We previously reported that spontaneous "mutation showers", clusters of spontaneous mutations, generally spanning less than 30kb, occur in mice at an estimated frequency of 1% or more of spontaneous mutations (PNAS 104(20):8403;May '07. The mutations are termed spontaneous since the mice were not intentionally exposed to any known mutagen. The unexpected clustering of the observed multiple mutations indicates that they occurred as a chronocoordinate event and is suggestive of a transient error-prone condition. The existence of mutation showers has implications for oncogenesis and evolution, raising the possibilities of "cancer in an instant" and of introns serving as sponges to absorb mutation showers. Herein we demonstrate that the yeast S. cerevisiae has four hallmarks of mutation showers, consistent with wide distribution of this phenomenon in eukaryotic organisms. Furthermore, investigation in mouse reveals that tandem base mutations (TBM's) are associated with "mutation drizzles", as they tend to involve a lower density and fewer clusters of mutations than when TBM's are not involved.

492/T

Dissociation between Gonadarche and Adrenarche in Patients with Glycogen Storage disease type 1a. *C.A. Stratakis, S.A. Boikos1.* SEGEN, DEB, NICHD, NIH, Bethesda, MD.

Two distinct processes take place during pubertal development in humans, adrenarche and gonadarche. In constitutional delay of growth and puberty, both events are delayed, with adrenarche occurring normally with advancing skeletal age, followed by gonadarche. Deficient adrenal androgen secretion has been demonstrated in chronic diseases, such as thalassemia major, with concurrently intact glucocorticoid and mineralocorticoid synthesis. Glycogen storage disease type Ia (GSD-Ia) is caused by an inherited defect of glucose-6-phosphatase. Severe failure to thrive is present in all untreated patients, but near-normal growth and pubertal development can be achieved with appropriate therapy. We report three patients with GSD-1a and absent adrenarche and delayed gonadarche [low testicular volume and lack of secondary sexual characteristics] at the age of 13 1/2 y, ., that were recently treated in our institution, with a six-month course of low-dose testosterone (T) enanthate (50 mg im, monthly). Gonadarche took place in all the cases after or during the treatment, as judged by TV and/or pubertal LH/FSH response to GnRH.. After a short course of a low-dose testosterone treatment, that apparently "induced" normal gonadarche, dissociation of adrenarche was evident in the first patient. We were able to study the adrenal steroidogenesis by an ACTH stimulation test in the next two patients: and show specific 3β-ol and sulfokinase deficiencies, as well a milder C17-20 lyase deficiency. We conclude that in GSD-1 there is delayed gonadarche, and delayed and deficient adrenarche. Induction of gonadarche with sex-steroids was successful, and did not compromise final height prediction. After pubertal induction, a dissociation of adrenarche was evident in our patients with GSD-1a, similar to what is seen in patients with thalassemia major. Defficient adrenal steroidogenesis may be responsible for the lack of normal development of pubic and facial hair in patients with GSD-1a.

493/T

Roberts syndrome in siblings, associated with ESCO2 gene mutation: outstanding intrafamilial variability of the clinical spectrum and the natural history. *M. Giovannucci Uzielli[1], G. Scarselli[1], H. Vega[2], E. Lapi[1], S. Stagi[3], N. Dayan[1], A. Zeffiri[1], M. Isoldi[1], S. Guarducci[1], L. Giunti[1].* 1) Dept. of Paediatrics, Human and Medical Genetics, University of Florence, Italy; 2) Johns Hopkins University USA and Universidad Nacional de Colombia; 3) Dept. of Paediatrics, Endocrinology Unit, University of Florence, Italy.

Roberts syndrome (RBS) (MIM#268300) is an extremely rare, autosomal recessive developmental disorder, characterized by growth retardation, craniofacial and eyes anomalies, and tetraphocomelia. RBS is caused by mutations in ESCO2, one of the two human homologs of Eco1, a gene essential for the establishment of cohesion in yeast. Irrespective of the position and type of mutation, the defect in RBS is most likely the loss of ESCO2 function, in particular of the acetyltransferase domain. Abnormal cellular characteristics of this disorder were first described by James German in 1979 as "heterochromatin repulsion, puffing and Premature Centromere separation (PCS)". This first report assumes a special significance, after the identification in subjects with Cornelia de Lange syndrome, of mutation in the cohesion regulator NIPBL, and more recently in the cohesion complex structural component SMC1A and in the complementary subunit of the cohesion heterodimer SMC3, another gene of the cohesion complex. RBS and CdLS represent the most important part of the Cohesinopathies. We report the observation, in the course of a long follow-up, of a family, with two siblings, children of consanguineous parents. The homozygous mutation c1595delT (pA532fsX4) is associated with a mild phenotype in the girl, who died a few days after birth. A severe clinical spectrum was observed, viceversa, in the boy, who lived for 16 years, though with a true craniofacial disruption and tetraphocomelia. In this child, weight and length were, at the time of death, only slightly higher than at the birth. Genotype-phenotype analysis.

494/T

High Frequency Of Central Nervous System Malformations Associated With Choanal Atresia. *T.A. Burrow, H.M. Saal, R.J. Hopkin.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Choanal atresia is a common craniofacial defect characterized by absence of the opening between the nasal cavity and the nasopharynx. Embryologically, this defect is a result of failed degeneration of the oro-nasal septum, which normally occurs around the sixth week of gestation. Presenting unilaterally and bilaterally, it occurs with an incidence of approximately 1 in 8,000 live births. Fifty percent of affected individuals have additional associated congenital anomalies. We are currently reviewing cases of choanal atresia and stenosis (CA/S) seen at Cincinnati Children's Hospital Medical Center over the past 10 years. To date we have completed the analysis of 38 individuals focusing on characterization of malformations of the central nervous system and neurologic deficits. Choanal atresia and stenosis were identified as isolated findings and in association with disorders of various etiologies, including teratogens, chromosomal abnormalities, single gene defects, and deformations. Among all affected individuals, 11 (29%), were noted to have some degree of developmental delay. Likewise, 13 individuals, 34%, were noted to have brain abnormalities on MRI. The most common brain abnormalities on MRI included inferior vermian hypoplasia, increased prominence of the ventricles and subarachnoid fluid space, hydrocephalus, and pituitary abnormalities, with three cases each. None of the individuals with isolated CA/S (3 cases), had associated developmental delays or brain abnormalities on MRI. Of the patients with developmental delay and/or brain anomalies, only 25% had CHARGE syndrome. Interestingly, choanal abnormalities were diagnosed in three individuals with holoprosencephaly sequence. Choanal atresia is the most common craniofacial defect affecting the nose and is frequently associated with developmental delay and brain malformations on MRI, particularly when occurring in connection with other features. This suggests overlapping pathways in embryology contributing to abnormal morphogenesis. We conclude that close developmental monitoring and brain imaging are indicated in cases of CA/S, particularly if additional anomalies are identified.

495/T

Early infantile epileptic encephalopathy with suppression-burst (Ohtahara syndrome) is caused by a longer polyalanine expansion mutation in the ARX gene. *M. Kato[1], S. Saitoh[2], A. Kamei[3], H. Shiraishi[2], Y. Ueda[2], M. Akasaka[3], J. Tohyama[4], N. Akasaka[4], S. Kumada[5], M. Kubota[6], K. Nakamura[1], K. Hayasaka[1].* 1) Dept Pediatrics, Yamagata Univ Sch Medicine, Yamagata, Japan; 2) Hokkaido University Graduate School of Medicine, Sapporo, Japan; 3) Iwate Medical University, Morioka, Japan; 4) Nishi-Niigata Chuo National Hospital, Niigata, Japan; 5) Tokyo Metropolitan Neurological Hospital, Tokyo, Japan; 6) Tokyo Metropolitan Hachioji Children's Hospital, Tokyo, Japan.

Early infantile epileptic encephalopathy with suppression-burst or Ohtahara syndrome (OS) is one of the most severe and earliest forms of epilepsy and often evolves to West syndrome (WS). The pathogenesis of OS remains unclear. *ARX* is a crucial gene for the development of interneurons in the fetal brain and polyalanine expansion mutations of *ARX* cause mental retardation or seizures including WS in male. Mutation analysis of *ARX* was performed for six sporadic male patients affected with OS by DHPLC and direct sequencing. We identified a hemizygous de novo 33 bp-duplication in exon 2, 298_330dupGCGGCA(GCG)9, which is thought to expand the original 16 alanine residues to 27 alanine residues (A110_A111insAAAAAAAAAAA) in the first polyalanine tract of the ARX protein, in two unrelated patients. Both patients started their seizures at the first day of life and had a small penis that was not seen in other patients. Their brain MRI showed the cerebral white matter changes, such as the dilatation of the lateral ventricles, thin corpus callosum, and delayed myelination. Although OS is mainly associated with brain malformations, *ARX* is the first responsible gene for idiopathic OS. The length of expansion in OS (11 alanine residues) was longer than that in WS (7 alanine residues) or non-syndromic mental retardation (1 to 3 alanine residues). Our observation that OS had longer polyalanine expansion than WS is consistent with the findings of earlier onset and more severe phenotypes in OS than in WS as observed in other polyalanine expansion or triplet repeat diseases. Hypogenitalism and white matter changes might be characteristic features for OS caused by the *ARX* mutation.

496/T

No association of sudden infant death syndrome with congenital central hypoventilation syndrome (Ondine's curse). *M. Osawa[1], A. Sasaki[2], F. Satoh[1], I. Hasegawa[1], R. Kimura[1], K. Hayasaka[2].* 1) Forensic Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan; 2) Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan.

Congenital central hypoventilation syndrome (CCHS), also known as Ondine's curse, is an autosomal dominant disorder, characterized by hypoventilation during sleep with an onset in infants. Characteristics of the clinical features suggest that undetected CCHS is potentially involved in cases of sudden infant death syndrome (SIDS). However, no evidence of real cases has been reported because it is difficult to make a postmortem diagnosis of CCHS. Recent studies indicate that the expansion of a polyalanine repeat in the PHOX2B gene is relevant to the pathogenesis of the disorder. However, it has been difficult to detect the repeated tract by conventional PCR because its high GC content (≈ 88%) inhibits amplifying reactions. In this study, a bisulfite treatment for DNA was developed to reduce the GC content, in which uracil is obtained by deamination of unmethylated cytosine residues. The converted DNA permitted direct PCR amplification using primers specific to the deaminated sequence of the coding strand, in which dropouts of expanded alleles were completely prevented. It yielded a product of 123 bp for the common 20-residue repetitive tract with converted T from original C by sequencing. In addition to the common 20-residue repeat, contracted alleles of 13- and 15-residue repeat were distributed at a frequency of 0.04 in the Japanese population group. The majority (90%) of clinically diagnosed CCHS patients carried heterozygous expansions of 25- to 33-residues at the polyalanine tract of PHOX2B. In contrast, analysis revealed no expansions in SIDS victims and healthy subjects. Table summarizes the detected number of chromosome (allele frequency) of PHOX2B in subjects of the CCHS, SIDS and control groups. These results suggest that the major pathogenesis of SIDS is distinct from that of CCHS.

497/T

Identification of a novel IRF6 variant in a Chinese family with Van der Woude Syndrome. *E.C. Tan[1], E.C.P. Lim[1], J. Cheng[2], V. Yeow[2].* 1) KK Research Centre, KK Women's and Children's Hospital, Singapore; 2) Cleft and Craniofacial Centre, KK Women's and Children's Hospital, Singapore.

Van der Woude syndrome (VWS) is a rare disorder with an autosomal dominant mode of inheritance. It closely mimics the more common non-syndromic CL/P except for the additional features of lip pits and hypodontia. For the non-syndromic form which is one of the most common congenital and craniofacial malformations in man, there is no known major genetic or environmental determinant to date despite intensive investigations. In contrast, mutations in the interferon regulatory factor-6 gene (IRF6) have been shown to co-segregate with the VWS phenotype. Using VWS families as a simpler model for the non-syndromic forms, identification of mutations and knowledge of how these mutations lead to oral clefting in VWS families will increase our understanding of the pathogenesis of malformation in craniofacial development. The proband is a Chinese boy who is 3 months old at the time of recruitment into the study. He has an affected maternal uncle (long deceased and genetic material unavailable) but his two parents are unaffected. Microsatellite analysis and DNA sequencing were performed on the genomic DNA from the proband and the parents. There is a G to T change in the 3rd exon or position 396 of the mRNA which will result in a non-synonymous substitution of arginine by tryptophan (R45W) within the DNA-binding domain. The proband is heterozygous for this variant which he inherits from his mother who is also heterozygous at this position. The sibling without the VWS phenotype is also heterozygous. We screened another 100 chromosomes in our control samples. All were negative for this variant. Although the variant is also found in the unaffected mother and sister, it cannot be ruled out that it might predispose to VWS in the presence of additional genetic or environmental factor which is only encountered in the proband and his maternal uncle but not in the mother or sister, or that penetrance is higher in males. Additional work is also needed to investigate the effect of the change from a hydrophilic to an aromatic- hydrophobic amino acid residue on the function of the protein.

498/T

Toriello-Carey syndrome in a patient with a de novo balanced translocation [46,XY,t(2;14)(q33;q22)] interrupting SATB2, a plausible candidate gene. *D.H. Tegay[1,2], K.K. Chan[3], L. Leung[3], C. Wang[4], G. Stone[5], R. Stanyon[5,6], H.V. Toriello[7], E. Hatchwell[1].* 1) Stony Brook University Medical Center, Stony Brook, NY; 2) New York College of Osteopathic Medicine, Old Westbury, NY; 3) Kwong Wah Hospital, Hong Kong, China; 4) Cold Spring Harbor Lab, Cold Spring Harbor, NY; 5) National Cancer Institute, Frederick, MD; 6) University of Florence, Florence, Italy; 7) Spectrum Health, Grand Rapids, MI.

Toriello-Carey Syndrome (TCS;OMIM#217980) is a multiple congenital anomaly syndrome characterized by common manifestations including corpus callosum agenesis, cardiac defects, cleft palate/Robin sequence, hypotonia, mental and postnatal growth retardation and distinctive facial dysmorphology (including micrognathia, telecanthus, small nose and full cheeks). Both autosomal recessive and X-linked inheritance have been proposed, but chromosomal abnormalities involving disparate loci have also been reported in a small number of cases.

We report a patient with classical features of TCS and an apparently balanced de novo translocation between chromosomes 2 and 14 [46,XY,t(2;14)(q33;q22)]. Flow sorted chromosomes were generated from lymphoblastoid cell lines and Phi29 amplified. Derivative chromosome 2;14 DNA was labeled with a fluor and hybridized against a mixture of differentially labeled non-derivative chromosome 2 and 14 DNA onto a tiling path BAC array. Translocation breakpoints were identified to within ~300kb and further PCR mapped. The breakpoint at 2q33.1 was found to directly interrupt the SATB2 (Special AT-rich sequence Binding protein-2) gene while the 14q22.3 breakpoint was not intragenic.

SATB2 functions as a transcription regulator at multiple sites and recent studies indicate important roles in craniofacial and CNS development. SATB2 mutation or deletion has been associated with both isolated and syndromic facial clefting, however, no other cases of TCS have been reported. Additionally, the results of SATB2 sequencing and MLPA currently being performed on our cohort of 20 TCS subjects will be presented.

499/T

Balancing gene dosage and ventricular outflowcontrasting cardiac malformations in deletion 17p11.2 syndrome (SMS) vs duplication 17p11.2 syndrome (PLS). *L. Potocki[1,3], J.A. Towbin[2,3], D. Dang[1,3], J.W. Belmont[1,3], J.R. Lupski[1,3].* 1) Molecular/Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics/Cardiology, Baylor Col Medicine, Houston, TX; 3) Texas Children's Hospital, Houston.

Congenital heart defects (CHD) affect 8-10/1,000 live births and are a leading cause of infant mortality. While both environmental and genetic factors have been implicated in the pathogenesis of CHD, the increased incidence of CHD in chromosomal abnormalities, multiple malformation syndromes, mendelian disorders, and the increased risk of CHD in first degree relatives, strongly support that genetic factors are the major cause of these anomalies. Left ventricular outflow tract (LVOT) malformations comprise a spectrum of defects that include aortic valve stenosis (AVS), bicuspid aortic valve (BAV), coarctation of the aorta (CoA) and hypoplastic left heart syndrome (HLHS). Multiple lines of evidence support a genetic etiology in the pathogenesis of these anomalies. Right ventricular outflow tract (RVOT) malformations comprise a distinct group of anomalies which include tetralogy of Fallot (TOF) and pulmonary valve atresia. The Potocki-Lupski syndrome (PLS)the homologous recombination reciprocal of the Smith-Magenis syndrome (SMS) microdeletionis a newly characterized microduplication syndrome and is the first predicted reciprocal microduplication syndrome described. The key clinical features of PLS include infantile hypotonia and failure to thrive, speech and language impairment, mental retardation, and autism. Greater than 50% of individuals have cardiovascular abnormalities including BAV, dilated aortic root, and septal defects. SMS is a distinct clinical entity. Interestingly the structural cardiac defects in SMS tend to involve the right side of the heart and include tetralogy of Fallot and anomalous pulmonary venous return. Herein we report an individual with the common (3.7Mb) PLS duplication who is status-post cardiac transplant in infancy due to HLHS, analyze the specific cardiovascular anomalies in our cohort of 58 SMS patients and 13 PLS patients, and review the literature regarding ventricular outflow tract anomalies in these reciprocal genomic disorders.

500/T

The Spectrum of Deletions in Kearns Sayre Syndrome. *T. Prior, R.E. Pyatt.* Pathology, 125 Hamilton Hall, Ohio State Univ, Columbus, OH.

The common features of Kearns Sayre Syndrome (KSS) include progressive external opthalmoplegia (PEO), pigmentary degeneration of the retina, and defects of cardiac conduction. The typical affected patient presents before the age of 20 with PEO, and pitosis. This is followed by the pigmentary retinal degeneration and heart block. Other features of the disorder may include ataxia, deafness, dementia, and diabetes mellitus. The most common type of mutation found in KSS is a deletion of mtDNA, and almost of all these deletions occur sporadically. About one-third of the cases of KSS are due to a common 4,977 bp deletion which is associated with direct repeats at the deletion junction. The severity of KSS depends on the extent of heteroplasmy and the tissue distribution of structurally altered mitochondrial genomes. An extreme form of KSS phenotype occurs when the frequency of deleted mtDNA in muscle cells is greater than 85%. Whereas, when lower levels of heteroplasmy for the deletion is observed, PEO is the only symptom. We present here the cumulative results from deletion analysis in KSS/PEO patients over a 3 year period. Thirty-five mutation positive cases from unrelated individuals were identified in muscle biopsy specimens. In addition to determining the approximate size of each deletion, the degree of heteroplasmy was also examined using scanning densitometry. These results describe one of the largest sets of mitochondrial deletions in KSS/PEO and illustrate the variability observed in the genetic analysis of this disorder.

501/T

WILLIAMS SYNDROME "PLUS" : A 4 MB DELETION IDENTIFIED IN A PATIENT WITH WILLIAMS SYNDROME, CONGENITAL ANOMALIES AND SEVERE DEVELOPMENTAL DELAY. *T. Narumanchi[1, 2], X. Hu[1], C. Dvorak[1, 2], D. Mercer[1], H. Andersson[1, 2], M. Li[1].* 1) Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA; 2) Dept. of Pediatrics, Tulane University Medical School.

We present the case of a 15 year old female recently diagnosed as having a 4 MB deletion on chromosome 7, including the genes for Williams syndrome, using an oligionucleotide microarray. The patient first presented in October 1992, at age 6 months for failure to thrive and dysmorphic features. She was subsequently found to also have had an ASD, pulmonary stenosis, tracheomalacia, and gastroesophageal reflux disease (GERD). A clinical diagnosis of Williams syndrome was made prior to the discovery of the Williams critical region. However, the patient had additional congenital anomalies and more severe developmental delay than typical for Williams syndrome and FISH analysis in 1995 failed to detect a deletion of the Williams critical region, confounding the diagnosis. The patient was reevaluated in March, 2007 with comparative genomic hybridization - microarray which showed a 4 Megabase deletion, extending from the Williams critical region to the centromeric band 7q11.22. At least 34 genes were deleted in the patient including all genes commonly deleted in Williams patients. Haploinsufficiency of the genes centromeric to the Williams critical region is presumed to be responsible for the severe phenotype of this patient. This case serves to remind us that the sensitivity of FISH test is not 100%, and highlights the usefulness of microarray-CGH in copy number abnormalities. We recommend that patients with Williams syndrome patients with unusual congenital anomalies and/or atypical developmental delay should have microarray-CGH evaluation for "Williams Syndrome PLUS".

502/T

Noonan and Cardio-facio-cutaneous syndromes: two clinically and genetically overlapping disorders. *M.L. Bondeson[1], A.M. Nystrom[1], S. Ekvall[1], M. Olsson-Engman[2], H. Enell[3], G. Anneren[1].* 1) Dept of Genetics & Pathology, Rudbeck Laboratory, Uppsala University Children's Hospital, Uppsala, Sweden; 2) Dept of Paediatrics, Regional Hospital of Karlskrona, Sweden; 3) Dept of Paediatrics, Regional hospital of Halmstad, Sweden.

Noonan and Cardio-facio-cutaneous syndromes are clinically related disorders associated with dysregulated RAS/RAF/MEK/ERK signalling. Noonan syndrome (NS), characterized by facial dysmorphism, heart defects and short stature is associated with mutations in the genes *PTPN11*, *SOS1* and *KRAS*. The clinically overlapping Cardio-facio-cutaneous (CFC) syndrome is distinguished from NS by the presence of ectodermal abnormalities in addition to the NS phenotype. The genetic aetiology of CFC was recently assigned to four genes, *BRAF*, *KRAS*, *MEK1* and *MEK2*. Here, we present a comprehensive mutation analysis of *BRAF*, *KRAS*, *MEK1*, *MEK2* and *SOS1* in 31 unrelated patients (7 CFC and 24 NS) without mutations in *PTPN11*. Mutations were identified in 6 CFC patients (1 in *BRAF*, 1 in *KRAS*, 1 in *MEK1*, 2 in *MEK2* and 1 in *SOS1*). Three of the mutations were novel. The *SOS1* mutation, identified in a patient with the original diagnose CFC, has previously been reported in a patient with NS. We also identified *BRAF* mutations in two patients diagnosed as NS. Both of the mutations have previously been reported in patients with CFC. To our knowledge, this is the first time mutations in *BRAF* have shown to be linked to the NS pathogenesis. Taken together, our results indicate that the molecular overlap between CFC and NS is more complex than previously suggested and that the syndromes might even present as allelic disorders. To facilitate diagnosis of these patients, we therefore propose that the recently designated name Neuro-cardio-facio-cutaneous syndromes (NCFC) should be used for diagnosis of patients presenting with any of the NCFC syndromes. As the genetic defect is established, the diagnosis can be refined to, e.g., NCFC-*SOS1*-associated.

503/T

Crisponi Syndrome and Cold-Induced Sweating Type 1: Two Syndromes - One Genetic Entity. *L. Crisponi[1], A. Meloni[2], M. Marongiu[1], F. Chiappe[2], M. Deiana[1], L. Marcia[1], G. Zampino[3], P. Nürnberg[4], G. Crisponi[5], F. Rutsch[6].* 1) INN/CNN, Cittadella Univ di Monserrato, Monserrato (CA), Italy; 2) University of Cagliari, Italy; 3) Departments of Pediatrics, Catholic University, Rome, Italy; 4) Cologne Center for Genomics, Cologne, Germany; 5) Casa di cura Sant' Anna, Cagliari, Italy; 6) General Pediatrics, University Children's Hospital, Muenster, Germany.

Crisponi syndrome (CS) is a severe autosomal recessive condition, characterized by abnormal, paroxysmal muscular contractions, hyperthermia and sudden death in most cases. Recently we identified *CRLF1* as the gene implicated in the pathogenesis of CS. We extended our cohort of patients affected by CS and up to now we detected 1 novel mutation. *CRLF1* is also involved in the pathogenesis of cold-induced sweating syndrome-1 (CISS1). CS and CISS1 belong to a group of conditions with overlapping phenotypes, also including cold-induced sweating syndrome type 2 and Stüve-Wiedemann syndrome. Since genotype/phenotype correlations are not clear for CS and CISS1,we performed functional studies on mutated CRLF1 constructs for the mutations pW76G, pP238RfsX6 and pK368X found in Crisponi patients, and tested the patients with CS for the presence of cold-induced sweating. We mutagenized the wt CRLF1 with the 3 indicated mutations. After transfection of the constructs in COS-1 cell lines, the mutant proteins derived from the missense and nonsense mutations were expressed and secreted, whereas the mutant protein derived from the frame-shift mutation was produced, but not secreted. The patients' phenotype did not differ from "protein-secreters" to "non-secreters". All surviving patients with CS developed scoliosis and cold-induced sweating. The presence of many overlapping clinical features in adolescence, including cold-induced sweating and scoliosis and the association of the diseases with mutations in the same gene, point to the fact that CS and CISS1 are two variations of the same genetic entity. However, the severity in the clinical phenotype of CS vs CISS1 does not seem to depend on the type of mutation, and more studies are in progress to clarify this difference.

504/T

From Stüve-Wiedemann syndrome to Crisponi syndrome. *N. Dagoneau[1], S. Bellais[1], B. Leheup[2], P. Blanchet[3], P. Sarda[3], L.I. Al Gazali[4], M. Di Rocco[5], A. Munnich[1], V. Cormier-Daire[1].* 1) Department of Medical Genetics and INSERM U781, Necker Hospital, Paris, France; 2) Department of Clinical Genetics, Children Hospital, Vandoeuvre les Nancy, France; 3) Department of Genetics, Arnaud de Villeneuve Hospital, Montpellier, France; 4) Department of Pediatrics, Faculty of Medicine and Health Sciences, Al Ain, United Arab Emirates; 5) Department of Pediatrics, Gaslini Institute, Genoa, Italy.

Stüve-Wiedemann syndrome (SWS) is characterized by bowing of the long bones with internal cortical thickening and flared metaphyses, trismus in response to stimuli and camptodactyly. These last features are shared by Crisponi syndrome which is distinct from SWS by the absence of congenital limb bowing. The clinical course of both syndromes is characterized by major feeding and respiratory difficulties and temperature instability usually leading to death in the first months of life.We have collected the samples of 45 SWS families and identified mutations in the Leukemia Inhibitory Factor gene (LIFR) in 33/45 SWS families. We have then excluded the LIFR in three families with Crisponi syndrome but identified mutations in the Cytokine Receptor-like Factor 1 (CRLF1) in all. Following this initial study, we identified homozygote CRLF1 mutations (c.178T>G, G60S) in two sibs from Morocco with Crisponi syndrome. We also considered CRLF1 as a candidate gene in the SWS patients without any LIFR mutation but we found no mutation. CRLF1 forms a heterodimer complex with Cardiotrophin Like Cytokine Factor 1 (CLCF1) and this heterodimer competes with Ciliary Neurotrophic Factor (CNTF) for binding to the ciliary neurotrophic factor receptor complex which is composed of CNTFR, gp 130 and LIFR. These findings suggest a key role of the CNTFR pathway in the function of the autonomic nervous system while the specific impairment of the LIFR pathway is presumably involved in the bone manifestations characteristic of SWS.

505/T

Molecular bases and clinical delineation of the Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *L. de Pontual[1], M. Rio[2], R. Redon[3], V. Malan[1], N. Boddaert[4], P. Plouin[5], NP. Carter[3], S. Lyonnet[1,2], A. Munnich[1,2], L. Colleaux[1], J. Amiel[1,2].* 1) INSERM U-781, PARIS, France; 2) Departement of Genetics; 3) 3The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 4) Pediatric Radiology and INSERM U-797; 5) Clinical Neurophysiology Unit and INSERM U-663.

Pitt-Hopkins syndrome (PHS) is a syndromic encephalopathy characterised by severe psychomotor delay, epilepsy and daily bouts of diurnal hyperventilation starting in infancy, mild postnatal growth retardation, postnatal microcephaly and distinctive facial features. A systematic 1Mb resolution genome wide BAC array in 4 PHS cases first identified a 1.8 Mb de novo microdeletion on chromosome 18q21.1 in 1 case. We subsequently identified de novo heterozygous missense mutations of a conserved amino acid in the basic region encoded by the TCF4 gene in the three additional PHS cases. These findings provide the first evidence of a human disorder related to class I basic helix-loop-helix transcription factor defects (also known as E-proteins). Haploinsufficiency is the most likely disease-causing mechanism but dominant-negative effect is an alternative hypothesis currently being tested for missense mutations of the basic domain. Expression analysis of the TCF4 gene during human embryonic development will also be presented. More recently, we identified further PHS cases by reviewing files for which PHS differential diagnoses had been excluded i.e. Rett, Angelman, and Mowat-Wilson syndromes. This novel series of patients will be presented. The facial gestalt of patients with PHS is extremely valuable for clinicians to consider the diagnosis before the onset of distinctive features such as bouts of hyperventilation and epilepsy that, although distinctive, may not be fully penetrant. EEG and brain MRI may also give valuable clues that will be discussed. Patients diagnosed with PHS display a broad spectrum of dysautonomic features that will be detailed. These data may shed new light on the normal processes underlying autonomic nervous system development and maintenance of an appropriate ventilatory neuronal circuitry.

506/T

Detailed analysis of the 17p11.2 region in 59 patients with Smith-Magenis syndrome. *M. Huizing[1], H. Edwards[1], C. Ciccone[1], M.P. Jones[1], S.C. Chandrasekharappa[1], C. Bendavid[2], J. Blancato[3], W.A. Gahl[1], A.C.M. Smith[1].* 1) NHGRI/NIH, Bethesda, MD; 2) Univ Rennes, France; 3) Georgetown Univ, Washington, DC.

Smith-Magenis syndrome (SMS) is characterized by distinct craniofacial and skeletal anomalies, speech/language delays, psychomotor and growth retardation, a striking neurobehavioral phenotype, and chronic sleep disorder related to an inverted circadian melatonin rhythm. Most cases are due to an interstitial deletion of 17p11.2; however, rare 'non-deletion' cases can be due to *RAI1* mutations. We performed a genotype-phenotype correlation on 59 SMS patients. Phenotype studies revealed some unique and variable clinical features, including hearing loss, low IgA levels, high cholesterol and skeletal features. We employed a dense map of 17p markers to determine the parent of origin of the deleted allele and found a slight skewing towards the maternal (63%) versus paternal (37%) allele, though this was not statistically significant. FISH analysis and quantitative real-time PCR (qPCR) were performed to determine the copy number of genes in the 17p11.2 area. qPCR assays for six genes of interest surrounding the SMS breakpoints were designed, including *RAI1* and *RASD1* (implicated in circadian rhythm), *MYO15A* (involved in hearing loss), *FLI1* (related to immune response), *PEMT* (functions in choline metabolism) and *TNFRSF13B* (implicated in IgA deficiency). The majority (56%) of patients had the common 17p11.2 deletion (3.5Mb), as expected. 11 patients (19%) had variable breakpoints, however, their clinical features could not be directly related to the copynumber of our 6 genes. 15 patients (25%) did not show a 17p11.2 deletion by FISH or qPCR, *RAI1* mutation analysis so far showed a novel mutation (P242L) in one of these patients. The non-deleted patients are being screened by whole genome CGH-array for possible novel chromosomal rearrangements. Our study emphasizes the value of a natural history study to recognize novel clinical features and outlier patients. We were unable to show a strong genotype-phenotype correlation. However, determination of exact breakpoints and the influence of genes outside the breakpoints on the resultant phenotype may shed more light on this.

507/T

Is Keutel syndrome genetically heterogeneous? *B. Moghaddam[1], A. Yuksel[2], M. Bober[3], K. Bolton[4], S. Chan[1], J. Liu[1], C. Nauta[1], J.A. Rousseau[1], S.A. Boyadjiev[1].* 1) Section of Genetics, Department of Pediatrics, University of California, Sacramento, CA; 2) Cerrahpasa Medical Faculty, Department of Medical Genetics, Istanbul, Turkey; 3) A.I. Dupont Hospital for Children, Wilmington DE, USA; 4) Division of Human Genetics, The University of the Witwatersrand, Johannesburg, South Africa.

Keutel syndrome is an autosomal recessive disorder characterized by abnormal cartilage calcification, peripheral pulmonary stenosis, midfacial hypoplasia, and brachytelephalangy. To date, 24 patients from 16 families have been reported and a total of four mutations in *MGP* have been identified. *MGP* encodes vitamin K-dependent matrix Gla protein that acts as a calcification inhibitor by repressing *BMP2*. We present the clinical manifestation of three unrelated and previously unreported patients from Turkey, South Africa and South Korea. A novel homozygous nonsense mutation c.79G>T (E27X) in *MGP* was identified in the family from Turkey. Despite the predicted complete loss of function mutation the clinical manifestation in all three affected was relatively mild, suggesting functional redundancy of the mechanisms governing calcification of cartilage. No mutations were identified in the remaining two cases. Some features of Keutel syndrome, such as midfacial hypoplasia and ectopic abnormal calcification, may be due to prenatal exposure to phenytoin or warfarin or to genetic disorders such as vitamin K epoxide reductase deficiency and some forms of chondrodysplasia punctata. Clinical and additional laboratory analysis of these two cases did not confirm evidence of any of these conditions that could mimic Keutel syndrome. This raises the possibility of genetic heterogeneity of Keutel syndrome.

508/T

Genome stability in SMC1A-mutated Cornelia de Lange Syndrome patients. *A. Musio[1], M. Paulis[1], M. Deardoff[2], M.L. Focarelli[1], K. Maninder[2], I. Krantz[2], P. Vezzoni[1].* 1) Human Genome Department, Istituto di Tecnologie Biomediche, CNR, Segrate, Milan, Italy; 2) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, USA.

Cornelia de Lange syndrome (CdLS) is a clinically heterogeneous developmental disorder characterised by facial dysmorphia, upper extremity malformations, cardiac defects, growth and cognitive retardation. Due to the often severe presentation of CdLS, most cases arise sporadically as a consequence of de novo mutations, while the milder familial cases are rare. Recent reports showed that mutations in NIPBL, which codes for a cohesin-associated factor, as well as in two cohesin subunits, SMC1A and SMC3, cause CdLS . These findings demonstrate that CdLS is a heterogeneous disorder, in agreement with the fact that the severity of the symptoms varies greatly among the patients, although the cellular basis of this diversity only now is beginning to be elucidated. Among the cohesin complex factors, SMC1A seems to play important and different roles. In fact, in addition to a structural function, it is involved in gene expression, in genome stability and in DNA repair and recombination. Recently, it has been observed that some CdLS patients carrying mutations in NIPBL gene show increased chromosome breakage and Premature Sister Chromatid Separation, suggesting that there may be some predisposition to chromosomal fragility in CdLS. This information is lacking in patients carrying mutations in the SMC1A gene. To address this point, Epstein-Barr virus immortalized lymphoid cell lines and primary human fibroblasts from CdLS patients have been established and analysed for both spontaneous and induced genome instability by standard cytogenetic methods. This work will allow us to further investigate the ability of CdLS cells to block the cell cycle and to repair damaged DNA providing, for the first time, a direct link between CdLS and genome instability.

509/T

Identification of new loci responsible for an SMS-like phenotype using whole genome array CGH. *S.R. Williams[1], S. Girirajan[1], D.B Shin[1], N. Nowak[3], D. Tegay[4], R. Fisher[4], E. Hatchwell[4], S.H. Elsea[1,2].* 1) Department of Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Department of Pediatrics, Virginia Commonwealth University, Richmond, VA; 3) Department of Biochemistry and Center of Excellence in Bioinformatics and Life Sciences, State University of New York at Buffalo and Department of Cancer Prevention, Roswell Park Cancer Institute, Buffalo, NY; 4) Department of Pathology, SUNY at Stony Brook, NY.

Smith-Magenis syndrome (SMS) is caused by either mutation or deletion of the *RAI1* gene on chromosome 17p11.2. Our data indicate that *RAI1* mutation analysis for individuals without 17p11.2 deletion but with a phenotype consistent with SMS reveals a *de novo* nucleotide change in only ~20% of cases, even though the patients strongly resemble SMS. We have analyzed 60 non-mutation/non-deletion cases for 44 clinical characteristics, providing significant support for the clinical indication of SMS. These data provide the basis upon which additional molecular studies can be undertaken in this well-characterized cohort. Thus, we have sought alternative approaches to help better understand this SMS-like phenotype on a molecular level and to identify new loci of interest. Array comparative genomic hybridization (aCGH) has proven to be an effective tool in the identification of copy number aberrations in patients where traditional methods have failed to make a molecular diagnosis. Here, we present data from our validated aCGH which directly detects *de novo* deletions or duplications. This approach to whole genome evaluation has resulted in the identification of new loci that better help to understand the phenotypic impact of the identified molecular aberration. In addition to these discoveries, we are able to rapidly screen our growing cohort of non-mutation/non-deletion SMS-like cases in an attempt to identify new copy number changes that may play a role in their phenotypic presentation. With this approach to genomic copy number screening, we hope to tease out the players and pathways that contribute to the SMS and SMS-like phenotypes.

510/T

Duplication 9p and Prader-Willi syndromes in an infant resulting from a de novo unbalanced 9;15 translocation. *M.T. Carter[1], F.D. Jacob[2], R. Ray[1], J.E. Allanson[1].* 1) Medical Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada; 2) Pediatric Neurology, University of Alberta, Edmonton, Alberta, Canada.

Duplication 9p has a well-described dysmorphic syndrome associated with it. The physical features include hypertelorism, down-slanting palpebral fissures, deep set eyes, down-turned corners of the mouth, and mild skeletal anomalies including hypoplastic terminal phalanges. We report an infant born with some of the typical features of duplication 9p syndrome, as well as the unusual features of extreme joint hyperlaxity with subluxation of the knees and elbows, arachnodactyly and total anomalous pulmonary venous return (TAPVR). Karyotype revealed a de novo unbalanced (9;15) translocation resulting in duplication of 9pter-9q13 and deletion of 15q distal to band q13. Methylation analysis and FISH studies revealed deletion of the SNRPN locus on the paternally derived chromosome 15, consistent with Prader-Willi syndrome. This infant represents the first reported case of duplication 9p syndrome with TAPVR, with the additional interesting finding of Prader-Willi syndrome resulting from an unbalanced 9;15 translocation.

511/T

Congenital diaphragmatic hernia (CDH) associated with deletion of chromosome 15q26: genotype -phenotype correlations. *A. de Klein[1], M. Klaassens[1,2], B. Eussen[1], R. Galjaard[1], D. Scott[3], B. Lee[3], B. Oostra[1], D. Tibboel[2].* 1) Dept Clinical Genetics, Erasmus MC, Rotterdam, Netherlands; 2) Department of Pediatric Surgery, Erasmus MC, Rotterdam, the Netherlands; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, USA.

Congenital diaphragmatic hernia (CDH, MIM 142340) is a severe birth defect characterized by a defect in the diaphragm, associated lung hypoplasia and postnatal pulmonary hypertension. Approximately 50% of the CDH cases are associated with other congenital anomalies and in 5-10% of the cases there is a chromosomal etiology. Deletion of 15q26 is the most frequently described structural chromosomal anomaly in patients with non-isolated CDH. recently we have reported two additional prenatally detected patients with a deletion of 15q26. The phenotype is similar to other patients with CDH caused by 15q26 deletions and includes intra-uterine growth retardation, left-sided CDH, cardiac anomalies and distinct dysmorphic features, as seen in Fryns' Syndrome. Here we would like te present a genotype -phenotype correlations of these 25 CDH and compare these with other known genotype-phenotype associations as for example the chromosome 1q anomalies. We believe that when these combination of birth defects are diagnosed, either pre- or post-natally, further investigations to identify or exclude a deletion of 15q26 or other CDH loci are indicated, since this will have major consequences for prognosis.

512/T

Delineation of a *de novo* chromosome 19(p13.1p13.2) duplication using comparative genomic hybridization (CGH). *A. Iglesias[1], M.J. Macera[2], J. Breshin[2], F. Cohen[2], A. Babu[2].* 1) Dept Pediatrics, Div Genetics, Beth Israel Medical Ctr, New York, NY; 2) Dept Medicine, Div Molecular Medicine & Genetics, Wyckoff Heights Medical Center, Brooklyn, NY.

An eight day old male with a history of polyhydramnios and neonatal hypotonia was seen because of feeding difficulties and arthrogryposis. The proband was born full term via cesarean delivery to a 27 year-old mother. He weighed 3015 g with a length of 51 cm and HC 34 (AGA). His apgar scores were 9/9. He had a rounded face with horizontal palpebral fissures, depressed nasal bridge, inverted V-shaped upper lip, a cupped right ear with a normal palate. Hip dysplasia was noted with distal arthrogryposis in his hands and talipes equinovarus (club foot). A neurological exam showed axial hypotonia and milder, but positive distal hypotonia. The suck/swallowing coordination was assessed as poor.

Chromosome analysis using GTG banding revealed one derivative chromosome 19 with additional material on the p arm. FISH using wcp19 painting probe and 19ptel telomere probe (Vysis) confirmed that the additional material originated from 19 and established the presence of a single19 p telomere on the der(19). The karyotype was 46,XY,?dup(19)(p13.1p13.2).ish dup(19)(?p13.1p13.2)(19ptel+,wcp19+). CGH analysis established the proximal breakpoint of the duplicated material at the middle of band p13.1 and the other at the distal end of band p13.2. This direct duplication occurred *de novo,* as both parents had normal karyotypes. The final karyotype was 46,XY,dup(19)(?p13.1p13.2).ish cgh dup(19)(p13.1p13.2). The proband is making good progress and is due for a follow up visit.

Duplications of chromosome 19p are rare as less than ten cases have been reported in the literature. The phenotypes are similar to those reported for trisomy 19 syndrome, however, not as severe. It is interesting to note that club feet, associated with trisomy 19, was observed in our proband and one of the dup(19)p cases. The use of CGH in such cases will help to improve the correlation of segmental imbalances and clinical manifestations.

513/T

Molecular cytogenetic analysis of a der(17)t(10;17)(q24;q25) chromosome in a child, by CGH. *M.J. Macera[1], G. Kupchik[2], S. Kinshpun[2], J. Breshin[1], F. Cohen[1], A. Babu[1].* 1) Div Molec Medicine & Genetics, Dept Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY; 2) Div Medical Genetics, Dept Pediatrics, Maimonides Medical Ctr, Brooklyn, NY.

Cytogenetic analysis on a peripheral blood specimen from a newborn received at birth, revealed a 46,XX,add(17)(q25).ish add(17)(wcp10+) karyotype. The mother and child were evaluated by us, twenty months later. The child displayed dysmorphic features and failure to thrive. Her physical exam was remarkable for epicanthal folds, depressed nasal bridge, midface hypoplasia, microcephaly, low set ears, full lips, pectus, flat occiput, overlapping toes and ulnar deviation of the fingers. An MRI of her brain showed mega cisterna magna extending in crescentric fashion external to the right and left cerebellum. There were small cortical heterotopia indenting the left occipital horn, with deficient white matter bilaterally and very small corpus callosum.

Chromosomal analysis upon her revisit, showed a 46,XX,der(17)t(10;17)(?q24;q25) karyotype. The mother's chromosomes were normal. The father's blood was unavailable. Comparative genomic hybridization (CGH) was performed to more precisely delineate the chromosome 10 breakpoint. The additional 10 material was determined to be 10q24.1→qter. Loss of chromosome 17q was not detected as the material missing was most likely below the level of detection of the assay. The rough estimate of CGH with a fixed diagnostic threshold is 10-12 Mb. The final karyotype was 46,XX,der(17)t(10;17)(?q24;q25).ish cgh der(17)t(10;17)(q24.1;q25)

Partial trisomy 10q, has been well defined although rare. In this syndrome, the additional chromosome material is usually derived from an unbalanced translocation, and often inherited from the father. The phenotype is comparable to the proband's clinical presentation, with the exception of a lack of hydronephrosis.

CGH analysis was extremely useful in cases such as this one where the father was not available for cytogenetic analysis.

514/T

Duplication of chromosome 12q24.11q24.23 identified by array-CGH in a patient with Noonan syndrome. *A. Patel[1], O. Shchelochkov[1], J. Wiszniewska[1], G. Weissenberger[1], P. Fernandes[1], A.C. Chinault[1], M.K. Kukolich[2], C. Eng[1], S.W. Cheung[1], V.R. Sutton[1].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Clinical Genetics, Cook Children's Hospital, Fort Worth, TX.

Noonan syndrome is an autosomal dominant disorder with an estimated incidence of 1 in 1000 to 2500 live births. It is characterized by short stature, short neck with webbing, cardiac anomalies, developmental delay of variable degree, cryptorchidism in males and characteristic facies. Gain of function mutations in the *PTPN11*, *KRAS* and *SOS1* genes that are components of the RAS-ERK signaling pathway are identified in about 68% of individuals with Noonan syndrome. We report the first case of a duplication of chromosome region 12q24.11q24.23 identified by arrayCGH that includes the *PTPN11* gene in a 3 year old girl with features of Noonan syndrome. The patient presented with postnatal onset failure to thrive, developmental delay, microcephaly, velopalatal incompetence, pectus excavatum, coarctation of aorta, atrial and ventricular septal defects, decreased muscle tone, and facial dysmorphic features consistent with Noonan syndrome, however, sequence analysis of *PTPN11* and *KRAS* did not identify a missense mutation. In addition, at three years of age her speech, gross and fine motor development was at the level of a 12-18 month old child. This degree of developmental delay was atypical for a patient with Noonan syndorme, raising concerns for a chromosomal abnormality. ArrayCGH showed an interstitial duplication of at least 8Mb including the *PTPN11* gene. The increased gene dosage of the *PTPN11* gene in the form of duplication is postulated to be comparable to the gain of function mutations seen in Noonan syndrome. We have shown that increased dosage of *PTPN11* can result in a Noonan syndrome phenotype. We suspect that duplications of one of the RAS-ERK pathway regions/genes may result in a Noonan syndrome phenotype in some of the remaining 30% of patients for whom no missense mutation was detected and should be considered part of a comprehensive evaluation for molecular defects in this pathway.

515/T

Identification of a novel microdeletion involving the entire *NEMO* gene at Xq28 in a patient with Incontinentia Pigmenti. *D. del Gaudio, P.A. Ward, L.L. Meyers, R.A. Lewis, D.L. Nelson, P. Fang, C.M. Eng.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Incontinentia pigmenti (IP) is a rare multi-systemic disorder characterized by skin, hair, teeth and central nervous system abnormalities. It is inherited as an X-linked dominant trait and is lethal in male fetuses. In females the clinical phenotype varies and is significantly modulated by skewed X-inactivation. Familial IP is caused by loss-of-function mutations in the *NEMO* gene (NF-kB Essential MOdulator or IKBKG) located on chromosome Xq28. NEMO is part of a regulatory complex required to activate the NF-kB transcription factor that is involved in a number of immune, inflammatory, and apoptotic pathways. Approximately 80% of IP patients harbor a common deletion encompassing exon 4 through exon 10 of the *NEMO* gene. This rearrangement appears to be mediated by flanking repeat sequences and can be identified by either Southern analysis or long-range PCR specifically targeted to the *NEMO* gene. Here we report the identification of a 33 year old female with typical IP, harboring a novel microdeletion encompassing the entire *NEMO* gene. Southern analysis indicates that this individual is negative for the common deletion; however, the normal sized fragment showed a significantly reduced intensity, indicating a possible heterozygous full gene deletion. Real time PCR and oligonucleotide-based array-CGH further defined the proximal deletion breakpoint at the 5' of the G6PD gene. X-inactivation study indicated that the patient had complete skewed X-inactivation. This is the first full *NEMO* gene deletion case reported to date. Further characterization of the deletion breakpoints will provide important clues towards understanding the putative mechanism responsible for the recombination event leading to the *NEMO* gene rearrangements, and potentially expand the mutation detection spectrum for IP.

516/T

Molecular characterization of deletion breakpoints in the CREBBP gene. *D. Simon[1], C. Rooryck[1,2], M. Stef[1], D. Lacombe[1,2], I. Coupry[1], B. Arveiler[1,2].* 1) Human Genetics Laboratory, Université Victor Segalen Bordeaux 2, Bordeaux, France; 2) Service de Génétique Médicale, CHU de Bordeaux, Bordeaux, France.

The Rubinstein-Taybi syndrome (RTS) is a rare autosomal-dominant disease caused by mutations in the CREB-binding protein (CREBBP), and more rarely the EP300, genes. RTS is known to be associated with CREBBP deletions in 10-15% of cases. Using array-CGH and quantitative multiplex fluorescent (QMF) PCR analysis we have shown that rearrangements occur in fact in about 20% of patients, and that the deletions' sizes range between 1245 bp and 6.5 Mb. We are now aiming to unravelling the mechanisms involved in these rearrangements. The repeated occurrence of breakpoints in some segments of the gene and surrounding region raises the possibility that particular sequences such as segmental duplications or repeated sequences are involved in these rearrangements. In order to investigate this, we have started sequencing the breakpoints of 13 CREBBP intragenic rearrangements. We have so far sequenced the breakpoints of six deletions the sizes of which range between 1245 and 48327 bp. One is a complex rearrangement involving a deletion and the insertion of a 435bp element located about 3 kb away from the deleted segment. No particular sequence was identified at or next to the corresponding breakpoints. In one case, the sequences flanking both breakpoints corresponded to AluSx and AluSc elements sharing 30 bases with 100% identity. In another case the identity was restricted to 5bp in an AluSx and a MIR3-type SINE, and in another one, 7bp were shared between an AluSx element sitting next to one of the breakpoints and an AluSg element located some 250 bp away from the other breakpoint. In the last two cases no homologous sequences could be identified. The other seven rearrangements are currently being characterized. The results obtained so far indicate that Alu sequences are frequently involved in CREBBP intragenic rearrangements. No segmental duplication was identified so far. We shall also characterize the extragenic breakpoints. From a mechanistic point of view, it will be interesting to see whether the large deletions are caused by different mechanisms from the smaller intragenic ones.

517/T

A WT1 exon 6 truncation mutation causes ambiguous genitalia in a patient with Denys-Drash syndrome. *A. Tsai[1], P. Chiang[2], S. Kopinsky[2], E. Spector[2].* 1) Div Clinical Gen & Metabolism, Childrens Hosp, Denver, CO; 2) DNA Diagnostic Lab, Department of Pedatrics, UCDHSC.

Denys-Drash syndrome (DDS) is a rare genetic disorder featuring the triad of congenital nephropathy, Wilms tumor, and intersex disorders (XY pseudohermaphroditism or XY female). DDS is associated with constitutional mutations in the Wilms tumor suppressor gene, WT1. Of 30 patients with reported mutations, 24 had mutations at exons 8 or 9; 21 of these were missense mutations in the zinc-finger region of WT1. Six patients had mutations outside exons 8 and 9. Only seven of the 30 were truncation mutations: one each in exons 1, 3 and 8; two each in exons 6 and 9. Unlike WAGR syndrome, with its complete deletion of one copy of WT1, DDS is most likely caused by a dominant-negative mode of action of mutant DDS proteins. We present here a new case of DDS with a novel nonsense mutation in exon 6, leading to a stop codon and hence a truncated protein. The patient was initially diagnosed with ambiguous genitalia/partial androgen insensitivity syndrome (46, XY). Orchiectomy was performed at age 5 days, and the patient was raised as a girl. At 8 months, she presented with a one-week history of vomiting, fever and abdominal distension. Huge bilateral Wilms tumors were detected. The entire coding region of the WT1 gene was sequenced from genomic DNA isolated from peripheral blood. A mutation was identified at exon 6 (Y339X; numbering is based on isoform B, NP_077742). Lessons learned: (1) Always consider a diagnosis of DDS in the presence of ambiguous genitalia and androgen insensitivity. Offer Wilms tumor protocol (quarterly renal ultrasound until age 12) until proved otherwise. Due to the rarity of DDS, our patient's ambiguous genitalia didn't initially raise suspicion of DDS, so she was not monitored for Wilms tumor. (2) The mutation identified represents the first report of an exon-6 truncation mutation in a patient with ambiguous genitalia. (3) Molecular diagnosis of WT1-related conditions like DDS, Frasier Syndrome, familial Wilms tumor and isolated diffuse mesangial sclerosis is now available clinically, and will greatly facilitate genotype-phenotype correlation studies.

518/T

Chromosome 22q11 instability: deletion and duplication in the same family. *S.C. Saitta, G.R. Jalali, D.M. McDonald-McGinn, E.H. Zackai, B.S. Emanuel.* Childrens Hospital of Philadelphia Philadelphia, PA.

We analyzed the parents of a proband with classical features of DiGeorge syndrome including a VSD, hypocalcemia, submucous cleft palate, and a typical chr22q11 deletion. Both parents were clinically normal, however analysis using MLPA showed that the 3 Mb region deleted in the child was duplicated in the father. Multiple recent reports have described reciprocal duplications of the DGS region,that present with variable mild phenotypes. In order to better define the mechanism(s) of rearrangement in this family, we first determined the parental origin of the proband's deletion using multiple polymorphic microsatellite markers from within chr22q11. This haplotype analysis showed that the proband's alleles were all of paternal origin, consistent with a deletion on the maternally-derived chr22. These findings suggest a de novo DGS deletion in the proband and an apparently unrelated microduplication in his father. We have previously demonstrated that seemingly recurrent deletions in first cousins were de novo and had occurred independently. Our findings highlight the genomic instability of the 22q11 region as predicted by its genomic structure. While the DGS deletion is the most frequently occurring microdeletion syndrome, increasing reports of the reciprocal microduplication of this region indicate that it may also have significant prevalence. With the advent of improved methods for detecting duplications, it becomes increasingly relevant to better define the associated clinical phenotypes, in order to provide accurate prognostic information and recurrence risk counseling.

519/T

Extended pedigree with multiple cases of XX sex reversal in the absence of SRY and of mutation at SOX9 and RSPO1 loci. *S.G. TEMEL[1], T. GULTEN[1], T. YAKUT[1], H. SAGLAM[2], N. KILIC[3], E. BAUSCH[4], W.J. JIN[4], M. LEIPOLDT[4], O. RADI[5], G. CAMERINO[5], G. SCHERER[4].* 1) Medical Genetics Department, Faculty of Medicine,Uludag University, BURSA, Turkey; 2) Pediatric Endocrinology Department, Faculty of Medicine, Uludag University, Bursa, Turkey; 3) Pediatric Surgery Department, Faculty of Medicine, Uludag University, Bursa, Turkey; 4) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany; 5) Istituto di Biologia Generale e Genetica Medica, Universita di Pavia, Pavia, Italy.

It is well established that testicular differentiation of the human embryonic gonad depends on the action of the Y-chromosomal gene SRY. However, exceptional cases such as SRY-negative cases of 46,XX testicular disorder of sexual development (DSD) and of 46,XX ovotesticular DSD document that testicular tissue can develop in the absence of the SRY gene. These SRY-negative XX sex reversal cases are very rare and usually sporadic, but a few familial cases have been reported. We present a large, consanguineous family with nine affected individuals with phenotypes ranging from 46,XX testicular DSD to 46,XX ovotesticular DSD, with predominance of male characteristics. Absence of SRY in peripheral blood was documented by fluoresence in situ hybridization (FISH) and PCR analysis in all nine affected individuals, and by FISH analysis on gonadal sections with testicular tissue in four affected individuals. By quantitative PCR, a duplication of the SOX9 gene was excluded. In addition, as linkage analysis showed that the nine affected members of the family do not share a common SOX9 haplotype, any mutation at the SOX9 locus could be ruled out. Also, no mutation was found within the RSPO1 gene, recently found to be mutated in familial XX sex reversal. Together, these findings implicate a mutation at a sex-determining locus other than SRY, SOX9 and RSPO1 as the cause for the XX sex reversal trait in this family.

520/T

Mutation spectrum of the RAS/MAPK pathway genes in Noonan, Costello and cardio-facio-cutaneous syndromes. *Y. Aoki[1], T. Niihori[1], Y. Narumi[1], H. Cavé[2], A. Verloes[2], H. Kawame[3], K. Kurosawa[4], H. Ohashi[5], N. Okamoto[6], G. Neri[7], R.C.M. Hennekam[8], G. Gillessen-Kaesbach[9,10], D. Wieczorek[9], M.I Kavamura[11], L. Wilson[9], K. Nishio[12], I. Kondo[13], P. Lapunzina[14], S. Kure[1], Y. Matsubara[1].* 1) Dept Medical Genetics, Tohoku Univ Sch Medicine, Sendai, Japan; 2) Hôpital Robert Debré (APHP), Paris, France; 3) Nagano Children's Hosp, Nagano; 4) Kanagawa Children's Med Ctr, Yokohama; 5) Saitama Children's Med Ctr, Saitama; 6) Osaka Med Ctr & Res Inst for Maternal & Child Health, Osaka, Japan; 7) Istituto di Genetica Medica, Rome, Italy; 8) Inst of Child Health, London, UK; 9) Univ Essen, Essen, Germany; 10) Univ. Schleswig-Holstein, Lübeck, Germany; 11) Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil; 12) Seirei Hamamatsu General Hospital, Hamamatsu; 13) Ibaraki Prefectural Handicapped Children's Ctr., Mito, Japan; 14) Hosp. Univ. La Paz, Madrid, Spain.

Noonan, Costello and cardio-facio-cutaneous (CFC) syndromes are autosomal dominant disorders characterized by a distinctive facial appearance, heart defects, musculocutaneous abnormalities and mental retardation. Recently we discovered proto-oncogene *HRAS* mutations in Costello syndrome and *KRAS* and *BRAF* mutations in CFC syndrome, establishing a new role of RAS/RAF/MEK/ERK pathway in human development. To elucidate the clinical and molecular characteristics of Noonan, Costello and CFC syndromes, we have so far analyzed *PTPN11*, *HRAS*, *KRAS*, *BRAF* and *MAP2K1/2* (MEK1/2) in 54 patients with Noonan syndrome (NS), 39 Costello patients and 78 CFC patients. Mutations in *PTPN11* were identified in 41% NS patients. *HRAS* mutations were detected in 21 patients with typical Costello syndrome. Mutations in *KRAS, BRAF* or *MAP2K1/2* were found in 60% of patients with CFC syndrome. We also identified four *SOS1* mutations in two NS patients, two typical CFC patients and one patient between NS and CFC phenotype, suggesting that mutations in *SOS1* are causative for NS and CFC syndrome. It is plausible to speculate that new genetic causes for Noonan-related disorders still remain to be unidentified in molecules in the RAS/MAPK pathway.

521/T

The Phenotypic Association of Transverse and Central Ray Limb Deficiencies. *A.M. Elliott, J.A. Evans.* Dept Biochem & Medical Gen, Univ Manitoba, Winnipeg, MB, Canada.

Central ray deficiency (Split Hand Foot Malformation, SHFM) can occur as an isolated anomaly or in association with other malformations. Classifications of SHFM include typical (central V-shaped cleft, often multimelic with positive family history) or atypical (central deficiency, sporadic, one affected limb). Typical SHFM is considered more "genetic" in nature and atypical more "vascular." The most severe expression of typical SHFM is considered to be fifth finger monodactyly. However, Maisels proposed the "Centripetal Suppression Theory" that explains split hand as a progressive insult to the developing hand plate ranging from a simple cleft with no tissue deficiency to the most severe formaphalangiaa terminal transverse defect (TTD) (Hand, 1970). Geneticists generally associate TTD with vascular insult. We performed a detailed clinical epidemiologic study to investigate the association of central ray deficiency and TTD. Isolated and syndromic patients were evaluated from the literature and the local (Manitoba) population. Inclusion criteria consisted of central ray deficiency and TTD in the same patient. This phenotypic combination was seen in both syndromic and isolated SHFM patients. Syndromic cases included those with long bone deficiency (e.g. tibial aplasia/ectrodactyly, femur-fibula-ulna complex). It was also associated with ulnar deficiency, hypoglossia-hypodactyly, chromosome anomalies and mutations in TP63. The underlying genetic defect, if any, has not been elucidated for many of these disorders. The finding of this phenotypic combination in the same patient suggests TTD are not exclusively vascular in nature and likely represent the most severe expression of SHFM, thus supporting Maisels' theory.

522/T

Colobomatous microphthalmia and a cyst associated with a nonsense *NF2* gene mutation. *T. Mononen[1], K. Kaarniranta[2], K. Tuppurainen[2].* 1) Dept Clinical Genetics, Kuopio Univ Hosp, Kuopio, Finland; 2) Dept Ophthalmology, Kuopio Univ Hosp, Kuopio, Finland.

Neurofibromatosis type 2 (NF2)-specific ocular findings include cataracts, epiretinal membranes, optic nerve sheath meningiomas, disc gliomas, and hamartomas. We report a rare association of optic disc coloboma, microphthalmia, and a retrobulbar cyst in an infant with NF2.

A male infant was born at 38 weeks gestation to a 31-year-old gravida 1 para 0 mother and a 35-year-old father after an uneventful pregnancy. Microphthalmia and strabismus of the left eye were observed at birth. Ophthalmologic evaluation revealed posterior lens opacities and optic disc coloboma in the microphthalmic left eye which appeared blind. There was a small, anomalous optic disc in the right eye and the visual acuity was decreased, 0.07 on Teller's line test. Magnetic resonance imaging revealed a microphthalmic left eye with optic disc coloboma and a retrobulbar cyst. In addition, a small schwannoma of the left vestibular nerve was observed. No hearing impairment was detected. The karyotype was normal male 46,XY. The molecular genetic testing of the *NF2* gene in leukocyte DNA revealed a nonsense mutation c.169C>T (p.Arg57X) confirming the diagnosis of NF2. The same mutation was not detected in the parents' blood samples and there was no family history of NF2.

The ocular abnormalities in this patient indicate that altered *NF2* gene dosage may cause developmental anomalies of the optic disc ranging from hypoplasia to coloboma, associated with microphthalmia and a cyst.

523/T

Atypical pattern of multiple malformations: pseudo-trisomy 13 syndrome with syngnathia? *C.C. Rebelo[1], C.M. Lourenço[1], L.C. Peres[2], J.M. Pina-Neto[1], V.E.F. Ferraz[1].* 1) Department of Genetics, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, MD; 2) Department of Pathology, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, MD.

The pseudo-trisomy 13 syndrome is characterized by variable multiple anomalies including holoprosencephaly, polydactyly, severe facial anomalies and other defects, resembling trisomy 13. Autosomal recessive inheritance is suggested for this disorder due to cases with consanguinity. We report on a fetus who was born with multiple malformations at 34 weeks of gestational age, from a nonconsanguineous couple. Ventilation of the baby was not possible due to choanal atresia and syngnathia. The clinical examination showed microcephaly, exorbitism, microphtalmos, facial asymmetry, microtia, microstomia, preaxial polydactyly, meningocele and abnormal external genitalia. On necropsy it was observed holoprosencephaly, syngnathia, hypoglossia, single umbilical artery, hemivertebrae, uterus bicornis. Heart and kidneys were normal at examination. Skin fibroblast karyotype was 46,XX. The present case may expand the spectrum of the pseudo-trisomy 13 syndrome, including syngnathia as an additional feature.

524/T

Hax1 gene mutation in an Iranian family affected to Kostmann disease and result of PND in the 5th pregnancy. *G. Vakili[1], Y. Shafeghati[1], H. Abolghasemi[2], M. Horwitz[3].* 1) Genetics Research Center, University of Social Welfare sciences and Rehabilita, Tehran, Iran; 2) Baghyatallah Hosp.and Medical Science University, Tehran, Iran; 3) Medical Genetic Div., Dept.Medicine, University of Washington, Seattle, USA.

Severe congenital neutropenia (SCN) or Kostmann syndrome is a rare type of neutropenia. It is inherited by autosomal recessive pattern. Consanguineous marriage mostly is a predisposing factor. Patients suffer from severe and recurrent bacterial infections (pneumonia, otitis media, abcesses, and.....). This disease was reported by Kostmann in a large consanguineous family from Northern part of the Sweden (1956). In this report we will present an Iranian family, with two affected children and two abortion. Parents are second cousins. Both of the sibs showed neutropenia from early infancy. They have had recurrent severe bacterial infections. The older one was a boy, who showed Myelodysplastic (MDS) changes after the age of 15 and died from AML when he was 16-year-old. The younger one is a 14-year-old girl just with the similar symptoms. The response of both of them was favorable to G-CSF. Mutation analysis of the ELA2 gene by direct DNA sequencing of PCR-amplified genomic DNA did not identify any abnormalities. In searching of mutations in other candidate genes, a homozygous mutation found in HAX1 gene in the proband, and each of the parents was heterozygous carrier for the mutation. The mutation was W44X, same as described by Klein and Welte in their Nature Genetics paper. After mutation detection in the family, the mother became pregnant. But the fetus was homozygote for the mutation.The family decided to terminate the pregnancy.

525/T

Phenotypic spectrum of *STRA6* mutations: from Matthew-Wood syndrome to non-lethal syndromic microphthalmia. *C. Golzio[1,5], N. Chassaing[6], J. Martinovic-Bouriel[2], S. Thomas[1], S. Mougou-Zrelli[2], B. Bessières[3], S. Odent[7], M. Bonnière[2], S. Delahaye[4,5], P. Calvas[6], A. Munnich[1,2,5], F. Encha-Razavi[1,2,5], S. Lyonnet[1,2,5], M. Vekemans[1,2,5], T. Attie-Bitach[1,2,5], H.C. Etchevers[1,6].* 1) INSERM U781, Hôpital Necker-Enfants Malades, Paris, France; 2) Hôpital Necker-Enfants Malades, Paris, France; 3) Institut de Puériculture, Dept. of Fetal Pathology, Paris, France; 4) Hôpital Necker-Enfants Malades, Dept. of Obstetrics, Paris, France; 5) Université René Descartes-Paris 5, Hôpital Necker-Enfants Malades, France; 6) INSERM U563, Hôpital Purpan, Toulouse, France; 7) Hôpital Sud, Dept. of Genetics, Rennes, France.

STRA6 encodes an integral cell membrane protein that favors RA uptake from soluble retinol-binding protein (Kawaguchi et al. 2007). Subsequently, RA affects transcription of developmental genes such as members of the fibroblast growth factor family. One transcriptional target of RA is *STRA6* itself (Bouillet et al. 1997). Molecular analysis of *STRA6* was undertaken in two unrelated consanguineous human fetuses we have previously described with Matthew-Wood syndrome [MIM 601186]. Each fetus had homozygous truncating mutations predicting a premature stop codon in *STRA6* transcripts. A third Matthew-Wood fetus presented compound heterozygosity of a missense and splicing mutation in *STRA6.* Compound heterozygous missense mutations have also been found in a middle-aged patient with severe bilateral microphthalmia, tetralogy of Fallot, and mild mental retardation but no apparent pulmonary defects. This patient is a member of a non-consanguineous family in which there are two other affected siblings with divergent phenotypes, including spina bifida occulta and autism. Including other reported cases (Pasutto et al. 2007), no genotype-phenotype correlations can yet be drawn. We propose that pathogenic *STRA6* mutations reduce RA uptake from maternal blood, leading to the impairment of a set of essential target genes and possibly explaining phenotypic diversity through a combination of environmental and innate variations.

526/T

Neural crest migration defects underlie craniofacial dysmorphology in Bardet-Biedl syndrome. *J.L. Tobin[1], M. Franco[2], E. Eichers[3], H. May-Simera[1], M. Garcia[3], J. Yan[3], M. Justice[3], J. Briscoe[4], R. Mayor[5], R. Lupski[3], P. Hammond[1].* 1) Molecular Medicine Unit, UCL Institute Child Health, London, United Kingdom; 2) Biomedical Informatics Unit, UCL Eastman Dental Institute for Oral Health Care Sciences, 256 Gray's Inn Road, London, WC1X 8LD, UK; 3) Dept of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA; 4) Developmental Neurobiology, National Institute for Medical Research, London, NW7 1AA, UK; 5) Department of Anatomy and Developmental Biology, University College London, WC1E 6BT, UK.

Facial recognition is central to the diagnosis of many syndromes and key craniofacial patterns may reflect common pathway etiologies. In the pleiotropic Bardet Biedl syndrome (BBS), a primary ciliopathy with intraflagellar transport dysfunction, patients have a characteristic facial "gestalt" that dysmorphologists have found difficult to characterize. Here, we use dense surface modeling to show BBS patients and mouse models both have midfacial flattening. Zebrafish morphants have defects of homologous facial structures and display hallmarks of disrupted Sonic Hedgehog (Shh) signaling. Fish epistasis experiments in concert with cell-based assays indicated the importance of Bbs proteins for Shh pathway transduction by mediating Gli3 processing. Furthermore, we discovered that Bbs proteins are required for neural crest cell (NCC) migration through their involvement with the planar cell polarity pathway. We propose a model whereby Bbs proteins mediate NCC migration and then modulate their responsiveness to Shh essential for normal patterning of the midline structures of the face. Finally, we observed for the first time, ciliated NCCs, supporting evidence for novel roles for Bbs proteins in NCC migration and Shh transduction. This is the first study to derive molecular pathomechanisms from characterization of facial dysmorphology which should provide the basis for investigation into other dysmorphic syndromes.

527/T

Genome-wide analysis shows unexpected CNV in monozygotic twins - its potential clinical implications. *J.T. den Dunnen[1], A.C.J Gijsbers[1], Y. Ariyurek[1], H.H. Thygesen[1], C.A.L. Ruivenkamp[1], D.I. Boomsma[2], E.P. Slagboom[3], M.H. Breuning[1], G.J.B. van Ommen[1].* 1) Center of Human and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland; 2) Biological Psychology, Vrije Universiteit, Amsterdam, Nederland; 3) Molecular Epidemiology, Leiden University Medical Center, Leiden, Nederland.

Array-based technologies now facilitate straightforward genome-wide screening of the human genome for the presence of deletions and duplications (Copy Number Variation, CNV). Recent data show a surprising variety and frequency of (likely) non-pathogenic CNVs. These are estimated to affect up to 10% of the human genome. In several genetic diseases these tools have also been successfully used to identify the genes involved or they are applied to identify new genes in cases with e.g. malformation syndromes and mental retardation. In the process of implementing genome-wide SNP arrays for CNV screening in clinical diagnosis we analysed blood-derived DNA samples of 10 monozygotic twin pairs. Based on shared CNVs patterns the individual twin pairs could be easily recognized. However, we also detected an unexpected number of unique differences within the monozygotic twin pairs. The number of CNVs identified depends mainly on the settings of the scoring algorithms used; in the size range of 0.3-1.2 Mb we detect 1-2 per twin pair. Preliminary validation data appear to confirm the findings while showing that the CNVs are not present in 100% of the cells. This suggests - not unexpectedly given the source material - somatic mosaicism, ie a postmeiotic emergence. This would have impact not only on the understanding of phenotypic diversity in MZ twins, but also on the use of somatic material (eg. lymphocyte DNA) for DNA diagnostics. A larger deletion / duplication identified in de novo cases might be somatic in nature. In these cases confirmation is thus in order using an independent second sample from another tissue.

528/T

A chromosome 19p deletion in a patient with SHFM, tetralogy of Fallot and a clinical phenotype of Angelman syndrome. *E. Aten[1], N.S. den Hollander[1], C.A.L. Ruivenkamp[1], J. Knijnenburg[2], H. van Bokhoven[3], J.T. den Dunnen[1], M.H. Breuning[1].* 1) Center of Human and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland; 2) Molecular Cell Biology, Leiden University Medical Center, Leiden, Nederland; 3) Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Nederland.

Congenital limb malformations are the second most common birth defects observed in infants. Split hand foot malformation (SHFM) also known as central ray deficiency, ectrodactyly and cleft hand/foot, can occur as an isolated malformation or in combination with other malformations such as the EEC (ectrodactyly-ectodermal dysplasia- cleft lip/palate) syndrome. This variability causes the phenotypic classification of SHFM to be very difficult. Management of affected patients and their families is further complicated by the genetic heterogeneity observed, with already five genomic loci implicated. Identification of genes involved in defects in human limb patterning has been limited, in part, by the enormous spectrum of phenotypes. Currently the five types are referred to as SHFM1 (7q21-q22), SHFM2 (Xq26), SHFM3 (10q24), SHFM4 (3q27), and SHFM5 (2q31). Molecular testing is only available for SFHM4 since patients mapped to this locus have been shown to carry mutations in the TP73L (p63) gene. We report a male patient with SHFM, tetralogy of Fallot and a clinical phenotype of Angelman syndrome. Using 1 Mb BAC array genome analysis we identified a deletion with a maximal deleted size of 1.4 Mb at chromosome 19p, confirmed by FISH analysis. Analysis of the parents of the patient showed that the deletion was de novo. To determine the borders of the deletion more precisely we used a 250K oligonucleotide SNP array (Affymetrix). The deletion was found to be 0.96 Mb in size, containing 27 genes. We screened 21 additional SHFM patients with ectrodactyly (TP73L mutation negative) for rearrangements in this region using a home-made MLPA assay but did thus far not detect other deletions or duplications. The 27 genes in the deleted region have been prioritised and are currently screened for possible mutations.

529/T

Pure Trisomy 3q29 Presenting as VATER Association. *M.W. Lee[1], A.R. Brothman[2], O.A. Abdul-Rahman[3].* 1) Pediatrics, University of Mississippi Medical Center, Jackson, MS; 2) Department of Human Genetics, University of Utah, Salt Lake City, UT; 3) Division of Medical Genetics, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS.

There are several cases of 3q duplications reported in the medical literature. The typical phenotype involves mental retardation, growth retardation, congenital heart defects, renal anomalies, and characteristic facial features. We report a case of a 9 year-old Caucasian female who initially presented with a diagnosis of VATER association that was later discovered to have a 3q29 duplication on microarray analysis. At birth, she was noted to have multiple gastrointestinal anomalies including a tracheoesophageal fistula, esophageal atresia, Meckel's diverticulum, malrotation, and imperforate anus. She was also noted to have vertebral anomalies, but no cardiac or renal defects. A karyotype was performed and did not identify any abnormalities. The patient was diagnosed with VATER until she presented for a follow-up evaluation at 9 years of age. At the follow-up visit, she was noted to have generalized growth deficiency, learning problems, and a history of developmental delay. A microarray analysis was performed using the Spectal 1MB Chip and demonstrated a duplication of chromosome 3q29. The duplicated region was estimated to be about 1.3 to 2.7 megabases and was confirmed by FISH. A review of the medical literature revealed no previously reported cases of a pure 3q29 duplication. Our patient showed very mild characteristics of patients with a larger 3q duplication. Although our patient had significant gastrointestinal and vertebral malformations typically seen in VATER association, none of the previously reported cases had similar findings. Therefore, we suspect that the duplication may have breakpoints within a gene critical for development of the gastrointestinal and skeletal systems. Such a gene may represent a candidate gene underlying at least some cases of VATER association. Based on our findings, we recommend that all patients with a diagnosis for VATER association who present with developmental delay or growth deficiency should undergo microarray analysis.

530/T

***FGFR2* Mutations in Turkish patients with craniosynostosis syndrome.** *O. ALPER[1], E. MIHCI[2], H. KAYSERILI[3], M.O. CALISKAN[1], S. TACOY[2], L.J. WONG[4], G. LULECI[1].* 1) DEPARTMENT OF MEDICAL BIOLOGY-GENETICS, FACULTY OF MEDICINE,AKDENIZ UNIVERSITY, ANTALYA, TURKEY; 2) DEPARTMENT OF PEDIATRICS,FACULTY OF MEDICINE,AKDENIZ UNIVERSITY, ANTALYA, TURKEY; 3) DEPARTMENT OF MEDICAL GENETICS,INSTITUTE OF CHILDREN'S HEALTH, FACULTY OF MEDICINE,ISTANBUL UNIVERSITY,ÇAPA,ISTANBUL, TURKEY; 4) DEPARTMENT OF MOLECULAR AND HUMAN GENETICS, BAYLOR COLLEGE OF MEDICINE, ONE BAYLOR PLAZA,HOUSTON,TEXAS, USA.

Fibroblast growth factor receptor 2 (*FGFR2*) gene mutations have been associated with the craniosynostotic conditions of Apert, Crouzon, Pfeiffer, Jackson-Weiss, Saethre-Chotzen, Beare-Stevenson Cutis Gyrata, and Antley-Bixler syndromes in various ethnic groups. Twenty unrelated Turkish patients (6 Apert syndrome, 6 Crouzon syndrome, 2 Pfeiffer syndrome, 2 Saethre/Chotzen syndrome, and 4 unclassified craniosynostosis) were screened for mutations in exons IIIa and IIIc of the *FGFR2* gene by polymerase chain reaction and direct sequencing. In the present study, *FGFR2* gene mutations are detected in all of 6 patients with Apert syndrome (4 with Pro253Arg; 2 with Ser252Trp) and 4 out of 6 patients with Crouzon syndrome (one with Cys342Tyr, three with Trp290Arg), one patient with Cys342Arg mutation in a Pfeiffer syndrome patient. We did not detect any *FGFR2* gene mutations in patients with Saethre-Chotzen syndrome or unclassified craniosynostosis. As this study provides a preliminary data in Turkish population, elucidation of the *FGFR2* mutations in patients with clinical features suggestive of Apert and Crouzon syndrome offers a significant benefit to those families in terms of genetic counseling and prenatal diagnosis.

531/T

Screening for 15q duplications/triplications using real-time PCR. *S. Bleoo, S. Chan, D. Hildebrand, N.J. Leonard, J.S. Bamforth, L. Vicen, M.J. Somerville.* Department Medical Genetics, University of Alberta & Stollery Children's Hospital, Edmonton, AB, Canada.

Duplications and triplications of the proximal arm of chromosome 15 have been reported in patients with developmental delay and in patients with autistic behaviour. As developmental delay is one of the most frequent indications for molecular testing in our lab, we designed a real-time PCR assay to detect copy number variations at this locus. Our assay included probes within the SNRPN, UBE3A and GABRB3 genes. This assay failed to detect any new cases of 15q duplication in our sample population which included 238 patients referred for developmental delay. However, this assay correctly identified a 15q triplication in a developmentally delayed male patient who demonstrated abnormal maternal dosage by Southern blot analysis for Angelman syndrome. This patient presented to genetics at age 30 months with hypotonia, and global developmental delay, including fine and gross motor skills, social and language skills. In addition to this patient, we have also been able to confirm two 15q duplication cases initially detected through cytogenetic analysis. One involves a fetus with a maternal 15q duplication; this fetus was subsequently terminated. Although there are very few reports that support the pathogenicity of paternal 15q duplications, our second case involved three brothers who each inherited a 15q11-13 duplication from their father. The father and all three of his sons have intellectual delays and the father has been diagnosed with schizophrenia. Therefore, we conclude that 15q duplications and triplications, although not common in our developmentally delayed population, are rapidly detected though a real-time PCR assay. Our data also provide evidence that some paternal 15q duplications are likely pathogenic.

532/T

An interstitial duplication of Xp22.31 defines a new candidate region for lissencephaly loci. *J.A. Martinez-Agosto.* Division of Medical Genetics, Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA.

We report on a 3 year old female with a history of lissencephaly, dysmorphic features, seizure disorder, microcephaly, central hypoventilation syndrome, poor swallow and suck coordination, ventricular septal defect, cardiomyopathy, gastroesophageal reflux, vesicoureteral reflux with hydronephrosis, multiple pneumonias, and sensorineural hearing loss. This patient also developed bilateral cataracts. Skull series was negative for craniosynostosis. At birth she was noted to be hypotonic and was diagnosed with central hypoventilation. An EEG showed epileptiform activity. Additional testing included very low chain fatty acids, mitochondrial and metabolic testing that were all normal, a karyotype that showed 46,XX, and fluorescent in-situ hybridization for subtelomeric deletions that were negative. On further testing, microarray analysis identified a duplication of Xp22.31. The duplicated region is proximal to, but does not include the STS locus and it is distal to, but does not include the KAL1 locus. The duplication does not include the XLAG/Arx locus, and it is distal to the Aicardi syndrome region. Microdeletions that include this region have been previously associated with variable phenotypes including mental retardation and dysmorphic features. In particular, two previous reports of large deletions including this region presented with a wide spectrum of physical features. This is the first report of a duplication of this region. Genes within this region include VCX-C/VCX3A, which is expressed in the fetus, brain, liver, skin, stomach and testis germ cells, and a new gene enriched in embryonic stem cells, CN268333. We suggest that these may represent candidate genes for lissencephaly and/or some of the additional congenital anomalies present in this patient.

.

533/T

A novel deletion in ROR2 causes combined brachydactyly type B and syndactyly type I in a Chinese family. *X. Zhang[1], D. Lv[1], Y. Luo[2].* 1) Departmen of Medical Genetics, Peking Union Medical College, Beijing, China; 2) China Medical University, Shenyang, China.

Brachydactyly type B (BDB, MIM 113000) is a dominantly inherited limb malformation with complete penetrance and variable expressivity. It is characterized by shortening or absence of distal phalanges of fingers/toes 2-5. Thumbs are less severely affected and often show broad and bifid distal phalanges. BDB can be caused by mutations in the ROR2 gene encoding a receptor tyrosine kinase. Syndactyly type I (SD1, MIM 185900) has complete or partial soft tissue syndactyly between fingers 3 and 4 and/or between toes 4 and 5. The SD1 locus has been mapped to chromosome 2q34-q36. We found a three-generation Han Chinese family with combined BDB and SD1. All the 12 affected individuals in the family showed typical BDB limb phenotypes and most of them also displayed complete webbing of fingers 3-4 and toes 2-3. Two-point linkage analysis was first performed using 3 microsatellite markers selected from the genomic region close to the ROR2 gene at chromosome 9q22 and 12 markers from the chromosome 2q34-q36 region. A maximum LOD score of 2.71 was obtained with the markers D9S1815 and D9S1841, suggesting a genetic linkage. Direct DNA sequencing of the PCR-amplified fragments revealed in the proband a heterozygous 1bp deletion in exon 9 of the ROR2 gene. This mutation showed perfect cosegregation with the disease phenotype in the family but not detected in 50 unrelated healthy controls. In summary, we have confirmed the link between the ROR2 gene and the combined BDB and SD1 in a Chinese family.

534/T

Screening of Interferon Regulatory Factor 6 (*IRF6*) in European Patients with Van der Woude / Popliteal Pterygium Syndrome or Non Syndromic Cleft Lip and Palate. *L. Desmyter[1], M. Ghassibe[1], N. Revencu[1,2], B. Bayet[2], C. Verellen[2], O. Boute[3], M. Lees[4], K. Devriendt[5], K. Claes[6], G. Mortier[6], M.C. Addor[7], M. Bouma[8], D. Genevieve[9], A. Goldenberg[10], A. Gözü[11], M. McEntagart[12], A. Sanchez[13], C. Vilain[14], L. Van Malderghem[15], M. Vikkula[1].* 1) de Duve Institute, Université catholique de Louvain, Belgium; 2) Cliniques universitaires St Luc, Belgium; 3) Hopital Jeanne de Flandre, France; 4) Institute of Child Health, UK; 5) KUL, Belgium; 6) UZ-Gent, Belgium; 7) C.H.U. Vaudois, Suisse; 8) Groningen university hospital, The Netherlands; 9) Hopital Necker-Enfants malades, France; 10) Hopital Charles Nicolle, France; 11) Plastik vr reconstrüktif, centrahl Uzmani, Turkey; 12) St George's hospital, UK; 13) Hospital clinic Mejia Lequerica, Spain; 14) U.L.B., Belgium; 15) IPG, Belgium.

Orofacial cleft is one of the most common birth defects in humans. It can be divided into syndromic and non-syndromic cleft (NSC). Based on epidemiological, embryological and genetic data, cleft lip with or without palate (CLP) is considered distinct from cleft palate only (CPO). Van der Woude (VWS) syndrome is a cleft syndrome characterized by pits in the lower lip present in 85% of cases. In addition to the signs of the VWS, the Popliteal Pterygium syndrome (PPS) includes popliteal and oral webs, syndactyly and genital abnormalities. The *IRF6* gene, localized to *1q32.2*, is mutated in patients with VWS and/or PPS. We screened 41 VWS and 13 PPS patients mainly from Europe by Denaturing High Performance Liquid Chromatography (DHPLC) and sequencing. We identified mutations in the coding region in the majority of our VWS patients and in all our PPS patients. More than 80% of the mutations are located in the conserved DNA and the less-well-conserved protein binding domains. This data confirms that mutations in the IRF6 gene are responsible for VWS and PPS syndrome in the majority of the patients. Since IRF6 has also been associated with NS-CLP, we screened 39 patients with familial history of NSC. However we have found no *IRF6* mutations in such patients. In contrast, some were identified in the novel CPO gene that we recently identified (see abstract by Ghassibe et al.).

535/T

Various Activating TIE2 Tyrosine Kinase Domain Mutations, Including the Recurrent R849W Substitution, Cause Cutaneomucosal Venous Malformation (VMCM) in a Paradominant Fashion. *N. Limaye[1], V. Wouters[1], M. Uebelhoer[1], A. Irrthum[1], L.M. Boon[1,2], J.B. Mulliken[3], J. Murphy[4], P. Rieu[5], L. Kangesu[6], A. Pennington[7], Y. Lacassie[8], J. Berg[9], S.A. Ivarsson[10], O. Enjolras[11], A. Dompmartin[12], E. Baselga[13], M. Vikkula[1].* 1) de Duve Institute, U.C. Louvain, Belgium; 2) Cliniques Universitaires St-Luc, Belgium; 3) Children's Hospital, Boston, USA; 4) Hospital for Sick Children, Canada; 5) Kinderchirugie, U. Nijmegen, Holland; 6) Essex Hospital, UK; 7) St-Vincent's Hospital, U. Melbourne, Australia; 8) Children's Hospital, LSU Health Sciences Center, USA; 9) Guy's Hospital, UK; 10) Universitetssjukhuset, Sweden; 11) Hôpital Lariboisière, France; 12) C.H.U-Department of Dermatology, France; 13) Hospital de la Santa Creu I Sant Pau, Spain.

Venous malformations, characterized by localized bluish lesions in the skin and mucosae, are predominantly sporadic, but 1-2% occur as an autosomal dominantly inherited trait, cutaneomucosal venous malformation (VMCM). Two causative kinase-domain mutations (R849W and Y897S) have thus far been identified, in the Ang receptor TIE2. We studied the TIE2 gene in twelve VMCM families: six bear the R849W change, five have novel tyrosine kinase domain mutations, and one has a carboxy-terminal end mutation. As with the known mutations, in vitro overexpression of these novel mutants results in ligand-independent TIE2 hyperphosphorylation. Interestingly, we also discovered a somatic deletion in VM tissue from a patient carrying the inherited R849W allele, which occurs in trans and partially deletes the TIE2 ligand binding domain. This is the first report of a somatic double-hit mutation in VMCM. Moreover, we show that the deletion-mutant is not hyper-phosphorylated, nor does it increase phosphorylation of the R849W allele. It may instead represent a local loss of wild-type TIE2, which would otherwise rescue the deleterious effects of the mutant, inherited allele. The focal development of VMCM is likely due to such combinations of predisposing hyper-phosphorylating germline mutations, with somatic second-hits, hallmarks of paradominant inheritance. (miikka.vikkula@uclouvain.be).

536/T

Origin and Mechanisms of Formation of Fetus-in-fetu: Two Cases with Genotype and Methylation Analyses. *S. Miura[1], K. Miura[1], K. Yoshiura[2], F. Hirahara[3], M. Yamanaka[4], N. Niikawa[2], H. Masuzaki[1].* 1) Dept OB/GYN, Nagasaki Univ Biomed Sci, Nagasaki, Japan; 2) Nagasaki Univ. Graduate School of Biomedical Science, Human Genetics, Nagasaki, Japan; 3) Yokohama City Univ Sch of Med, OB/GYN, Kanagawa, Japan; 4) Kanagawa Childrens Hospital, OB/GYN, Kanagawa, Japan.

Fetus-in-fetu (FIF) is a condition in which a host infant has parasitic fetiform mass within its body cavity. We describe here results of molecular genetic analysis in two cases (FIF-1 and FIF-2) of fetus-in-fetu. In FIF-1, a male host had in his retroperitoneal cavity two fetiform masses with vertebral columns, and in FIF-2, a fetiform mass with the vertebral column was present in a cranial cavity of a male host. Genotyping of each case using microsatellite markers revealed that the host infant and its fetus(es) inherited one copy each of parental alleles and shares identical genotypes. These findings were confirmed by SNP analysis using Affymetrix GeneChip Human Mapping 50K Array, and support a monozygotic twin theory for FIF. Analysis of the methylation status was done in both cases at the differentially methylated region (DMR) within the human IGF2-H19 locus after bisulfite treatment, methylation-specific PCR and cloning of PCR products. Normally, only the paternal allele is methylated and the maternal allele unmethylated in DMR. However, in FIF-1, seven (46.7%) of 15 clones from a fetiform mass and six (66.7%) of nine clones from the other mass showed unmethylated paternal allele, while the methylation status of a host infant and its fetiform mass in FIF-2 was same in all clones examined and showed the normal patterns. These data suggest that in FIF-1, two isolated blastocysts both originated from one zygote may have been implanted into the other host blastocyst during an establishing process of methylation, and such abnormal implantation may have occurred in FIF-2 after the establishment of methylation. This is the first case of FIF showing different methylation patterns between a host infant and fetiform mass.

537/T

Williams-Beuren syndrome with cardiomyopathy and cerebellar hypoplasia; proposing a severe infantile form. *N. Okamoto[1], T. Yamagata[1], S. Nakamura[1], K. Ichihashi[1], Y. yada[1], N. Takahashi[1], H. Shiraishi[1], M.Y. Momoi[1], N. Matsumoto[2], T. Mizuguchi[2].* 1) Pediatrics, Jichi Medical University, Shimotsuke, Tochigi, Japan; 2) Dept Hum Genet, Yokohama City Univ Grad Med, Yokohama, Japan.

Williams-Beuren syndrome (WBS) is caused by hemizygous deletion of chromosome 7q11.23. The phenotype of WBS consists of characteristic "elfin" face, cardiovascular anomaly, growth deficiency and mental retardation. Supravalvular aortic stenosis is specific to WBS and other cardiac anomalies are also reported. We report a patient with WS who had atypical and severe manifestations. Case report: A Japanese boy was born at 35 weeks of gestation by caesarean section. His facial appearance showed blepharophimosis, broad and depressed nasal bridge, anteverted nares, full cheeks, long philtrum, prominent lower lips and micrognathia. He also presented with typical features of WS such as redundant soft skin, hypoplastic right foot nails, contracture of hip and knee joints, right inguinal hernia and hoarse voice. As cardiac anomalies, he had aortic hypoplasia, localized narrowing of the descending aorta, small VSD, ASD and PDA. Additionally he showed hypertrophic cardiomyopathy that induced left ventricular outflow obstruction, and severe mitral insufficiency. As CNS abnormalities, he showed congenital hydrocephalus, cerebellar and brain stem hypoplasia and sensory deafness. He showed intractable tonic convulsions since three months of age. Hypothyroidism was also noted. He died at 1 year and 5 months of age for progressive cardiomyopathy. His serum transferrin isoelectric focusing pattern was normal and Congenital Disorder of Glycosylation syndrome was excluded. Microarray CGH containing 4200 BACs revealed a unique abnormality, approximate 1.0-Mb deletion in 7q11.23, ranging from RP11-614D7 (72,182,285-72,371,583) to RP11-137E8 (73,389,371-73,574,238). Discussion: The deletion detected in this patient was typical as WBS. However, some of his major marformations, such as central nervous system dysplasia, deafness, hypertrophic cardiomyopathy and hypothyroidism, were atypical as WBS. WBS should include such severe infantile form.

538/T

Molecular characterization of tuberous sclerosis patients in Taiwan. *D. Chu[1], M. Huang[1], J. Lin[2], C. Wang[2], C. Hou[2].* 1) Graduate Inst Medical Biotech, Chang Gung Univ, Sch Med Tech, Tao-Yuan, Taiwan; 2) Department of Pediatrics, Chang Gung Memorial Hospital, Taiwan.

Background: Tuberous sclerosis (TS, MIM#191100) is an autosomal dominant disorder characterized by hamartomatous lesion in multiple organs, especially in the skin and brain. Other common clinical features include epilepsy, learning difficulties, and behavioral problems. TS patients display genetic heterogeneity, with the existence of two different causative genes on chromosomes 9q34.3 (TSC1) and 16p13.3 (TSC2), respectively. Mutations in either of these two genes lead to loss of tumor suppressor function. Materials and Methods: one hundred and thirteen peripheral blood samples were obtained from TS cases (37 families) or their family members. Genomic DNA was extracted. Denaturing high performance liquid chromatography (dHPLC) was conducted to screen possible genetic lesions in TSC1 and TSC2 genes. Direct DNA sequencing was then performed to confirm dHPLC findings. Results: data showed that genetic lesions were found in 22 patients, including 2 missense, 1 nonsense, 1 insertion, and 1 deletion mutations in the TSC1 gene, while 4 missense, 1 nonsence, 7 deletions and 2 insertions in TSC2 gene. In addition, we performed real-time quantitative PCR to determine the quantities of hamartin and tuberin mRNA in patients with mutations. Data showed that there was no significant difference between the hamartin or tuberin mRNA levels. Clinically, 95% of the study cases presented epilepsy, 62% of them showed kidney symptom, 48% of them had heart striated muscle tumor. Benign angiomyolipomas, the most common TS lesion, were found in 62% of these TS cases. Cardiac rhobdomyoma was observed in 48% of TS cases. All cases with TSC2 gene mutations were mentally retarded. No evidences of correlation between TSC1 and TSC2 mutations and other spectacular clinical phenotypes in these patients studied were observed. Conclusions: The genetic lesions leading to TS and clinical features are highly heterogeneous. It is practical to screen TSC1and TSC2 genes with dHPLC followed by DNA sequencing to aid accurate diagnosis of TS.

539/T

Case Report: a Novel Mutation in VDR Gene in an Iranian family with two affected children with Vitamin D Resistant Rickets and Alopecia totalisA. *N. Momenin[1], Y. Shafeghati[1], S.T. Esfahani[2], W. Wuyts[3].* 1) Genetics Research Center, University of Welfare Sciences & Rehabilitation, Tehran, Iran; 2) Children`s Hospital Medical Center,Tehran Medical University,Tehran,Iran; 3) Medical Genetics Center,University of Antwerp,Antwerp,BelgiumA.

Background- Hereditary vitamin D resistant rickets type II (HVDRR II) is a rare autosomal recessive disorder, most often caused by mutations in the VitD receptor gene. It is usually presented with rachitic changes not responsive to VitD treatment. Circulating levels of 1,25(OH)2 VitD3 is elevated, thus differentiating it from Vit D dependent rickets type I. Alopecia of the scalp or whole of the body is seen in some families with VitD dependent rickets type II. This is usually associated with a more sever phenotype. Materials and Methods- In this report, we present our findings on a family exhibited the typical clinical features of alopecia totalis, renal tubular acidosis, mild generalized aminoaciduria, refractory rickets in two siblings. The proband is now an 18-month-old boy. He is the 3rd offspring of a healthy couple. At the end of the first month of his life alopecia occurred and progressed to total loss of his scalp hair, along with refractory rickets. The family have had 2 older children, the oldest was a boy and had had similar disease and died at the age of 2 years and 8 months. Results- Alkaline phosphatase was high, PTH was high. Other routine biochemical tests were WNL, but 1+ glycine detected in his urine. Skin biopsy performed and the result was alopecia areata. Mutation analysis for VDR gene by direct sequencing analysis of all coding exons showed a Homozygous c.122G>A(p.Cys41Tyr) variant in exon 2 as a novel point mutation that several arguments point to a pathogenic effect. Conclusion- The older child of the family was a boy which had similar disease and died because of its complications at the age of 32 month. The 2nd child is a healthy 5-year-old girl. Parents are relatives. We should be aware of this very rare disease, whenever we see a patient who is suffering from refractory rickets with alopecia.

540/T

Deficiency of a member of the immunoglobulin superfamily causes a form of C (Opitz trigonocephaly) syndrome. *T. Kaname[1, 11], K. Yanagi[1], Y. Chinen[2], Y. Makita[4], N. Okamoto[5], H. Maehara[3], I. Owan[3], F. Kanaya[3], Y. Oike[6], T. Yamamoto[7], K. Kurosawa[7], Y. Fukushima[8, 11], J.M. Opitz[9], K. Yoshiura[10, 11], N. Niikawa[10, 11], K. Naritomi[1, 11].* 1) Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan; 2) Dept Pediatrics, Univ Ryukyus, Nishihara, Japan; 3) Dept Orthopedics, Univ Ryukyus, Nishihara, Japan; 4) Dept Pediatrics, Asahikawa Medical College, Asahikawa, Japan; 5) Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; 6) Dept Mol Genet, Kumamoto Univ, Kumamoto, Japan; 7) Kanagawa Children's Medical Center, Yokohama, Japan; 8) Dept Medical Genetics, Shinshu Univ, Matsumoto, Japan; 9) Dept Pediatrics, Pathology, Obstetrics and Gynecology, and Human Genetics, Univ Utah Schl Med, Salt Lake City, UT; 10) Dept Hum Genet, Nagasaki Univ Graduate School of Biomedical Sciences, Nagasaki, Japan; 11) SORST, Japan Science and Technology, Kawaguchi, Japan.

The C syndrome is characterized by trigonocephaly associated with unusual facies, psychomotor retardation, redundant skin, joint and limb abnormalities, and visceral anomalies. In an individual with the C syndrome harboring a balanced chromosomal translocation, t(3;18)(q13.13;q12.1), we identified a gene (*OTCS*), which encodes a member of the immunoglobulin superfamily, was disrupted at the 3q13.3 breakpoint. In mutation analysis of nine karyotypically normal patients with the C or C-like syndromes, we identified a missense mutation in exon 6 of the *OTCS* gene in one patient. The missense mutation was not found among 420 normal Japanese individuals. Cells with the mutated OTCS protein lost adhesion and growth activities in vitro. These findings may indicate that OTCS mutations cause a form of the C syndrome by interfering with cell adhesion and growth.

541/T

An illustrative case of a mosaic deletion of FMR1 in a mildly affected male. *M. Ikeda[1], B. Coffee[1], D. Budimirovic[2], L. Hjelm[1], W. Kaufmann[2], S.T. Warren[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Kennedy Krieger Institute, Johns Hopkins University, Baltimore, MD.

Fragile X syndrome (FXS) is a common form of inherited mental retardation and is most often due to the loss of the FMR1 gene expression by expansion of a 5' UTR CGG-repeat. However, other sequence specific mutations such as missense changes and deletions involving FMR1 can also abrogate gene function. We report here the characterization of a 1 Mb mosaic deletion encompassing both FMR1 and FMR1NB in an 11 year-old male showing cognitive delay and social avoidance. Although a normal length 23 CGG repeat allele was present, PCR amplification of the repeat tract and a Southern blot probing for FMR1 in patient DNA returned signal intensities that were decreased relative to controls. Use of a comparative genomic hybridization microarray for the X-chromosome, probing at an average intermarker distance of 330 bp, identified a possible 1 Mb deletion spanning FMR1 and the next downstream gene, FMR1NB. A PCR based assay, using primers immediately outside the boundaries of the deletion, amplified a breakpoint junction fragment and confirmed a deletion of 1,013,395 bp. The fragment sequence revealed L1 elements immediately flanking the breakpoint with a common 4 bp sequence at each edge, indicating non-homologous end joining utilizing this microhomology. This case illustrates issues regarding FMR1 gene testing. The patient had a relatively mild FXS phenotype (IQ of 73 but without facial features of FXS) due to his 10% mosaicism of the intact X-chromosome in lymphocytes. Moreover, this large deletion resulting in weaker, but normally sized fragments on PCR and Southern blot testing could be interpreted as normal. Thus, for patients with mild FXS phenotypes and normal clinical testing results, FMR1 copy number variation should also be considered.

542/T

Mutation spectrum of the Iduronate-2-Sulfatase gene and its implications for the molecular diagnosis of Mucopolysaccharidosis Type II in Korean patients. *Y.E Kim[1], C.S Ki[2], E.K Kwon[3], M.J Kwak[3], S.J Kim[3], K.H Paik[3], K.M Pyun[3], M.J Lee[1], S.H Chu[1], A.H Kim[1], D.K Jin[3].* 1) Clinical Research Center, Samsung Biomedical Research Center; 2) Departments of Laboratory Medicine and Genetics Samsung Medical Center, Sungkyunkwan University School of Medicine; 3) Departments of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine.

Mucopolysaccharidosis type II (MPS II) or Hunter syndrome is a rare X-linked lysosomal storage disorder caused by the deficiency of iduronate-2-sulfatase (IDS) that is required for the catabolism of dermatan and heparan sulfates. MPS II is caused by heterogeneous mutations that occur in the IDS gene ranging from point mutations to gross deletions and recombinations. We have previously identified IDS gene mutations in 23 out of 25 Korean patients with MPS II. In the present study. We attempted to elucidate the presence of mutations in 21 additional patients with MPS II as well as in 2 patients in whom mutations had not been detected in a previous study. Bidirectional sequencing analysis identified 17 mutations in the 23 patients: 7 missense, 1 nonsense, 4 deletion, and 5 splicing mutations. Among these, 7 were novel mutations including 3 missenses (Ser61Pro, Pro197Arg, and Pro261Ala), 3 deletions (c.344delA, c.420delG, and c.1112delC), and 1 splicing mutation (c.1180-1G/C). This data along with the data from a previous study has lead to the complete identification of the causative mutations in 46 Korean patients with MPS II.

543/F

Vitiligo and hearing loss: Report of a new case. *L.Hdez Gomez[1], G.Juarez Garcia[2], D.Gomez Torres[3], M.Izqdo. Ortiz[4], E.Hdez Gomez[5].* 1) Dept. Genetics and Audiology, Instituto Nacional Rehabilitacion, Mexico, DF; 2) Dept. Genetics and Neuropsicologia, Instituto Nacional Rehabilitacion, Mexico, DF; 3) Dept. Investigación, Instituto Nacional Rehabilitacion, Mexico, DF; 4) Dept. Neurotology, Instituto Nacional Rehabilitacion, Mexico, DF; 5) Facultad de Estudios Superiores Iztacala. Universidad Nacional Autonoma de Mexico Biologia.

Vitiligo is a common, often inherited, acquired disorder resulting from destruction of functional melanocytes (MCs). It affects all ethnic groups and has a worldwide occurrence of 0.3-1.0%. Functional MCs in patients with vitiligo disappear from the involved skin by a mechanism(s) that has yet to be identified. Traditionally, there have been three hypotheses to explain vitiligo: the immune, the neural and the autocytotoxic hypotheses A number sign is used with this entry because of evidence that susceptibility to vitiligo, like a number of other autoimmune disorders, is associated with more than 1 gene, loci for susceptibility to autoimmune disease, particularly vitiligo, have been mapped to chromosomes 17p13, 1p31,7,8,and 4. There is convincing evidence that vitiligo is a systemic disorder influencing the whole pigmentary system, including melanocytes in the inner ear. Cochlear melanocytes and also melanin-containing cellular elements of the auditory system may be affected in vitiligo and interfere with the conduction of action potentials. We report a Mexican woman affected with hearing loss and vitiligo. Female patient 62-year-old, hearing loss and vitiligo onset at 59-year-old. Audiometric test showed sensorineural bilateral middle hearing loss. Speech audiometric test showed neural deficit. Timpanogram: curves A bilateral. Stapedial reflex: absent bilateral. ABR performed reported without response at 100 dB. Trascient evoked otoacoustic emissions absent bilateral. Vestibular response to caloric is markedly reduced. Posturographic testing sensory analisys and motor control test abnormal.

544/F

Facioauriculovertebral spectrum. Mexican family with probable Autosomal recessive inheritance. *C.F. Martinez-Cruz[1,2], G. Garcia-Sanchez[3], M. Diaz-Garcia[3], S.G. Juarez-Garcia[4].* 1) Servicio de Comunicacion Humana,Departamento de Seguimiento Pediatrico, Instituto Nacional de Perinatologia, Mexico, D.F; 2) Servicio de Pediatría. Instituto Mexicano del Seguro Social. HGZ 53. México, D.F; 3) Servicio de Genética.Instituto Nacional de Rehabilitación, México, D.F; 4) Servicio de Audiologia.Instituto Nacional de Rehabilitación, México, D.F.

Facioauriculovertebral spectrum (fav), is a complex of malformations, mainly of craniofacial structures develop from the first and second branchial arches, generally unilateral, with eye anomalies, neurological defects, mental retardation, various forms of spinal, heart and renal anomalies (Gorlin)most patients have conductive or sensorineural hearing loss. Expression varies within families and it is usually sporadic (Rollnick) We presented a Mexican family with facioauriculo vertebral spectrum with variable expressivity and probable autosomal recessive inheritance. Parents unaffected, nonconsanguineus. All three children are affected. Propositus, a male 19 years old, right microtia-atresia, severe hearing impairment, hemifacial microsomy. Sister, 14 years old, right microtia-atresia type II, severe hearing impairment and cleft palate. Sister, 12 years old, left preauricular tag with normal hearing. Three relatives in second degree with folded helix.

545/F

Otosclerosis. Family History and Audiological Findings in 103 Mexican Patients. *D.M. Mendoza-Ugalde[1], G. García-Sánchez[1], C.F. Martínez-Cruz[2,3], R. Baez-Reyes[4].* 1) Servicio de Genetica, Direccion de Investigacion.Instituto Nacional de Rehabilitacion, México, D.F; 2) Servicio de Comunicacion Humana.Departamento de Seguimiento Pediatrico.Instituto Nacional de Perinatologia. México D.F; 3) Servicio de Pediatría. Instituto Mexicano del Seguro Social. HGZ 53. México, D.F; 4) Servicio de Genetica,Departamento de Investigacion.Instituto Nacional de Perinatologia. México D.F. email. gsanchezg03@yahoo.com.mx.

INTRODUCTION: Otosclerosis is caused by abnormal bone homeostasis of the otic capsule, resulting in hearing impairment. The etiology of the disease remains unclear and environmental as well as genetic factors have been implicated. The most common age of presentation is among the 2ª and 5ª decade of the life and is rare in the infancy. Is more common in Caucasian and more frequent in women (2:1). To date, seven loci (OTSC1-7) have been reported, but none of the responsible genes have been cloned.The diagnosis is based on the clinic, audiometric and radiological studies.OBJECTIVE: Identify the hereditary patter in 103 Mexican patients with otosclerosis and atudy the audiological findings. MATERIAL AND METHODS: Included 103 Mexican patients with clinical, audiological, radiological and surgical diagnosis of otosclerosis. We carried out to all patients pedigree, wich included at least three generations. RESULTS: The patients had a minimum age of 12 years and maximum of 69 years. 25% men and 75% women. We studied a total of 206 ears: 199 with hearing loss, (100 rights and 99 lefts) and 17 ears with normal hearing. Time of evolution from 1 to 44 years, age of onset between 6 and 59 years old. The type of hearing loss more frequent was mixed with ascending configuration. Vestibular sintomatology was referred in 38.9%. Stapedectomy was performed in 73 patient (67.6%). Hereditary patter was observed in 49 patients (45.4%): Autosomal dominant (28.76%) and autosomal recessive (16.6%). CONCLUSIONS: In this sample the otosclerosis is a genetically heterogeneous disorder, being the autosomal dominant inheritance the most frequent cause.

546/F

Increased Elastolysis Contributes to the Phenotype of Cutis Marmorata Telangiectasia Congenita. *S. Jain[1,5,6], A. Hinek[2,6], M. Baghetti[3,6], H. Tresurer[2,6], G. Taylor[4,6], M. Silver[4,6], D. Nykanen[3,6], D. Chitayat[1,6].* 1) Clinical Genetics & Metabolics; 2) Cardiovascular Research; 3) Cardiology; 4) Pathology; 5) Hospital for Sick Children; 6) University of Toronto, Toronto, Canada.

Cutis Marmorata Telangiectasia Congenita (CMTC) is a cutaneous vascular anomaly presenting at birth with levido reticularis, phlebectasia, and telangectasia. Multiple anomalies and involvement of other systems is noted in 30-80% of cases. Macrocephaly-CMTC has been described as a new syndrome and findings include macrocephaly, hypotonia, developmental delay, hemihypertrophy, connective tissue defect and toe syndactyly. We report a child with CMTC, born with extensive generalized phlebectasia, nevus vascularis reticularis, skin ulcers and developed intracerebral hemorrhage, retinal detachments, hypothyroidism and pulmonary hypertension. Initially serum copper levels of 29.8 umol/L(normal=10.5-23umol/L) and serum ceroloplasmin of 522 mg/L(normal=269-473 mg/L) were noted. The patient died at the age of 20 months. Autopsy confirmed multiorgan telangiectasia, ectatic capillary and venous proliferation and severe medial thickening of the small pulmonary arteries. Fibroblast studies revealed poor deposition of well assembled elastic fibers despite initially synthesizing normal amounts of tropoelastin, microfibrillar scaffolds, collagen type I and fibronectin. Additional assays indicated a heightened elastolytic activity of serine elastase(s) in patient's fibroblasts. Moreover, while incubation of CMTC fibroblasts with copper sulfate increased their elastolytic activity, addition of calcium chloride or α1-antitrypsin, but not phenantrolin and C-64 (inhibitors of metalo- and serine- proteases, respectively) reduced their elastolytic activity to normal levels. We also found that treatment with copper sulfate eliminated beneficial effect of α1-antitrypsin in cultures of CMTC and normal fibroblasts. We postulate that an increased concentration of copper can inactivate α1-antitrypsin, thereby creating deficiency of this natural inhibitor of serine elastases. This, in turn may facilitate elastolysis, leading to the connective tissue and vascular disorders observed in our patient with CMTC.

547/F

Spine abnormalities is correlated with back pain in young persons with Ehlers-Danlos Syndromes. *S. Bangura[1], B.F. Griswold[1], L. Sloper[1], R. Raza[3], C.A. Francomano[2], N.B. McDonnell[1].* 1) LCI, NIA/NIH, Baltimore, MD; 2) GBMC, Baltimore, MD; 3) Harbor Hospital, Baltimore, MD.

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue characterized by joint, skin and vascular abnormalities. Joint laxity, dislocations and chronic pain are commonly recognized features of EDS in young persons, however, the correlation of pain with spinal pathology has not been studied systematically. We investigated abnormalities of the spine in 26 consecutive subjects younger than 25 with a diagnosis of hypermobile or classical EDS enrolled in a natural history study of hereditary disorders of connective tissue at the National Institutes of Health. The age range was 12-25, and there were 14 females and 12 males. Magnetic Resonance Imaging (MRI) of the lumbar spine was obtained in all subjects, and additional limited thoracic and cervical studies were obtained on some of the subjects. In 15 of these young patients, abnormal signal was detected in lumbar discs, with eccentric placement or diminished size of nucleus pulposus. Degenerative disc disease, bulging or herniated discs, and facet joint athrosis was present in at least one level in 20/25 patients. Two patients have severe lumbar spinal stenosis. Dural ectasia was associated with the presence of Marfanoid body habitus and scoliosis, however none of the patients have cardiac or ocular findings that suggested the diagnosis of Marfan syndrome. Schmorl's nodes were seen in all male subjects. All subjects with spinal pathology had complaints of back/neck pain. The results suggest that MRI investigations are likely to identify spinal pathology in young EDS patients who have significant back or neck pain.

548/F

Low Serum Testosterone in Men with Marfan Syndrome. *M. Burchett[1], B.F. Griswold[2], L. Sloper[2], C.A. Francomano[3], S. Basaria[4], N.B. McDonnell[2].* 1) Centre Col, Danville, KY; 2) LCI, NIA/NIH, Baltimore, MD; 3) GBMC, Baltimore, MD; 4) CRB, NIA/NIH, Baltimore, MD.

Marfan syndrome is a heritable disorder of connective tissue associated with cardiovascular and skeletal, and ocular abnormalities. Abnormalities of sex hormones in Marfan syndrome have not been noted previously. In six consecutive adult male subjects with Marfan syndrome, ages 25-52, enrolled at the National Institutes of Health, serum total testosterone values below the age norms were detected in research based testing. The lowest value was 155 ng/dL in a 31 year old man. Steroid hormone binding globulin (SHGB) was not elevated in any of the subjects. The subjects had normal sexual development and infertility was not noted. All subjects had decreased muscularity, a feature noted commonly in Marfan syndrome, as well as osteroporosis or osteopenia. One subject had suffered two vertebral compression fractures. Investigation of the pituitary axis in the Marfan syndrome cohort is ongoing in the study. Replacement therapy with exogenous testosterone was not initiated due to concern for cardiovascular consequences such as increase in blood pressure and progression of aortic root enlargement. Low serum sex hormones in Marfan syndrome may be a contributor to reduced bone density and increased fracture risk.

549/F

Bronchiectasis and Mycobacterium Avium Complex(MAC) infection is associated with Hereditary Disorders of Connective Tissue. *B. Griswold[1], L. Sloper[1], C.A. Francomano[2], N.B. McDonnell[1].* 1) LCI, NIA, Baltimore, MD; 2) GBMC, Baltimore, MD.

Bronchiectasis is an abnormal stretching and enlarging of the respiratory passages and may predispose the patients to respiratory infections. A previous study noted a relationship between bronchiectasis and the presence of scoliosis, however did not discuss the role of heritable disorders of connective tissue (HDCT). In a cohort of 95 patients with Ehlers-Danlos syndrome (EDS) enrolled at a natural history study of HDCT at the National Institutes of Health, we identified four patients with bronchiectasis and infection with Mycobacterium avium complex (MAC) in the setting of intact immune function. The organism was identified by specimens obtained during bronchoscopy. MAC consists of two speciesM. avium and M. intracellulare, and is found in water supplies, house dust, soil, farm animals, birds, and cigarette components. It rarely causes pathology in the immunocompetent host. Two of the subjects were women, ages 39 and 50, with hypermobility type of EDS and Marfanoid body habitus, mild scoliosis and dolicocephaly. The third patient was a 50 year old woman with classical EDS, with atrophic scars, doughy skin and joint hypermobility and no evidence of scoliosis. The fourth patient was a teenage boy with hypermobile EDS, stretchy skin without atrophic scars. He had congenital stenosis of the lumbar spine, however, did not have scoliosis. Two patients were diagnosed with bronchiectasis and MAC prior to enrollment in the study, and the others developed the infection during the follow-up period.

550/F

Familial Hypochondroplasia and Epilepsy due to a FGFR3 mutation (C1620A) in a father and two children. *P.A. Levy[1,2], K. Cherian[3], Q. Pan[2].* 1) Department of Pediatrics, Children's Hospital at Montefiore, Bronx, NY; 2) Department of Pathology, Montefiore Medical Center, Bronx, NY; 3) Department of Neurology, Montefiore Medical Center, Bronx, NY.

Hypochondroplasia is an autosomal dominant skeletal dysplasia with short stature, disproportionate shortening of the limbs (micromelia), small hands and feet and macrocephaly. It is similar to but milder than achondroplasia. Hypochondroplasia, achondroplasia, thanatophoric dwarfism, and Muenke syndrome are all caused by mutations in FGFR3. Most patients with hypochondroplasia are heterozygous for one of two mutations in FGFR3, (either C1620A or C1620G). This falls within the tyrosine kinase domain and together, these mutations are present in 70 percent of the hypochondroplasia patients studied. FGFR3 is expressed in brain as well as bone and plays a role in brain development. Medial temporal lobe dysgenesis has been reported in three patients with hypochondroplasia and a C1620A mutation. We present a family with hypochondroplasia, seizures, as well as temporal lobe abnormalities. Our patients had seizures associated with apneic episodes in the newborn nursery. The older sibling, a male had seizures soon after birth and had MRI findings suggestive of a temporal lobe abnormality. EEG findings of both children found a focus for the seizures in the temporal lobe. Our patients had unilateral foci, the boy in the left temporal lobe and his younger sister in the right temporal lobe. DNA sequencing was done on the mother, father and their two affected children, looking for the C1620A mutation in exon 11 of the FGFR3 gene on chromosome 4 p16.3. The father and his two children were positive for this mutation. The mother and a control were normal. There is a lack of agreement on a definitive set of diagnostic criteria for hypochondroplasia, and it is difficult to diagnose radiologically. Testing for the two known mutations is warranted to help confirm the diagnosis. The association of temporal lobe dysgenesis and seizures with the C1620A mutation is probably underdiagnosed. DNA testing would identify patients at risk for seizures and help to better define the clinical picture of hypochondroplasia.

551/F

Abnormalities of the Spine and Reduced Bone Density in Vascular Ehlers-Danlos Syndrome. *N. Obeng-Adjei[1], B.F. Griswold[1], L. Sloper[1], R. Raza[3], C.A. Francomano[2], W. Chen[1], J. Yang[1], N.B. McDonnell[1].* 1) LCI, NIA/NIH, Baltimore, MD; 2) GBMC, Baltimore, MD; 3) Harbor Hospital, Baltimore, MD.

Type III collagen is present in fiber bundles in bone cortex, with highest concentrations at the Haversian canal surface and at the bone-periosteal interface. Vascular Ehlers-Danlos syndrome (VEDS) is caused by mutations in COL3A1 encoding procollagen III, and is associated with reduced life expectancy due to arterial or hollow organ rupture. The prevalence of spine abnormalities and reduced bone density in VEDS has not been reported previously in the literature. In fourteen consecutive patients (ages 14-55) with mutations in COL3A1 enrolled in the hereditary disorders of connective tissue study at the National Institutes of Health, we noted a high prevalence of dural ectasia (8/14) in research magnetic resonance imaging (MRI) of the lumbar spine. Lumbar or cervical spinal stenosis due to disc herniation was seen in 7/14 participants. Posterior fossa volume was reduced in 5/14 subjects, however herniation of the cerebellar tonsils into the foramen magnum was not seen. All subjects over 18 had reduced bone density; 6/12 had osteoporosis and 6/12 had osteopenia. The results suggest that assessment of bone density is indicated for VEDS patients starting in young adulthood and treatment needs to be initiated to reduce fracture risk. Lower back or neck pain in VEDS may be a result of spinal stenosis or dural ectasia, and needs to be evaluated with MRI imaging.

552/F

Spondyloepiphyseal dysplasia, Omani type. A second family and expansion of the phenotype. *S. Robertson[1], M. van Roij[2].* 1) Department of Paediatrics, Otago Medical School, Dunedin, New Zealand; 2) Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands.

Two siblings, the offspring of a second-cousin union, are presented manifesting a spondyloepiphyseal dysplasia that is progressive from early childhood and results in severe kyphoscoliosis and arthropathy in association markedly variable brachydactyly. Owing to the novelty of the phenotype an Affymetrix 50K genome wide scan was performed seeking regions that were homozygous by descent from a common ancestor of the parents of the two affected children. A region of >15Mb was identified at 10q22 incorporating the CHST3 locus, encoding chondroitin 6-O-sulfotransferase, previously been shown to be mutated in the recessive condition, Spondyloepiphyseal dysplasia (Omani type). Both children were shown to be homozygous for the mutation 857T>C predicting the substitution L286P. The residue 286L is tightly conserved through evolution implying a high likelihood that the observed mutation is causative of the observed phenotype. Clinical descriptions of SED Omani type have been restricted to two Omani families with short stature, progressive kyphoscoliosis, severe arthropathy with joint dislocations but only minimal manifestations in the hands. Our observations extend the phenotype associated with mutations in CHST3, report the first family outside Oman, and add to the wide spectrum of skeletal anomalies that can arise from the dysregulation of the sulfation of cartilage.

553/F

Spondylocostal dysostosis with preaxial polydactyly: a new entity? *M.J. Rodovalho-Doriqui[1], L.R. Giuliani[2], C.M. Lourenço[1], C.D. Martinhago[1], J.M.de Pina-Neto[1].* 1) Medical Genetics Division, Clinical Hospital of Ribeirão Preto - São Paulo University, Ribeirão Preto-SP, Brazil; 2) Pediatrics/Medical Genetics Division, Federal University of Mato Grosso do Sul, Campo Grande-MS, Brazil.

Spondylocostal dysostosis (SCDO) is a genetic Mendelian disorder with autosomal recessive inheritance. Findings include segmentation and formation defects throughout cervical, thoracic and lumbar spine such as hemivertebrae, block vertebra and unsegmented bars with fusion of the ribs at the costo-vertebral junction. Zeller et al (1982) described an unusual association between spondylocostal dysostosis and preaxial polydactyly in a 22 old month boy. We report two patients with spondylocostal dysostosis and preaxial polydactyly. Case 1. Male, 17 years old, the first child of a young, healthy and non-consanguineous couple. He had normal psychomotor and pubertal development. Physical examination showed stature bellow the 3rd percentile, short neck, scoliosis and preaxial polydactyly of the left hand. The radiological findings consisted of hemivertebrae, cervical and thoracic fused vertebrae and costal fusion in superior portion of left hemithorax. Case 2. Female, 1 year and 7 months old, the first child of healthy and non-consanguineous parents. She had normal psychomotor development. Her clinical exam showed stature bellow the 3rd percentile, severe scoliosis and preaxial polydactyly of her right hand. Her x-rays showed segmental defects of thoracic and cervical spine and marked costal anomalies. Abdominal ultrasonography, blood karyotype and echocardiography of both patients were normal. Radiographs of the spines of other members of both families were normal. The rare association between spondylocostal dysostosis and polydactyly suggests a particular pattern of malformation whose etiology seems different from the classical SCDO caused by mutations in DLL3 and MESP2 genes.

554/F

Heterozygous *LRP5* mutations in children with fractures. *A. Saarinen[1], M. Mäyränpää[2], A.-E. Lehesjoki[1], O. Mäkitie[1,2].* 1) Folkhälsan Institute of Genetics and Department of Medical Genetics, University of Helsinki, Finland; 2) Metabolic Bone Clinic, Hospital for Children and Adolescents, University of Helsinki, Finland.

Background: Mutations in *LRP5*, coding for the low density lipoprotein receptor-related protein 5, have been shown to cause a variety of disorders including autosomal recessive Osteoporosis-pseudoglioma syndrome (OPPG), autosomal dominant High Bone Mass disorder, and Familial Exudative Vitreoretinopathy (FEVR). While homozygous *LRP5* mutations result in OPPG, characterized by severe osteoporosis and blindness, heterozygous *LRP5* mutations may result in milder osteoporosis. In this study we assessed *LRP5* genotypes and skeletal phenotypes in a cohort of Finnish children with fractures. **Patients and Methods:** The study included all children aged 0-16 yrs who were assessed at the Helsinki University Hospital during a 12 month period because of a new fracture and had experienced i) at least two low-energy long bone fractures before the age 10, ii) three low-energy long bone fractures before the age 16 or iii) one low-energy vertebral fracture. Children with previously diagnosed osteogenesis imperfecta were excluded from the study. Patients were assessed for bone mineral density (BMD) by DXA, for vertebral morphology by spinal radiographs and for relevant blood biochemistry. DNA samples were obtained and the 23 exons and exon-intron boundaries of the *LRP5* gene were analyzed by sequencing. **Results:** Seventy-five children fulfilled the inclusion criteria and DNA samples were obtained from 66 of them. Three different heterozygous missense mutations were identified. In addition to these mutations, a variety of rare polymorphisms was identified. Only some of these were observed in control samples (N= 500) suggesting that they may have functional implications. **Conclusions:** Three heterozygous missense mutations and several rare polymorphisms in *LRP5* were found in 66 children with fractures. These results are in accordance with previous findings suggesting that even heterozygous *LRP5* mutations may result in symptomatic osteoporosis and confirm the importance of intact Wnt-signalling for normal bone development.

555/F

Heterotopic ossification as a clue to the underlying pathogenesis of osteogenesis imperfecta type V. *L.H. Seaver[1,2], J. Brumblay[1], D.K. Kwock[2], K.N. Kon[1], R.M. DiMauro[1].* 1) Kapi'olani Medical Center for Women and Children, Honolulu, HI; 2) Department of Pediatrics, John A. Burns School of Medicine, Honolulu, HI.

Osteogenesis imperfecta (OI) type V is a recently recognized type of brittle bone disease characterized by mild to moderate fracture tendency, hyperplastic callus (HC) formation, ossification of the interosseous membrane (IOM) of the forearm, radial head dislocation, normal sclerae, radiodense metaphyseal band, unique bone histology and autosomal dominant inheritance. The genetic basis is unknown. The purpose of this report is to call attention to this rare type of OI and suggest that the heterotopic ossification is a clue to the underlying pathogenesis.

A previously healthy 6-month-old female presented with an acute rib fracture. Skeletal survey revealed additional healing rib fractures, osteopenia, vertebral compression of T9 and T11, slender diaphyses with flared metaphyses, subtle wormian bones and ossification of the interosseous membrane of the forearm bilaterally. Physical examination revealed length and weight at 10th centile, OFC 50th centile, large anterior fontanelle, slightly grey sclerae, but no radial head dislocation. Family history, physical examination and forearm radiographs of the parents were unremarkable. Collagen 1 synthesis and secretion was normal.

OI type V is unique, in that it is characterized by heterotopic ossification in the setting of osteopenia and bone fragility. Ossification of the IOM is striking and present in all reported cases. Similar changes can be seen in skeletal fluorosis, which is associated with heterotopic ossification of soft tissues and osteopenia in some cases. In vitro and animal studies have shown fluorosis stimulates activation and proliferation of osteoblasts and osteoblast-like tissue. HC can occur spontaneously in OI type V, without antecedant fracture. These observations suggest that the genetic basis and pathogenesis of OI type V may be due to a defect in regulation of bone development and mineralization by osteoblasts.

556/F

Bifurcated femur with absent tibia: case report and expansion of the phenotype of Wolfgang-Gollop syndrome. *W.V. Wurm[1], C.A. Friedrich[2].* 1) Dept Pediatrics, University Mississippi Medical Center, Jackson, MS; 2) Dept Preventive Med, Div Med Genet, UMMC.

A premature neonate (25 wks GA) was evaluated because of bronchopulmonary dysplasia and left leg anomalies. Her mother received no prenatal care. A sibling was healthy. Examination revealed expected findings for gestational age, no coloboma, no cleft lip or palate, a small skin tag over the right clavicle, and no ectrodactyly or upper limb or right leg abnormalities. The left lower extremity showed a medial protuberance of the distal left femur, left talipes equinovarus with the foot rotated almost 180 degrees (pointing posteriorly), and broad great toes. X-rays revealed a bifurcation of the femur with an absent tibia. No spine or rib anomalies were seen. Head and renal ultrasound examinations showed no abnormalities. A high-resolution karyotype revealed no abnormalities including no deletion at 8(q11.23q13.3). A complete genomic hybridization microarray assay revealed no deletions or duplications (ARUP Laboratories, Constitutional Chip V3 and Spectral Genomics 1 MB Chip). Unilateral bifid femur with absent tibia has been reported rarely with unclear inheritance. In some patients an upper extremity is also affected, and ectrodactyly has been reported commonly. Several families with affected sibs and unaffected consanguineous parents have been reported, but in one family multiple generations were affected. One patient had bilateral affected lower limbs. One patient with multiple other congenital anomalies had a deletion of 8q. Congenital heart defects, lissencephaly, cleft palate and palate, and tracheoesophageal fistula have been reported. Despite her extensive localized malformation she did not have any of the associated findings described in previous patients. The etiology of Gollop-Wolfgang Complex (OMIM 228250) remains unknown.

557/F

Handedness and APOE Genotype in School-Aged Children. *C.S. Bloss[1], D.C. Delis[2, 3], M.W. Bondi[2, 3], D.P. Salmon[4].* 1) University of California, San Diego and San Diego State University Joint Doctoral Program in Clinical Psychology, CA; 2) Veterans Affairs San Diego Healthcare System, CA; 3) Department of Psychiatry, University of California, San Diego, School of Medicine, CA; 4) Department of Neurosciences, University of California, San Diego, School of Medicine, CA.

Recent developmental studies have found evidence for an advantageous effect of the ε4 allele of the apolipoprotein E gene (APOE) during human prenatal, perinatal, and infancy periods of life, and possibly a detrimental effect of the ε2 allele. To further explore the role of APOE genotype in early brain development, hand dominance, which can be an indicator of early atypical brain and/or cognitive development, was assessed in a sample of school-aged children. A total of 147 children enrolled at a public school reported their hand dominance for writing and underwent buccal swab testing to determine their APOE genotype. A chi-square test was used to determine whether hand dominance differed as a function of APOE genotype. Notably, a significantly higher percentage of ε2-positive children were left-hand dominant for writing (29.2%) versus ε3/3 homozygote (8.8%) and ε4-positive (6.1%) children ($p < .05$). This finding raises the possibility that the ε2 allele may be associated with factors that give rise to atypical hemispheric dominance and/or that it may serve as a risk factor for certain disorders found to be more prevalent in left-handed individuals (e.g., developmental learning disorders). While the ε2 allele may serve as a risk factor with respect to early brain development, thereby contributing to its relatively low prevalence in the population, it appears to have protective properties against the development of certain disorders later in life, such as Alzheimer's disease.

558/F

Neuroimaging findings in macrocephaly-cutis marmorata telangiectatica congenita. *R.L. Conway[1], B.D. Pressman[1], W.B. Dobyns[2], M.G. Butler[3], E. Zackai[4], S.C. Saitta[4], L. Campbell[4], C.L. Clericuzio[5], J.M. Milunsky[6], H.E. Hoyme[7], J. Shieh[7], J.B. Moeschler[8], B. Crandall[9], J.L. Lauzon[10], D. Viskochil[11], B. Harding[12], J.M. Graham[1].* 1) Cedars Sinai Medical Ctr., Los Angeles, CA; 2) Univ. of Chicago, IL; 3) Children's Mercy Hospital, Kansas City, MO; 4) Children's Hospital of Philadelphia, PA; 5) Univ. of New Mexico School of Medicine, Albuquerque, NM; 6) Boston Univ. School of Medicine, Boston, MA; 7) Stanford Univ. Medical Ctr., Stanford, CA; 8) Dartmouth Medical School, Lebanon, NH; 9) UCLA School of Medicine, Los Angeles, CA; 10) Alberta Children's Hospital, Canada; 11) Univ. of Utah School of Medicine, Salt Lake City, UT; 12) Great Ormond St. Hospital for Children, London, UK.

To investigate neuroimaging abnormalities found in the overgrowth syndrome Macrocephaly-Cutis Marmorata Telangiectatica Congenita (M-CMTC) we analyzed available brain MRI or CT scans from 17 unpublished patients and compared their findings with features identified through a review of published cases. Common findings included white matter irregularities, ventriculomegaly and brain asymmetry. A distinctive feature in more than half was cerebellar tonsillar herniation (CTH), usually associated with rapid brain growth and progressive posterior fossa crowding during infancy; in 4 such cases this was an acquired event. Concurrent with the development of CTH, ventriculomegaly and dilated dural venous sinuses were seen along with prominent Virchow-Robin spaces in many patients. We postulate the constellation of these features suggests a dynamic process of mechanical compromise in the posterior fossa, perhaps initiated by a rapidly growing hindbrain which causes congestion of venous drainage and compromised cerebrospinal fluid reabsorption. We also found numerous examples of focal cortical dysplasia and polymicrogyria, a high frequency of cavum septum pellucidum or vergae, thickened corpus callosum, wide optic nerve sheaths, and one case of venous sinus thrombosis. One case had a perifalcine mass resembling a meningioma at age 5 years. This is the second apparent occurrence of this tumor in M-CMTC, adding to the unique CNS abnormalities in this syndrome.

559/F

22q13 deletion syndrome with severe language delay without social communication disturbance in a 8 years old girl with moderate mental retardation. *K. Dahan[1], X. Pepermans[1], X. Schlogel[2], N. Lannoy[1], C. Sibille[1].* 1) Dept Genetics, UCL Saint-Luc Hosp, Brussels, Belgium; 2) Pediatric Dept, UCL Saint-Luc Hosp, Brussels, Belgium.

The terminal 22q13.3 deletion syndrome is characterized by neonatal hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech, autistic behavior, and minor dysmorphic features. Even if deletions were extremely variable in size, extending from 160 kb to 9 Mb, the minimal critical region should include the three subtelomeric genes (*RABL2B*, *ACR*, *SHANK3*). A less severe phenotype as well as discordance for the minimal telomeric region prompt us to report the present observation. The proband is an 8 years old girl with a severely impaired speech and moderate mental retardation (overall IQ 56). Hypotonia is evident without tendency to overgrowth and dysmorphic facial features. Behavioral disturbances are poor concentration and hyperactivity in a very engaging child. We used FISH, MLPA and High-resolution SNP analysis and found a *de novo* deletion of 22q13.3. The proximal deletion breakpoint was mapped between *ECGF1* and *CPT1B* and removed 172 kb of the terminal 22q13, including *ARSA*, *SHANK3* and *ACR*. The terminal deletion breakpoint was located upstream the 3'UTR of *RABL2B*. The parental origin was paternal. We show for the first time that a *de novo* deletion of the paternal chromosome 22q13 respecting the integrity of the *RABL2B* gene may cause severe language delay in a girl with moderate mental retardation without disturbance in social communication.

560/F

Molecular analysis of spinal muscular atrophy by gene dosage analysis of survival motor neuron genes in Korean population. *J.H. Kim[1], J.H. Lee[2,4], M.H. Lee[2,4], B.J. Kim[3,4], J.W. Kim[1,4], C.S. Ki[1,4].* 1) Departments of Laboratory Medicine, Samsung Medical Center; 2) Pediatrics, Samsung Medical Center; 3) Neurology, Samsung Medical Center; 4) Sungkyunkwan University School of Medicine, Seoul, Korea.

Purpose: Spinal muscular atrophy (SMA) is an neurodegenerative disorder mainly caused by homozygous deletion of survival motor neuron (SMN) genes. The causative gene is SMN1 gene, but the disease types and severity are influenced by SMN2 gene dosage and adjacent gene deletion. So we investigated SMN1, SMN2, and adjacent gene copy numbers to determine the gene dosage distributions in normal individuals and the patient groups. Methods: We investigated a total of 55 individuals (15 SMA patients, 6 carriers, and 34 controls) with a commercially available multiple ligation-dependent probe amplification (MLPA) kit. The 15 patients were tested for the homozygous deletion of SMN1 gene by PCR-RFLP and were composed of 9 patients with homozygous deletion of SMN1 gene and 6 patients with clinical diagnosis of SMA but without homozygous SMN1 gene deletion. Six carriers were parents of the SMA patients with homozygous SMN1 gene deletion. Direct sequencing of SMN1 gene was performed for a patient with a heterozygous SMN1 gene deletion. Results: The SMN1 gene dosage was 2 copies in all controls but the SMN2 gene dosage was variable: 1 copy in 10 individuals (30%) and 0 copy in one individual. In SMA patients showing homozygous SMN1 gene deletion, the SMN2 gene dosage was usually conserved. One patient, previously not detected with PCR-RFLP, was diagnosed as a compound heterozygote with a heterozygous SMN1 gene deletion and a frame shift mutation in the SMN1 gene. Three SMN2 gene copy numbers were seen in two patients of SMA type I and IV, respectively. Conclusion: The gene dosage analysis in SMA seems important for the detection of compound heterozygotes and carrier status. However, it is not clear the genotype-phenotype correlation between the copy numbers of SMN2 gene and disease severity. Further analysis for the patients with clinical SMA with neither heterozygous nor homozygous deletion of SMN1 gene are needed.

561/F

Clinical characterization of three patients with NF1 and suspected glomus tumors. *J.L. Sloan[1], C. Park[1], A. Moshyedi[2], L. Yao[3], C.R. Lee[4], D.R. Stewart[1].* 1) GDRB, NHGRI, Bethesda, MD; 2) NIH Clinical Center, Bethesda, MD; 3) Dept. of Radiology, NIH Clinical Center, Bethesda, MD; 4) Lab. of Pathology, NCI, Bethesda, MD.

Glomus tumors are benign tumors that arise from the glomus body, a ubiquitous contractile neuromyoarterial receptor that controls blood pressure and temperature. They are classically solitary, located in the distal phalanx and present with temperature hypersensitivity and severe, localized, paroxysmal pain. Multi-focal glomus tumors in patients with neurofibromatosis type 1 (NF1) have been reported, suggesting a possible association. Adults with NF1 were recruited to the NIH for a study on disease variability. Of the ~75 participants in our cohort, 3 reported a history of fingertip pain. Patient 1 was a 35-year-old female with classic glomus tumor symptoms. MRI revealed tumors in 3 of 6 symptomatic digits. The tumors in the 3 fingers were extirpated and confirmed histologically to be glomus tumors. She had complete resolution of pain in 2 fingers and partial in the other digit. Patient 2 was a 35-year-old female who reported pain in her left 4th digit and recently developed pain in her right 1st digit. No abnormalities were observed on physical exam or by bilateral hand MRI. Patient 3 was a 50-year-old male with a 15-year history of pain in his left arm with symptoms consistent with reflex sympathetic dystrophy (RSD). MRI of both hands revealed lesions consistent with glomus tumors of the left 2nd and 4th digits and right 1st digit. These tumors were removed and pathology confirmed glomus tumors in all digits. Our experience supports the hypothesis of an association between NF1 and glomus tumors. Three of our 75 patients reported fingertip pain, suggesting that this complication of NF1 may be more common than previously anticipated. In sporadic cases, multi-focal tumors are very rare. In all 3 NF1 patients, glomus tumors were either suspected or confirmed in multiple fingers. There was also variability in the natural history of the probable glomus tumors ranging from mild temperature sensitivity and pain to RSD. The molecular pathogenesis of glomus tumors in NF1 is under investigation.

562/F

A case of spinocerebellar ataxia type 17 (SCA17) associated with homozygous 46/47 repeats of the TBP gene. *P. Tarantino[1], E.V. De Marco[1], F. Annesi[1], D. Civitelli[1], S. Carrideo[1], M. Caracciolo[1], E. Pisano[2], G. Annesi[1].* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Neurology Unit, Annunziata Hospital- Cosenza, Italy.

Spinocerebellar ataxia type 17 (SCA17) is a dominant progressive neurodegenerative disorder, caused by a triplet repeat expansion within the TATA-binding protein gene (TBP); normal expansions range from 29 to 42 repeats, whereas abnormal expansions range from 43 to 63 repeats. A reduced penetrance is associated to 43-48 repeats. The disease is characterized by progressive limb and gait ataxia, dysarthria, motor, cognitive and psychiatric abnormalities. In this study, we describe a new homozygous SCA 17 patient from Southern Italy. We observed a patient, son of consanguineous parents, affected by autosomal dominant ataxia, pyramidal and extrapyramidal signs and peripheral neuropathy. He was investigated for repeat expansions in the genes of the spinocerebellar ataxias SCA1, SCA2, SCA3 SCA6, SCA7, SCA8, SCA12, SCA17 and DRPLA. Genomic DNA was amplified with fluorescent primers spanning the SCA expansions. PCR products were separated onto a capillary sequencer. We identified an abnormal CAG/CAA repeat expansion of 46/47 size, within the TBP gene, confirmed by direct sequencing. This is the third case of homozygous expansion described in a patient with SCA17. The first case reported, carrying a homozygous 47 repeat expansion, showed a very severe phenotype with a late onset but rapidly progressing ataxia associated with dementia caused by an apparent partial isodisomy 6. The second case, carrying a homozygous 48 repeat expansion, presented rapidly progressive dementia and chorea implying a HD-like disease. Moreover, previous studies report that homozygotes for SCA2, SCA3 and SCA6 disease show earlier onset and more severe manifestations than heterozygotes. Currently, clinical analysis of the patient and genetic and clinical analysis of other family members are ongoing to evaluate the genotype-phenotype correlation in this family. The addition of one more homozygous case is useful to clarify the molecular mechanisms underlying the gene dosage effects in the polyQ diseases.

563/F

The relationship between congenital malformations and pediatric malignancies. *M. Akgul[1], O. Cogulu[2], S. Aksoylar[2], A. Alpman[1], B. Durmaz[2], C. Gunduz[3], G. Koturoglu[2], N. Cetingul[2], F. Özkinay[2].* 1) Medical Genetics, Ege University, Izmir, Turkey; 2) Department of Pediatrics, Ege University, Izmir, Turkey; 3) Department of Medical Biology, Ege University, Izmir, Turkey.

Multipl environmental and genetic factors are responsible from the development of cancer. Common pathways may play a role in tumorogenesis and congenital malformations. Down syndrome is the most popular genetic syndrome associated with increased incidence of malignancy whereas central nervous system and urinary system abnormalities were reported as the highest risk group in regard to development of cancer. However the relationship between cancer formation and congenital malformations remains obscure. The aim of our study was to detect the distribution and incidence of congenital minor and/or major malformations in cases diagnosed with malignancies during childhood. A total of 64 pediatric cases diagnosed with different types of cancers and 60 age matched control group without any chronic disorders were enrolled in the study. Each patient was simultaneously subjected to dysmorphological examination by two clinical geneticists. Detected malformations, clinical and laboratory findings of the patients were recorded. Of the total cases, 28 had leukemia, 10 had lymphoma and 26 were diagnosed to have solid tissue tumors. The sex ratio was 1.0 and the average age was 9.84±5.77 in the patient group and 8.23±4.56 in the control group. Epicanthus was found in 32.81% of the patients and 13.33% of the controls (p=0.01), whereas blue sclera was recorded in 12.50% of the patients and 1.66% of the controls (p=0.03). High palate was observed in 65.62% of the patients and 23.33% of the control group (p<0.01) and attached ear lobe was significantly higher in the patient group (28.12%) than the control group (5.00%) (p=0.0006). In conclusion, our results may support the idea that cancers and congenital minor anomalies share many common molecular pathways and factors, and, thus, further studies related to congenital anomalies may guide for the clarification of tumorigenesis in cancer.

564/F

Neonatal hepatoblastoma in Beckwith-Wiedemann syndrome: which role for imprinting alteration of the 11p15.5 region? *L. de Sanctis, MC. Russo, C. Marinaccio, A. Testa, D. Farinasso, L. Costa, F. Cresi, M. Silengo, L. Silvestro, R. Miniero.* Dept. Pediatric Sciences, Univ Torino, Torino, Italy.

Beckwith-Wiedemann syndrome (BWS) is an "overgrowth" disease caused by alteration in the 11p15.5 region (paternal UPD, hypo/hypermethylation of the differential methylated regions, duplications, traslocations, point mutations), where H19, IGF2 and other imprinted genes involved in growth reside. BWS is characterized by macrosomia with hemihypertrophy, macroglossia, omphalocele or umbilical hernia, neonatal hypoglicemia, ear and renal abnormalities. 10-15% of BWS children early develops an intrabdominal neoplasia: Wilms' tumor (67%), hepatoblastoma (11%), rabdiomiosarcoma (5%) and neuroblastoma (4%). Hemihypertrophy is the clinical sign significantly associated to an increased relative risk (IRR) of neoplasia. A genotype/epigenotype correlation has recently been evoked between paternal UPD and H19 hypermethylation with cancer IRR. G.M., born at term, 4290g, came to our observation for respiratory distress syndrome, showing macroglossia, macrosomia with hemihypertrophy, umbilical hernia, neonatal hypoglicemia, suggestive for BWS, for which molecular analysis of the 11p15.5 region has been performed. During pregnancy increased alphafetoprotein (AFP) levels were recorded, polihydramnios, hypergrowth and renal US hyperechogenicity. At birth AFP was 58.690 kUI/l, abdominal US showed bilateral renal dysplasia. At 24 days it has been repeated for the worsening clinical conditions showing a 8x5 cm mass in hypochondrium, confirmed by TC scan. AFP was 245532 kUI/l; fine needle biopsy allowed to define a hepatoblastoma, for which a reductive pre-surgery chemiotherapy was begun. Molecular analysis revealed a paternal UPD. This case report: - is the first report of a very early onset of hepatoblastoma in BWS - confirms the usefulness of serial AFP determinations as hepatoblastoma marker - underscores how a definite and early molecular characterization of the 11p15.5 region alteration can drive the physicians in the correct management of each epigenetic BWS subgroup.

565/F

Rhabdomyosarcoma in Costello syndrome: Clinical review and molecular studies. *K.W. Gripp[1], L. Nicholson[1], D.L. Stabley[2], K. Sol-Church[2].* 1) Medical Genetics, A.I. duPont Hosp, Wilmington, DE; 2) Biomed. Research, A.I. duPont Hosp, Wilmington, DE.

Costello syndrome (CS) is a rare anomaly and tumor predisposition syndrome, with rhabdomyosarcoma (RMS) being the most common malignancy. Tumor screening was proposed. CS is due to germline mutations in *HRAS*, an oncogene at 11p15.5. The CS causing missense *HRAS* mutations are identical to those found in isolated tumors, and result in gain-of-function. While it appears obvious that a germline mutation in an oncogene should be the first "hit" in tumorigenesis of CS pts, Kratz (2007) showed that in the development of isolated RMS loss of heterozygosity (LOH) for 11p15.5 precedes *HRAS* changes. In light of the discovery of *HRAS* mutations in CS and the new information on RMS initiation, we reviewed RMS in presumed CS pts.

Results: Screening didn't identify any RMS, but 2 pts became symptomatic between screenings. While all reported pts presented with RMS ≤ age 6, we report here a 16 year old presenting with RMS. In 12/20 pts information about the *HRAS* change is known: G12S in 9 (75%); G12A in 2 (16%); G12C in 1. This is comparable to the distribution in CS overall with G12S in 80% and G12A in 9%. Previously we reported that most, but not all, CS mutations occur in the paternal germline (Sol-Church 2006). This may be relevant to the RMS risk in this population. Information on the parental origin of the mutation is available on 2, both paternal. LOH for 11p15.5 was previously reported in 5 tumors (Kerr 2003), but parental status of LOH was not identified. We now prove LOH and loss of the maternal allele in 2 novel cases. If LOH of the maternal allele with overexpression of the paternal allele is the initiating event, then maternally derived *HRAS* mutations may imply a reduced RMS risk. No pts with maternal mutation had RMS.

Conclusions: While most CS associated RMS occurs in young children, adolescents are at risk. Our data suggest a similar tumor risk associated with G12S and other *HRAS* changes. Parental origin of the germline mutation may impact RMS risk.

566/F

Hereditary cancer syndrome found by aCGH in a patient being evaluated for a Prader-Willi-like syndrome. *B. Heald[1, 2], R. Moran[1], M. Milas[3], C. Garner[3], C. Burke[4], B. Torchia[5], J. Coppinger[5], C. Eng[1, 2, 6].* 1) Genomic Medicine Instit, Cleveland Clinic, Cleveland, OH; 2) Taussig Cancer Center, Cleveland Clinic, Cleveland, OH; 3) Dept of Surgery, Cleveland Clinic, Cleveland, OH; 4) Dept of Gastroenterology, Cleveland Clinic, Cleveland, OH; 5) Signature Genomics Laboratory, Spokane, WA; 6) Dept of Genetics, Case Western Reserve University College of Medicine, Cleveland, OH.

A 22-year-old woman was referred for a genetics evaluation. Several features consistent with Prader-Willi syndrome (PWS) were observed, including mental retardation, short stature, obesity, hypotonia, and small hands and feet. However, the patient lacked many of the key behavioral features of PWS. The patient was adopted and no information is known about her family history. Routine karyotype and chromosome 15 methylation studies were normal. Array comparative genomic hybridization (aCGH) identified a deletion of 5q22 encompassing the APC tumor suppressor locus, resulting in familial adenomatous polyposis (FAP) with mental retardation. A colonoscopy revealed hundreds of polyps throughout the colorectum. Ultrasound of the thyroid detected nodules confirmed on biopsy and operative resection to be papillary carcinoma of the morula type, a type of cancer found in less than 2% of patients with FAP. Only 16 other patients are described in the literature with interstitial chromosome 5 deletions encompassing APC. All patients had mental retardation, dysmorphic facial features, and other developmental abnormalities. The presentation of FAP in these patients is similar to that described in patients with mutations in APC. Our patient is the only case in which the deletion was detected on aCGH and not routine karyotype. The implementation of aCGH into clinical practice has the ability to identify syndromes in patients that were previously unsuspected. The power of this improved technology lies in its genomic assessment of copy number changes at a higher resolution than routine karyotyping. In addition to addressing the cognitive needs of this patient, early identification of a cancer predisposition syndrome and recommendations for appropriate surveillance were made.

567/F

Role of WD repeat proteins DMXL1 and DMXL2 in health and disease. *M.R. Hegde, L.H. Chin.* Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA.

Prader-Willi syndrome (PWS) is a developmental disorder characterized by mental retardation (MR), infantile, hypotonia, poor suck reflex, growth retardation, and childhood onset of pronounced hyperphagia resulting in morbid obesity. PWS is a classic imprinting disorder with most cases resulting from paternal deletions of 15q11-q13 or maternal uniparental disomy 15. However, not all patients who present with PWS-like phenotype have chromosome 15 involvement, suggesting genetic heterogeneity. We have recently identified 6 novel missense mutations in the DMXL1 gene on chromosome 5 in 12% (6/52) of patients with a PWS-like phenotype who previously tested negative for known chromosome 15 etiologies. While the function of DMXL1 is unclear, it is a member of the highly conserved WD repeat protein family, found in all major eukaryotic taxa. Indeed, all six mutations replace an amino acid conserved in DXML1 from human to yeast. A highly similar gene, DMXL2, maps to chromosome 15q21 and a microdeletion of the region including the DMXL2 gene has been reported in a small number of patients with MR, hypotonia, growth retardation and obesity. We therefore hypothesize that mutations in either DMXL1 or DMXL2 may present with a PWS-like phenotype and may account for a sizable fraction of the genetic heterogeneity. We are currently conducting extensive mutation and functional analysis of the mutations in DMXL1 and DMXL2 genes and their proteins. It is hoped that this study will define a novel genetic disorder resembling PWS and provide initial clues to the mechanism of the disorder by biochemical and model system studies.

568/F

A NEW CANDIDATE REGION FOR COFFIN-SIRIS SYNDROME? *R.S. Simão[1], C.M. Lourenço[1], L.C. Veiga Castelli[2], L.A.F. Laureano[1], L. Martelli[1,2].* 1) Medical Genetics Division, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil; 2) Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

Coffin-Siris Syndrome (CSS) is a rare genetic Mendelian disorder characterized by mental retardation, growth restriction, absent fifth-digit fingernails or hypoplastic fifth-finger terminal phalanx and coarse facies. Since the first description, over 60 cases have been reported. All previous patients with well-documented Coffin-Siris Syndrome were chromosomally normal, and the chromosomal location of the gene is unknown. We report the description of an infant with severe typical findings of Coffin-Siris Syndrome who also presented a large de novo duplication of the long arm of chromosome 3, that was confirmed by spectral karyotype analysis. The parental karyotypes were normal and none of the relatives have any sign of Coffin-Siris Syndrome. The breakpoints 1q21.3 and 7q34 have been suggested as possible locations for a Coffin-Siris gene but, to our knowledge, this report is the first to describe a child with Coffin-Siris Syndrome features and chromosomal aberrations that may indicate another candidate region for genetic mapping of this syndrome.

569/F

Genetic links between maternal diabetes/obesity and neural tube defects. *H. Zhu[1], W. Lu[1], L. Suarez[2], M. Canfield[2], G.M. Shaw[3], R.H. Finnell[1].* 1) Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, TAMU-HSC, Houston, TX; 2) Department of State Health Services, Austin, TX; 3) California Birth Defects Monitoring Program, Berkeley, CA.

BACKGROUND: Neural tube defects (NTD) are common, costly, and deadly human congenital anomalies. One of the most promising clues to the causes of NTDs is that women who use multivitamins containing folic acid during early pregnancy are at reduced risk for NTD, however, the etiologies and mechanisms remain largely unknown. Maternal diabetes is an established risk factor for NTD. Pre-pregnancy obesity also increases the risk of NTD. A possible explanation for the association between maternal obesity and NTD risk is that obese women have alterations in glucose tolerance. There are many studies of the genetic variants that increase susceptibility to type 2 diabetes and obesity. We hypothesized that these variations may also increase the women's risks for having NTD-affected pregnancies. Under the condition of maternal hyperglycemia, the fetal genes regulating glucose transportation may also have impact on NTD risk. METHODS: We conducted a candidate gene association study to test the aforementioned hypotheses. DNA samples were derived from a population-based case-control study from a Texas-Mexico border Hispanic population. Variants in several candidate genes (TCF7L2, ENPP1, UCP2, LEP and SLC2A2) were interrogated using TaqMan SNP assays. Odds ratios and 95% confidence intervals were used to estimate the risk effect of the variants. RESULTS: A diabetes-associated allele in SNP rs7903146 in TCF7L2 gene is associated with increased risk of NTD-affected pregnancy among this Hispanic population (OR=4.0, 95% CI: 1.1~14.9, P=0.02). This SNP has been consistently reported as a strong predictor for type 2 diabetes and obesity in multiple populations. In addition, a mild protective effect was observed when the minor allele was present in a nonsynonymous SNP in infant SLC2A2 gene. CONCLUSION: Our observations provided preliminary evidence supporting the hypotheses that genetic variations associated with maternal diabetes/obesity and embryonic glucose transportation may increase the NTD risk.

570/F

Arterial Tortuosity Syndrome: clinical and molecular findings in 12 newly identified families. *B. Callewaert, A. Willaert, J. De Backer, B. Loeys, P.J. Coucke, A. De Paepe.* Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Background: Arterial tortuosity syndrome (ATS) is a rare autosomal recessive connective tissue disease, characterized by widespread arterial involvement with elongation, tortuosity and aneurysms of the large and middle-sized arteries. Recently, mutations in SLC2A10 were identified in this condition. This gene encodes the facilitative glucose transporter GLUT10 and was previously suggested as a candidate gene for diabetes mellitus type 2. Methods: Twelve newly identified ATS families with 16 affected individuals were clinically and molecularly characterized. In addition, extensive cardiovascular imaging and glucose tolerance tests were performed in both patients and heterozygous carriers. Results and conclusions: All 16 patients harbor bi-allelic SLC2A10 mutations and haplotype analysis suggests founder effects for all 5 recurrent mutations. Facial resemblance was obvious and all patients had involvement of the skin and skeleton. Remarkably, patients were significantly older than those previously reported in literature (p=0.04) and only one affected relative died, most likely of an unrelated cause. Although the natural history of ATS in this series was less severe than previously reported, it does indicate a risk for ischemic events. Two patients initially presented with stroke, respectively at age 8 months and 23 years. Tortuosity of the aorta or large arteries was invariably present. Two adult probands (aged 23 and 35 years) had aortic root dilation, 7 patients had localized arterial stenoses and 5 had long stenotic stretches of the aorta. Heterozygous carriers did not show any vascular anomalies. HbA1c levels and glucose tolerance tests were normal in 6 patients and 8 heterozygous individuals of 5 families. As such, overt diabetes is not related to SLC2A10 mutations associated with ATS.

571/F

The phenotypic variability of laminopathies: a trap for the clinicians. *V. Drouin-Garraud[1], L. Guyant-Maréchal[2], A. Bedat-Millet[2], F. Anselme[3], G. Savoure[3], A. Laquerrière[4], P. Richard[5], T. Frebourg[1].* 1) Department of Genetics, University Hospital and Inserm U614, Faculty of Medicine, Institute for Biomedical Research, Rouen, France; 2) Department of Neurology, University Hospital, Institute for Biomedical Research, Rouen, France; 3) Department of Cardiology, University Hospital, Institute for Biomedical Research, Rouen, France; 4) Department of Pathology, University Hospital, Institute for Biomedical Research, Rouen, France; 5) Molecular Cardiogenetics, La Pitié-Salpétrière University Hospital, AP-HP, Paris, France.

Since the identification of *LMNA* mutations in the autosomal dominant form of Emery-Dreifuss muscular dystrophy, *LMNA* mutations have been shown to result in a wide range of phenotypes including autosomal recessive form of EDMD, dilated cardiomyopathy, familial partial lipodystrophy of the Dunnigan type, mandibuloacral dysplasia, Charcot-Marie-Tooth neuropathy, Hutchinson-Gilford Progeria or Werner Syndrome, lethal restrictive dermopathy and other complex phenotypes. We report our clinical experience on the inter- and intra-variability of the *LMNA* mutation phenotype based on the extensive investigation of 38 patients from 13 families. Fourteen patients from 5 families showed a muscular disease associated with cardiac involvement, and 2 had clinical involvement since infancy. Eighteen patients from 3 families had isolated severe cardiac disease and these families were dramatically affected by an autosomal dominant form of sudden death. Three patients had partial lipodystrophy. Finally, 3 patients from 2 families showed an unusual complex phenotype associating cardiac involvement, mild dysmorphic facial features and acrogeria. Most of the patients had a severe cardiac disease. This series highlights that the diagnostic of laminopathies should be considered in patients with supraventricular arrhythmia and conduction system disease, even in the absence of dilated cardiomyopathy. The frequency of ventricular arrhythmia justifies to perform presymptomatic diagnosis in order to implant in mutation carriers cardioverter defibrillators which prevent sudden death.

572/F

Influence of gender on phenotypic manifestations and their age of onset in 1013 probands with Marfan syndrome or related phenotypes with FBN1 mutations: an international study. *L. Faivre[1], G. Collod-Beroud[2], B. Loeys[3], A. Child[4], C. Binquet[5], E. Gautier[5], B. Callewaert[3], E. Arbustini[6], K. Mayer[7], M. Arslan-Kirchner[8], C. Beroud[2], C. Bonithon-Kopp[5], M. Claustres[2], L. Ades[9], J. De Backer[3], P. Coucke[3], U. Francke[10], A. De Paepe[3], C. Boileau[11], G. Jondeau[12].* 1) Centre de Génétique, CHU Dijon, France; 2) INSERM, U827, Montpellier, France; 3) Medical Genetics, Ghent University, Belgium; 4) Cardiological Sciences, St George's Hospital, London, UK; 5) Centre d'investigation clinique - épidémiologie clinique, CHU Dijon, France; 6) Molecular Diagnostic Unit, Policlinico San Matteo, Pavia, Italy; 7) Human Genetics, Martinsried, Germany; 8) Humangenetik, Hannover, Germany; 9) Marfan Research Group, Westmead Children's Hospital, Sydney, Australia; 10) Genetics and Pediatrics, Stanford University, USA; 11) Génétique moléculaire, Hôpital Ambroise Paré, Boulogne, France; 12) Centre de Référence Marfan, Hôpital Bichat, Paris, France.

The cardinal features of Marfan syndrome (MFS) involve the ocular, cardiovascular and skeletal systems. Taking advantage of the data of a large international study including 1013 probands with a pathogenic FBN1 mutation, we analysed the influence of gender on the patients phenotypes. Using the Kaplan-Meier method for features for which the age at diagnosis was available and the Mantel-Haensel test for other features, we did not find any significant difference for age at diagnosis of MFS or related disorder, survival, skeletal, lung and dural involvements in males as compared to females. However, significant differences were found for the cumulative probability of aortic surgery in patients with aortic dilatation. Indeed, 46% of males had surgery for aortic dilatation before or at 40 years compared to 34% in females (p=0.0002). A marginally significant result was found for the cumulative probability of ascending aortic dilatation, with a probability of 80% (99.9%-CI=35%-57%) before or at 40 years in males compared to 70% in females (99.9%-CI=23%-48%)(p=0.0036). In conclusion, the gender of a patient might influence the risk of developing ascending aortic dilatation as well as its severity.

573/F

Elastin Gene Mutations: Genotype-Phenotype Correlations in Supravalvular Aortic Stenosis. *V. Hucthagowder[1], L. Jonggadipo[1], P. Kaplan[2], B.A. Kozel[1], D. Kathy Grange[1], M.C. Johnson[1], Z. Urban[1,3].* 1) Department Pediatrics, Washington University, St. Louis, MO, USA; 2) Division of Biochemical Genetics, Children's Hospital, Philadelphia, PA, USA; 3) Department of Genetics, Washington University, St. Louis, MO, USA.

The goal of this study was to better define the genetic epidemiology of familial supravalvular aortic stenosis (SVAS). We recruited 50 probands with SVAS and screened the elastin gene (ELN) for mutations using genomic DNA by a combination of denaturing high performance liquid chromatography and direct DNA sequencing. Additional family members were genotyped for mutations discovered and clinical data was collected from 34 participants using questionnaire and a review of medical records. Skin fibroblasts were collected from 11 participants and ELN expression was analyzed by quantitative RT-PCR and sequencing. Selected mutations were evaluated using luciferase reporter assays. We identified 15 novel and 6 previously described ELN mutations. The majority (81%) of the mutations were predicted to cause premature termination codons. Four of these premature termination mutations were shown to activate a nonsense-mediated decay pathway resulting in null alleles. Two further mutations caused reduced transcription by disrupting the elastin promoter. In addition to obstructive vascular disease and structural heart defects, patients showed facial and connective tissue characteristics previously described only in Williams syndrome. Male patients had significantly (p=0.026) more severe SVAS requiring surgery than females. Asymptomatic mutation carriers showed significantly higher residual elastin expression than patients with SVAS. We conclude that despite significant allelic heterogeneity the functional haploinsufficiency of the elastin gene is a unifying disease mechanism for familial SVAS. Our clinical data showed significant connective tissue involvement in these patients in addition to cardiovascular disease. Finally, gender and residual elastin gene expression emerge as significant modifying factors of SVAS.

574/F

Aortic Root Disease and Myotonic Dystrophy in Two Siblings: A Unique Family with Maternal Connective Tissue Disease and Paternal CTG Expansion in *DMPK*. *J. Platt[1], T. Mozaffar[2], M.V. Zaragoza[1].* 1) Center for Molecular and Mitochondrial Medicine and Genetics and Dept. of Pediatrics, Division of Genetics and Metabolism, University of California, Irvine; 2) Dept. of Neurology, University of California, Irvine.

Diseases of the aorta including dilation and dissection are significant features of inherited defects of connective tissue including most notably, fibrillin 1 in Marfan Syndrome. Myotonic Dystrophy type 1 (DM1) is an autosomal dominant, multisystem disorder characterized by skeletal muscle weakness, myotonia, cataracts and cardiac conduction abnormalities. DM1 is caused by CTG expansion in the gene Myotonic Dystrophy Protein Kinase (*DMPK*). We describe a family consisting of two siblings both with aortic root disease, minor skeletal abnormalities, progressive muscle atrophy, weakness and early-onset cataracts. Their mother has significant aortic root disease. Their father has frontal balding, bilateral cataracts and adult-onset diabetes. DNA testing for Myotonic Dystrophy revealed mutations in *DMPK* for both siblings (>250 and >350 CTG repeats) and for their father (64 CTG repeats). DNA sequence analysis of *Fibrillin 1* (*FBN1*) detected two heterozygous, maternally inherited nucleotide changes: E1283A in exon 31 and IVS58-21G>A in intron 58. The *FBN1* sequence variants have not been previously described as disease-causing mutations; thus, E1283A most likely represents a novel mutation in *FBN1* for aortic root disease. Clinical and molecular evaluation of this unique family provides insight on the genotype-phenotype associations in two individuals with both connective tissue disease and myotonic dystrophy.

575/F

Lethal Cardiomyopathy in Child with Partial Trisomy 22q11.23 and Homozygous MYBPC3 mutation. *N. Powell[1], L. Gole[2], U. Surti[2], S. Madan-Khetarpal[1].* 1) Medical Genetics, Children's Hospital of Pittsburgh, Pittsburgh, PA; 2) Pittsburgh Cytogenetics Laboratory, Magee-Womens Hospital of UPMC, Pittsburgh, PA.

We report an Amish male infant having a lethal cardiomyopathy with two genetic abnormalities: a duplication of 22q11.23 identified by array comparative genomic hybridization, but not seen on a 700 G-band karyotype, and a presumably pathogenic homozygous mutation of the MYBPC3 gene which codes for cardiac myosin binding protein C. The family history includes a brother born three years earlier with hypertrophic cardiomyopathy, who passed away at 17 days of life, and several paternal relatives with similar cardiac history. This brother had a genetic and metabolic evaluation which was non-diagnostic, not including array CGH or hypertrophic cardiomyopathy gene testing. Both parents and his 21 month old brother have been asymptomatic but have not undergone formal cardiology evaluation. Most patients with an MYPBC3 mutation have a mild course and prognosis, with lethality rarely being reported. The duplication 22q11.23 may be clinically significant or a normal variant in the population since it is distal to the DiGeorge Syndrome I/Velocardiofacial Syndrome region and the Cat-Eye Syndrome critical region at 22q11.2. This patient is a unique individual with lethal cardiomyopathy having both partial trisomy 22q11.23 and a MYBPC3 mutation.

576/F

Familial Small Bowel Perforation in Siblings with Thin, Hyperextensible Skin, Tissue Fragility, Minor Joint dislocations, occasional Hypermobility and Co-Occurance of Intestinal Disease. *C.A. Bay[1], R. Kelleher[2], S. Morrill-Cornelius[1], R.G. Cadle[1], B.D. Hall[1].* 1) Clinical/Biochemical Genetics, Univ Kentucky, Lexington, KY; 2) Central Baptist Hospital, Lexington, KY.

We have observed a sibship of 4 affected individuals with small bowel perforation, skin hyperextensibiity, easy bruisability, tissue friability, and occasional mild joint dislocation/hypermobility, most closely resembling the Ehlers-Danlos class of connective tissue disorders. Of the 4 individuals with bowel perforation, one died of postoperative complications at age 23. Each affected individual had been diagnosed with an additional intestinal diagnosis of either diverticulitis (3) or celiac disease (1). The proband is a 46 yo white female who perforated her small bowel at age 45. Surgeons noted extremely friable tissue, with no associated areas of hemorrhage. PMH + for episodic diverticulitis starting in 20's; increased skin distensibility, easy bruisability, and recurrent dislocation of one shoulder. PE + for normal stature, thin face, not pinched. Skin was extremely soft and hyperextensible, normal scars. She was not hypermobile. Family history was notable for 4 of 7 siblings (3F:1M)with bowel perforations at age 20's - 45. Consanguinity was denied. Both parents: + for diverticulitis, but no connective tissue signs/symptoms. Affected siblings: loose, redundant skin with easy bruisability. One affected female could hyperextend her fingers and wrists, but no other joints; One had postoperative abdominal wall hernia, another Addison's disease. Laboratory investigations to date include: normal type I and III procollagen studies for EDS IV (U of Washington Collagen Dx Laboratory); normal lysyl hydroxylase, serum copper and renal ultrasound. Echocardiogram:normal aortic root diameter; thin and pliable pulmonic valves, rest unremarkable. Ophthalmic exam: normal. All 4 individuals in this sibship who experienced bowel perforation had similar cutaneous findings, and had an additional intestinal disease (diverticulitis, celiac disease). This suggests that the co-occurance of severe intestinal disease in a family with thin, friable tissues, can result in bowel perforation.

577/F

Phenotipic Variability of Diphalia. Three cases report from the Hospital para el Niño Poblano. México. *L. Cuellar[1], J. Aparicio[2,6], P.M. Barrientos[3], H.M.L Hurtado[4], G.R. Vargas[5].* 1) Urology and Pediatric Surgery; 2) Genetics; 3) Endocrinology; 4) Cytogenetics; 5) Pathology, Hospital Para El Nino Poblano; 6) Estomatology, Benemérita Universidad Autónoma de Puebla, México.

Code Words: Diphallia, hipospadias, homebox genes. INTRODUCTION. Diphallia, or penile duplication (PD), is a medical condition in which a male infant is born with two penises. It has been estimated that one out of 5 million live births in the United States results in a diphallic birth defect. When diphallia is present, a different kind of other congenital anomalies such as renal, vertebral and anorectal duplication are observed. There is also a higher risk of spina bifida. Infants born with PD and its related conditions have a higher death rate from various infections associated with their more complex renal or colorectal systems. CLINICAL CASES. A study was performed in three male patients 2 months, 4 and 16 years respectivelly. All patients were diagnosed with real diphallia, well developed with urinarious meatus, and both testicles, one of the case a vessel duplication was observed, all of the patients has a normal cariotype, 46XY. Pathology studies were performed to the surgered penises. CONCLUSION. It is thought diphallia occurs in the fetus between the 23rd and 25th days of gestation when an injury, chemical stress, or malfunctioning homeobox genes hamper proper function of the caudal cell mass of the fetal mesoderm as the urogenital sinus separates from the genital tubercle and rectum to form the penis. This rare condition has been documented in pigs and other mammals. It is commonly mistaken that all sharks have this condition, but in reality they have a pair of "claspers" which serve a reproductive function. REFERENCES. 1. Sergio F. Camacho-Gutierez y cols. Genitourinary reconstruction in a case of penis duplication associated to bladder duplication, perineal hypospadias and bowel sequestration. Rev Mexicana de Urologia.2004:64:135-138. 2. Wecker SS: Pene gemino quidam, Obs Med Admirab Moust Lib Y: De partibus Genitalibus, Francoforth, 1609. 3. Hollowell JG: Embryologic considerations of diphallus and associated anormalies, J Urol 117:728, 1977. jmapar@prodigy.net.mx.

578/F

Update on the NIH Study on ARPKD/CHF and other Ciliopathies. *M. Gunay-Aygun[1,2], E. Font-Montgomery[1], M. Parisi[3], D. Adams[1], H. Edwards[1], L. Lukose[1], P. Choyke[4], R. Fischer[1], I. Bernardini[1], J. Bryant[1], B. Gochuico[1], L. Guay-Woodford[5], H. Heller[6], P. Mohan[7], K. Daryanani[8], W. Gahl[1,2].* 1) MGB, NIH/ NHGRI, Bethesda, MD; 2) Intramural Office of Rare Diseases, NIH; 3) University of Washington, Seattle, WA; 4) NCI, NIH; 5) University of Alabama, Birmingham AL; 6) NIDDK, NIH; 7) CNMC, Washington, DC; 8) NIH Clinical Center.

Human ciliopathies are a group of distinct syndromes with overlapping features caused by defects of the cilia or its basal body/centriole. These include the autosomal dominant (ADPKD) and recessive (ARPKD) polycystic kidney diseases, nephronophthisis (NP), Joubert (JS) and related cerebello-oculo-renal syndromes (CORS), and Bardet-Biedl (BBS), Meckel-Gruber (MGS), Oral-Facial-Digital (OFD), and Alstrom syndromes (AS). ARPKD, the most common pediatric ciliopathy, is characterized by progressive renal insufficiency and congenital hepatic fibrosis (CHF). Although a subset of the patients with JS/CORS, BBS, OFD, and AS are known to have kidney and liver involvement, the nature of kidney and liver disease in these syndromes is poorly defined, largely because pertinent data are limited and retrospective. We have recently expanded the ongoing NIH natural history study on ARPKD/CHF (www.clinicaltrials.gov, trial NCT00068224) to include other ciliopathies. Here we present MRI and high resolution ultrasound (HR-US) results, correlated with liver and kidney function data, on 88 patients with 95 admissions (60 ARPKD/CHF, 6 JS/CORS, 8 ADPKD/CHF and 14 unknown type of PKD/CHF). In ARPKD/CHF, kidney size and extent of cyst involvement on imaging did not correlate with creatinine clearance, except for the very mild patients. MR cholangiogram and HR-US were the most useful imaging modalities for biliary abnormalities and mild kidney involvement, respectively. Three JS/CORS patients had enlarged kidneys with diffuse cystic changes diagnosed perinatally, indistinguishable from ARPKD. We continue to enroll patients to this study to define the full phenotypic spectrum of ciliopathies and to produce comprehensive longitudinal data to provide the groundwork for more focused studies and future therapeutic interventions.

579/F

Third patient with paternal isodisomy for chromosome 7 and cystic fibrosis. *C. Le Caignec[1], B. Isidor[1], U. de Pontbriand[2], V. David[2], M.P. Audrezet[3], C. Ferec[3], A. David[1].* 1) Service de Genetique Medicale, CHU, Nantes, France; 2) Clinique Médicale Pédiatrique, CHU, Nantes, France; 3) Laboratoire de Génétique Moléculaire, CHU, Brest, France.

Many patients with maternal isodisomy of chromosome 7 (isoUPD7) have been described, mainly with intrauterine and postnatal growth retardation or with Silver-Russell syndrome. In contrast, only two cases of paternal isoUPD7 and cystic fibrosis have been reported. Here, we describe the third patient with paternal isoUPD7 and cystic fibrosis. At 3 years of age, the young girl had bronchitis with chronic respiratory disease and exocrine pancreas insufficiency. At clinical examination she had no dysmorphic features and normal growth and psychomotor development. A positive sweat chloride test confirmed the clinical diagnosis of cystic fibrosis. Molecular analysis of the CFTR gene showed homozygosity for the F508del mutation. Her father was heterozygous for the F508del mutation, while unexpectedly her mother did not carry the mutation, but was homozygous for the normal allele. For 16 informative microsatellite markers along the length of chromosome 7, the child was homozygous for one of the paternal alleles, whereas these alleles were absent from the mother. These results confirmed the paternal isoUPD7. At 6 years of age, her height and weight remained normal at +1 SD. She had normal psychomotor development. To date, only two cases of paternal isoUPD7 and cystic fibrosis have been published. The first patient (Pan et al. 1998) had two different recessive disorders, namely cystic fibrosis and primary ciliary dyskinesia with dextrocardia and situs inversus totalis. Pre and postnatal growth were normal. A homozygous F508del mutation with paternal isoUPD7 was identified in this patient. The second patient (Fares et al. 2006) developed severe postnatal growth retardation but this was most likely secondary to his serious medical problems. The child was homozygous for the G542X mutation but molecular analysis of his parents showed paternal isoUPD7. Together with our report, these findings support the hypothesis that paternal isodisomy for human chromosome 7 may have no phenotypic effect on growth.

580/F

Intrafamilial Variability in Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF). *L. Lukose[1], E. Font-Montgomery[1], D. Adams[1], H. Edwards[1], A. Garcia[1], J. Bryant[1], P. Choyke[3], T. Heller[5], P. Mohan[6], K. Daryanani[7], L. Guay-Woodford[4], W. Gahl[1], M. Gunay-Aygun[1,2].* 1) MGB, NHGRI, NIH, Bethesda, MD; 2) Intramural Office of Rare Diseases, NIH; 3) NCI, NIH; 4) University of Alabama,Birmingham, AL; 5) NIDDK, NIH; 6) CNMC, Washington DC; 7) NIH CC.

ARPKD/CHF is characterized by progressive renal insufficiency and CHF complicated by portal hypertension (PH). It is caused by mutations in PKHD1, which encodes fibrocystin. The majority of ARPKD/CHF patients present early in childhood, mostly perinatally with enlarged microcystic kidneys, oligohydramnios and hypoplastic lungs. A minority present later in childhood or in adulthood with PH. A subset of patients also have macrocysts of the bile ducts (Caroli's syndrome) predisposing to cholangitis. Chronic renal insufficiency, hypertension, recurrent cholangitis, esophageal varices and hypersplenism are the major sources of morbidity and mortality. The severity and rate of progression of the kidney and liver disease can be variable even within the same family. As part of an ongoing NIH study on ARPKD/CHF and other ciliopathies (www.clinicaltrials.gov, NCT00068224), we have evaluated 60 ARPKD/CHF patients. In this group, 5 families had 2 and 1 had 4 affected sibs. In one family, one of the sibs was diagnosed prenatally and required kidney transplantation at age 18, whereas the NIH evaluation of her 3 sibs at ages 28, 23 and 21, revealed cysts confined to the renal medulla on high resolution ultrasound; their creatinine clearances were 94, 76, and 122 ml/min/1.73 m2, respectively. In another family, the proband presented at birth with enlarged kidneys, whereas his asymptomatic 12-year old sister, who had normal abdominal ultrasound at age 2, manifested cysts confined to the renal medulla. In another sibship, the 7-year old proband presented with splenomegaly at age 3 and had a severely echogenic liver, marked splenomegaly and grade III esophageal varices. His 9-year old asymptomatic sister had a mildly echogenic liver and borderline splenomegaly. This wide intrafamilial variability suggests the presence of strong genetic modifiers.

581/F

Inheritance patterns of pectus excavatum based upon pedigree analysis. *V. Proud[1], L. Horth[2], K. Segna[3], E. Maple[2], R. Kelly[4], D. Nuss[4], M. Stacey[3].* 1) Medical Genetics, CHKD,EVMS, Norfolk, VA; 2) Dept. Biological Sciences, Old Dominion University, Norfolk, VA; 3) Center for Pediatric Research, Eastern Virginia Medical School, Norfolk, VA; 4) Dept Surgery, Children's Hospital of the King's Daughters, Norfolk, VA.

Pectus excavatum (PE) is the most common congenital chest wall malformation, affecting 1/400 children. A chest cavity depression manifests as a result of displacement of the sternum. Additional traits are often associated with PE, including cardiac, musculoskeletal, neural, ocular, skin, and pulmonary-related traits. Thus far, only familial tendency has been reported for PE, based upon clinical observations. Here, we report on the genetic inheritance patterns of PE, based upon pedigree analysis of families harboring PE. We address the presence of secondary traits as they relate to the inheritance of PE. About half of the PE cases demonstrate evidence of sex-linkage, with the majority of these being X-linked recessive inheritance patterns. At least one sixth of the PE cases appear to be a result of a homozygous recessive, autosomal genotype. The remaining cases of PE may be explained by polygenic inheritance or spontaneous mutation, or are cases where we cannot definitively predict one inheritance pattern over another. Secondary traits were evaluated as evidence for a semi-dominant, autosomal PE-associated allele(s), so individuals expressing secondary traits, but not PE, were considered heterozygous for one or more PE-related allele(s). Pedigree data supports the theory that an individual expressing secondary traits, but not PE, may be heterozygous for one or more PE-associated autosomal allele(s). Mutations in Ch10q and in the Col2A1 gene have been associated with PE in other studies, thus the potential for polygenic effects exists.

582/F

A common pathogenetic role for vitamin K-dependent inhibitors of calcification in PXE and the PXE-like syndrome: novel insights in ectopic mineralization. *O.M. Vanakker[1], L. Martin[2], D. Gheduzzi[3], B.P. Leroy[1], B. Loeys[1], P.J. Coucke[1], L. Schurgers[4], C. Vermeer[4], I. Pasquali-Ronchetti[3], A. De Paepe[1].* 1) Ctr Medical Genetics, Ghent Univ Hosp, Belgium; 2) Dpt of Dermatology, Porte-Madeleine Hosp, Orléans, France; 3) Biomedical Sciences Dpt, Univ of Modena and Reggio Emilia, Italy; 4) VitaK & CARIM, Dpt of Biochemistry, Univ of Maastricht, The Netherlands.

INTRODUCTION: Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder, characterized by oculocutaneous and cardiovascular manifestations, due to mineralization and degradation of elastic fibers. The causal ABCC6 gene encodes an ATP-dependent transmembrane transporter; however, the pathogenetic link with the elastic fiber abnormalities remains unknown. We recently identified a novel PXE-like syndrome, resembling PXE and associated with a deficiency of vitamin K (VK)-dependent clotting factors. We have shown it to be caused by mutations in GGCX, encoding a γ-carboxylase, important for activation of VK-dependent proteins, several of which are calcification inhibitors. As such, this disease provides novel possibilities to unravel further the pathogenetic events causing PXE and the PXE-like syndrome and to expand our knowledge on elastic fiber homeostasis. METHODS AND RESULTS: ELISA experiments revealed imbalanced ratios of active and inactive osteocalcin (OC) and matrix gla protein (MGP) in 3 PXE-like patients, with an increase of inactive protein. Immunohistochemistry revealed increased staining of inactive and active MGP, OC and fetuin in 3 PXE-like skin biopsies compared to controls. In 9 PXE patients, but not in 3 patients with elastofibroma/elastosis (elastic fiber dystrophy), identical results were obtained. CONCLUSION: We have shown that dysfunction of (VK-dependent) regulators of calcium metabolism may form a common final pathway in the PXE-like syndrome and PXE. Our findings, appearing specific for ectopic mineralization, represent a major advance in our understanding of this important pathophysiological process, of elastic fibre homeostasis and hence of the pathogenesis of PXE, opening potential avenues for therapeutic agents, such as vitamin K.

583/F

Clinical features in children with microdeletions of the NF-1 gene detected by array CGH. *J.F. Atkin[1], R. Moran[2], E. Edelman[2], C. Rigelsky[2], B. Burton[3], J. Coppinger[4], L.G. Shaffer[4].* 1) Molecular/Human Genetics, Columbus Childrens Hospital, The Ohio State University, Columbus, OH; 2) Cleveland Clinic Foundation, Genomic Medicine, Cleveland, OH; 3) Childrens Memorial Hospital, Dept Genetics, Chicago, IL; 4) Signature Genomics Laboratories, LLC, Spokane, WA.

Approximately 4-5% of individuals with NF-1 have deletions of the entire gene. These individuals are reported to have earlier onset and more significant cutaneous neurofibromas, higher incidence of malignancy, more severe developmental delay/mental retardation, distinctive dysmorphic facial features and congenital anomalies. In some cases, features of connective tissue disease and overgrowth are seen. We report 4 patients detected by array CGH analysis to have a deletion covering the entire gene. These patients had CGH array testing because of birth defects and/or developmental delay and/or dysmorphic features, not for features of NF-1. Only 1 of 4 patients had features that met the diagnostic criteria of NF-1, 6 or more cafe au lait spots and inguinal freckling. No parents were affected. None of the patients had neurofibromas or significant delays. All were of normal size including head circumference. Cafe au lait spots present in all 4 were not of significant number or size. None had malignancies. Consistent clinical findings include normal development or mild delays, specific facial dysmorphic pattern, normal growth parameters, and caft au lait spots (around 5-10 that did not present originally). The age range at original genetics evaluation was 7 weeks to 2 years. The current age range is 4 months to 3.5 years. One chld has polyvalvular heart disease and increased skin folds, one has mild coarctaion of the aorta and shawled scrotum. One has strabismus. No other birth defects were found. Except for the facial features, none of these patients fit the phenotype previously reported for entire gene deletions. This may be related to age of diagnosis. Long term follow-up is needed. Further reports are needed to more precisely assess the microdeletion phenotype and to help with prognosis for these families as well as for surveillance guidelines.

584/F

Implications of a Novel *SOX9* Mutation on the Sexual Phenotype of a Fœtus with True Hermaphroditism and Acampomelic Campomelic Dysplasia. *M. Beaulieu Bergeron[1,2,4], G. Scherer[5], J.-C. Fournet[1,2,4], E. Lemyre[3,4], N. Lemieux[1,2,4].* 1) Département de Pathologie et biologie cellulaire, Université de Montréal, Canada; 2) Département de Pathologie; 3) Département de Pédiatrie and; 4) Centre de Recherche, CHU Sainte-Justine, Canada; 5) Institute of Human Genetics and Anthropology, University of Freiburg, Germany.

Campomelic dysplasia (CD) is a rare disease caused by a mutation in *SOX9*, a gene involved in both chondrogenesis and sexual development. Along with severe skeletal malformations, CD causes sex reversal in 75% of 46,XY patients. We report here on a fœtus presenting with the acampomelic form of CD and true hermaphroditism. The 22 weeks-old fœtus, who has a homogenous 46,XY constitution in both fibroblasts and amniocytes, was found to have a left testis, a right ovary and normal male external genitalia. Analyses revealed a novel mutation of *SOX9*, caused by a *de novo* cytosine insertion in codon 381. Since a case of true hermaphroditism in CD was previously published, we compared the two mutations. Although both mutations were caused by a cytosine insertion inducing a frameshift, the published mutation generates a truncated protein missing almost entirely its third exon while our novel mutation is translated in a protein that has its transactivation domain replaced by an aberrant amino acids sequence. We also examined if the abnormal sexual development of the fœtus was caused by hidden 45,X/46,XY mosaicism in the gonads. FISH experiments performed on formalin-fixed and paraffin embedded gonadal tissues revealed no significant mosaicism in the testis or the ovary. So far, no genotype-phenotype correlation has been found for any of the *SOX9* mutations, and some individuals bearing identical mutations are known to have a variable sexual development. Recent evidence suggests that the sexual phenotype of patients with CD could be influenced by polymorphisms in the *SOX8* gene. Indeed, experiments in knock-out mice showed that *Sox8* could partially substitute for *Sox9*. Further experiments will be needed to explain the effects of this *SOX9* mutation on sexual development.

585/F

Gastrointestinal Disorders in Patients with Hypermobile or Classical Ehlers-Danlos Syndromes. *A. Gustafson[1], B.F. Griswold[2], L. Sloper[2], M. Lavallee[4], C.A. Francomano[3], N.B. McDonnell[2].* 1) Brown Univ, Providence, RI; 2) LCI, NIA/NIH, Baltimore,MD; 3) GBMC,Baltimore, MD; 4) IUSM, South Bend, IN.

Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue that are characterized by joint, skin, and vascular abnormalities. Most literature reports of gastrointestinal (GI) involvement in EDS have been limited to patients with vascular EDS. Patients with vascular EDS have abnormalities of type III collagen, a constituent of the bowel wall, and thus prone to GI complications and bowel rupture. Patients with other types of EDS, however, also report GI dysfunction. Complete physicals and medical histories were obtained from 90 patients with hypermobile or classical types of EDS enrolled in the National Institutes on Aging protocol 2003-086, "Clinical and Molecular Manifestations of Heredity Disorders of Connective Tissue." We found a high prevalence of GI manifestations in this cohort of patients, including severe chronic constipation (17%), irritable bowel syndrome (12%), acid reflux or gastroesophageal reflux disease (14%), and/or chronic abdominal pain (22%). Gastroparesis was noted in four subjects. The prevalence of each of these disorders was significantly higher in our cohort ($P<.0001$) compared with the general population. In our cohort, lack of tissue integrity may cause structural abnormalities, decreased blood vessel wall strength, and/or altered motility or absorption, which may all contribute to development of GI disorders.

586/F

Alert to asymtpmatic arterial hypertension in Williams-Beuren syndrome in childhood. *R. Honjo, E.A. Furusawa, D.R. Bertola, L.M.J. Albano, L. Suzuki, V.H.K. Koch, C.A. Kim.* Instituto da Criança, São Paulo, SP, Brazil.

Williams-Beuren Syndrome (WBS) is caused by a microdeletion at 7q11.23 and is characterized by a distinctive facial appearance and overfriendliness behavior. Among the most important genes deleted in WBS, the ELN gene, encoding elastin, is thought to be responsible for the vascular abnormalities. Supravalvar aortic stenosis is the most common cardiovascular malformation in WBS. However, other peripheral systemic vascular stenosis can represent an important complication, leading to arterial hypertension and stroke. The hypertension found in ~40% of WBS patients is not often associated with visible vascular stenosis on duplex US and appears to fall in the category of essential hypertension with no well-defined structural cause, since anatomic renovascular hypertension is an infrequent finding. Here we describe a patient with WBS who developed hypertension due to a vascular stenosis in a very precocious age, reinforcing the need to supervise the blood pressure in WBS soon in childhood. CASE REPORT: female, 10yo, referred to the Genetics Unit due to myocardial hypertrophy and dysmorphism at age 6. The facial appearance, mild motor and speech delay, and overfriendliness behavior suggested a diagnosis of WBS. At age 8, in a follow-up consultation, it was detected a high blood pressure, although the patient was asymptomatic. The duplex US showed an increase acceleration velocity in the renal arteries, suggesting stenosis. The angiotomography revealed a narrowed aorta, from the descending segment to the emergency of the superior mesenteric, renal arteries, and celiac trunk. The patient underwent an aortorenal and ileorenal bypass procedure. Two years later, she experienced arterial hypertension once again. The angiotomography did not show abnormalities concerning the grafts but there was a significant worsening of the aortic stenosis and its branches. DISCUSSION: The current case shows us the importance of blood pressure supervision since childhood in all WBS patients, even in asymptomatic ones, and the need to check over vascular stenosis if arterial hypertension is detected, once a surgical procedure may be indicated and prevent other renal complications.

587/F

Atypical Presentations of Noonan Syndrome with Hematologic Disease. *R. Jethva[1, 2], J. Ganesh[2], I. Krantz[1], S. Saitta[1], L. Campbell[1], P. Kaplan[2].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Section of Metabolic Disease, Children's Hospital of Philadelphia, Philadelphia, PA.

Noonan syndrome (NS) is an autosomal dominant disorder characterized by typical facial features, skeletal anomalies, cardiac defects, and developmental delay. It is genetically heterogenous and has variable clinical expression. The classic physical features include short stature, triangular facies, curly and coarse hair, low-set ears with thickened pinnae, ptosis, epicanthal folds, down-slanting palpebral fissures, webbed or short neck, low posterior hairline, excess nuchal tissue, and skeletal anomalies. In newborn infants, however, facial features may be subtle. These infants often have normal birth growth parameters and the phenotype may be limited to generalized edema and excess nuchal tissue. Approximately 80% of affected individuals have a cardiac anomaly, including pulmonic stenosis and hypertrophic cardiomyopathy. Patients with NS may have many other systemic complications. Specifically, hematologic findings can include hepatosplenomegaly, thrombocytopenia, coagulopathy, and leukemia. We report three cases of NS that presented with hematologic features diagnosed as juvenile myelomonocytic leukemia (JMML). Each infant had an atypical physical phenotype, which led to delays in diagnosis. Although each individual lacked the classic features, there were other findings suggestive of NS. In addition to having JMML, all three cases had severe failure to thrive, developmental delay, and at least one other common feature of NS, including pulmonic stenosis and wide-spaced nipples. We illustrate that patients with NS and JMML may not present with classic phenotypic features, thus making the diagnosis of NS more difficult. These cases highlight the importance of considering NS in infants with postnatal growth failure, developmental delay, hematologic disease, and usually at least one other common feature of NS. Making the diagnosis is particularly important because the prognosis of JMML in patients with NS is reported to be significantly better than in non-NS patients with JMML.

588/F

Skull defects, alopecia, and distinctive facies; a new syndrome? *A. Kariminejad[1], B. Bozorgmehr[1], M.R. Ashrafi[2], M.H. Karimi-Nejad[1].* 1) Clinical Genetics, Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran; 2) Markaz Tebi Hospital, Tehran, Iran.

We describe the first and only child of first cousin parents with skull defect, alopecia, sparse eyebrows and eyelashes, hypertelorism, epicanthal folds, wide and flat nasal bridge, notched and hypoplastic alae nasi, short palpebral fissure, and high forehead. Skin appears normal except for very sparse body hair. On examination of the skull, fontanels are wide and skull ossification defect on the frontoparietal region can be detected. Three dimensional maxillofacial and skull MRI revealed large calvarial defect in parietal region. A round smaller defect is noted in the parieto-occipital suture. Superior sagittal suture is widely patent. The association of alopecia and ossification defects of skull has previously been reported by Pinherio et al. (1983). They reported four sisters from a sibship of thirteen with hypotrichosis, enamel hypoplasia, dystrophic nails, supernumerary nipples, pigmented nevi and bony deficiency in the frontoparietal region. Hyperkeratosis on the palms and mild xeroderma on the limbs was also present. Our patient does not have the enamel hypoplasia, dystrophic nails, supernumerary nipples, pigmented nevi, hyperkeratosis or xeroderma, and has dysmorphic features absent in the reported cases. The association of skull defect and alopecia and dysmorphic features has not previously been reported. We suggest that the association of these features may characterize a new autosomal recessive syndrome.

589/F

Clinical delineation of sleep disturbance in Cornelia de Lange syndrome (CdLS). *A.D. Kline[1], G. Saba[2], R. Morse[3], W.C. Duncan[4], A.C.M. Smith[3].* 1) Harvey Inst Human Gen, Greater Baltimore Medical Ctr, Baltimore, MD; 2) Division of Human Gen, Univ of Md. Hosp., Baltimore, MD; 3) NHGRI/NIH, Bethesda, MD; 4) NIMH/NIH, Bethesda, MD.

Significant progress has been made in recent years characterizing the clinical and molecular aspects of CdLS. The specific facial features, multiple malformations, developmental delays and behavioral issues are due to mutations in genes related to the cohesin complex, important in cell division and regulation of gene expression. Although other multiple malformation syndromes are associated with sleep disturbance, characterization of sleep issues in CdLS has been largely unknown. A parental questionnaire, regarding sleep behavior, sleep environment, and other behavioral or medical concerns, was received from 74 caretakers of patients attending the national CdLS Foundadtion conference or a regional multidisciplinary aging clinic, or through the Foundation newsletter. Fifty-nine (80%) of the 74 individuals experienced at least one indicator of sleep disturbance, including difficulty falling asleep (51%), frequent nocturnal awakenings (65%), consecutive days without sleep (30%), and frequent daytime napping (14%). Parental perceptions of sleep disturbance varied. There is a higher prevalence of reported sleep disturbance in individuals with gastroesophageal reflux and self-injurious behavior (SIB); SIB was associated with significantly decreased night sleep. Frequent nocturnal awakenings were positively correlated with anxiety. An increased severity of sleep disturbance in adolescence occurred in 39%. In addition, 53% of individuals 18 years and older experienced consecutive days without reported sleep, compared to 36% of those below 18 years. Symptoms of possible sleep apnea have been noted in the older population, and sleep apnea was documented on one previous sleep study. Additional studies, using measures of sleep such as wrist actigraphy on mutation-positive individuals or polysomnography, are being conducted currently to more objectively characterize sleep disturbance in CdLS. This may lead to improved treatment and management, likely to benefit individuals with CdLS as well as their caregivers.

590/F

Cerebral infarction in a 3-year-old patient with progeria. *R. Kosaki[1], M. Uno[2], K. Mizuguchi[3], Y. Abe[3], T. Nagasawa[3], O. Migita[1], T. Tanaka[1], T. Okuyama[1], K. Kosaki[4], A. Oka[3].* 1) Dept Strategic Medicine, Natl Ctr Child Health & Dev, Tokyo, Japan; 2) Dept Interdisciplinary Medicine,Natl Ctr Child Health & Dev, Tokyo, Japan; 3) Dept of Neurology ,Natl Ctr Child Health & Dev, Tokyo, Japan; 4) Dept of Pediatrics,Keio Univ.School of Medicine,Tokyo Japan.

Hutchinson-Gilford progeria syndrome (HGPS) is an autosomal dominant disorder caused by mutations in LMNA and is characterized by prematurely accelerated aging that manifests at age one or two years. The life expectancy was 6-20 years, with the average of 12.6 years. Significant morbidity and mortality is associated with various cardiovascular complications, most notably coronary insufficiencies, due to premature atherosclerosis. Here we report a HGPS patient who developed cerebral infarction at unusually early age of 3 years and 5 months. The Japanese girl was born at 38 weeks gestation with birth weight of 2022g. Clinical diagnosis of HGPS was made at 18 months of age on the basis of the characteristic features including growth failure, loss of subcutaneous fat and sparse hair. At the age of 3 year 2 months, she complained of a mild headache. Three months later, she developed left-sided clonic convulsion and left arm paresis. Magnetic resonance imaging on the brain showed acute infarctions at the right frontal cortex. Magnetic resonance angiography disclosed decreased perfusion in the right middle cerebral artery and in the anterior cerebral artery. On ultrasound examination of the carotid arteries, right carotid artery was poorly visualized. She was diagnosed as having anterior cortical watershed infarction of the right hemisphere due to occlusion of the right internal carotid artery. The left arm paresis improved and she has been medicated with aspirin and dipyridamole. Review of the literature revealed 9 HGPS cases who had cerebrovascular accident. The onset of the patient herein reported is earlier than that of any of the 9 previously reported cases. We would recommend monitoring carotid patency of HGPS patient as early as three years of age so that preventive prescription could be initiated in a timely manner.

591/F

Persistent müllerian duct and jejunal atresia: evidence for a new syndrome. *G. Morin[1,5], C. Jeanpetit[1], C. Belville[2], J. Ricard[3], H. Bony-Trifunovic[4], B. Boudailliez[4], J.P. Canarelli[3], J.Y. Picard[2], M. Mathieu[1,5].* 1) Clinical Genetics Unit, Amiens University Hospital, Amiens, France; 2) INSERM U782, Clamart, France; 3) Pediatric Surgery Service, Amiens, France; 4) Pediatric Endocrinology, Amiens, France; 5) Prenatal Diagnosis Center, Amiens, France.

Persistent Müllerian Duct Syndrome (PMDS) is a rare form of male pseudohermaphrodism characterized by the retention of Müllerian derivative in an otherwise normally virilized male. Approximately half of the cases are secondary to mutations in the anti-Müllerian hormone gene (AMH). Most of the other cases are in relation with mutations of the anti-Müllerian hormone receptor gene (AMH-RII). In these two situations the mode of inheritance is autosomal recessive and the genital abnormality appears isolated. In rare cases, persistent Müllerian duct can be associated with additional features: lymphangiectasia, mental retardation, microphtalmia, hypospadia, lipoatrophic diabetes, vitamin D resistant rickets. In 1997 Klosowski et al reported the case of a patient with PMDS and jejunal atresia, but negative for mutation of AMH and AMH-RII genes. We report on a second patient bearing this association. This boy is born after a pregnancy characterized by polyhydramnios and bowel dilatation at the third trimester. After spontaneous delivery at 35 weeks of amenorrhea he presented evidences for intestinal occlusion. Surgical laparotomy revealed jejunal atresia and the presence of a uterus. Blood karyotype showed a normal male 46,XY formula. Total and free testosterone and anti-mullerian hormone were at normal ranges. Screening for mutations in the whole coding sequence of anti-mullerian gene ant its receptor (AMH-RII) was negative. These two similar observations suggest the existence of a distinctive entity, probably of genetic origin, with peculiar molecular mechanism. The same geographic origins in North of France of the two patients suggest a foundation effect.

592/F

Mental Retardation, Ataxia with Vermis Hypoplasia and Distinctive Facial Appearance, Report of Two Cases. *J. Prieto[1,2], G. Contreras[1], P.M Hurtado[1].* 1) Inst de Genetica Humana, Univ Javeriana, Bogota Cundinamar, Colombia; 2) Hospital la Victoria, Secretaria Distrital de salud, Bogota, Colombia.

Case report: We decribed two siblings born of consanguineous parents with similar characteristics. Case 1: boy 5.5 years old that comes to our service with a history of global retardation. He is born from a third pregnancy with normal prenatal care. Institutional vaginal childbirth. Weight: 2950 grs, Size: 52 cms, PC: 33 cms. Apgar 8/10 and 10/10. He has had a global retardation and has not achieved the goals for his age. Case 2: older sister of case 1, a girl now 13 years old, has also mental retardation and history of psychomotor developmental delay. No inconvenient is register in her pregnancy. Her weight at birth was 2800 grs and size: 48 cms. Also, for both sibs we described a nonprogressive ataxia, mental retardation, hypertonic in arms and legs and speech delay. At the actual physical examination we found: In the boy short stature, microcephaly, anteverted narins, umbilical hernia, fifth finger both hands shorter; For the girl the positive findings are short stature, microcephaly, prominent helix, sinofris, micrognathia, fifth finger both hands shorter. Paraclinics Vermis hypoplasia documented in both by magnetic resonance imaging (MRI), and also for the girl volume loss in the brain stem at the pons level and cerebellar changes with 4th ventricle enlarged. In our cases, there were no specific clinical signs or positive data in the screening tests with regard to metabolic diseases. For the girl there is karyotype report 46, XX. Discussion Vermis hypoplasia is found in association with a variety of neurologic and systemic disorders. Although the description we found in the literature the vermis hypoplasia is found in patients with different phenotype to the one we described here. We didn't find in the literature a Case report similar to ours, including vermis hypoplasia, mental retardation, nonprogressive ataxia, hypertonic arms and legs, microcephaly, in association with speech delay and motor development impairment. Counting the consanguineous relationship of the parents of this two siblings, we concluded this is an autosomal recessive entity.

593/F

Nevo-like phenotype, not associated to lysyl hydroxylase deficiency: a new form of overgrowth syndrome? *G. Scarselli, M. Ottaviani, N. Dayan, A. Zeffiri, E. Lapi, S. Guarducci, M.L. Giovannucci Uzielli.* Dept. Paediatrics, Genetics, University of Florence, Firenze, Italy.

We report on three unrelated patients, one female and two males, affected with Nevo syndrome, a rare autosomal recessive disorder, characterized by increased pre- and postnatal length, generalized joint laxity, muscular hypotonia, hyrsutism and moderate mental retardation. In two cases, the parents are consanguineous. For the three cases, pregnancy and delivery were referred at term, with birth weight and length >97th centile. Severe hypotonia was also referred for the three subjects, at birth and during the first 2 years of life. The three patients displayed tall stature, dolicocephaly, kyphosis, large hands and feet, spindle-shaped fingers, hirsutism and hypermobility of the large joints. The psychomotor developmental milestones were delayed: independent walk at the age of 2 years, and delayed cognitive and language development with a progressive, slow improvement. One of the patients was included in a long follow-up programme. At age 28 year, sudden rupture of the left iliac artery occurred, and repaired surgically. The aortic diameter is extremely reduced , especially at the abdominal level, with a manifest dilation at the iliac fork. In 2005, Nevo syndrome was recognized as allelic to the EDS VIA, an inherited connective tissue disorder characterized by a deficiency of lysyl hydroxylase due to mutations in PLOD1 gene. In this patient, the ratio of total urinary lysylpyridinoline (LP) to hydroxylysyl pyridinoline (HP) was normal, excluding mutations in the PLOD1 gene. The same dosage is programmed for the two other subjects, who present a clinical spectrum strictly overlapping to that of the oldest of our patient.

594/F

Co-occurrence of 4p16.3 deletions with both paternal and maternal duplications of 11p15: modification of the Wolf-Hirschhorn syndrome phenotype by genetic alterations predicted to result in either Beckwith-Wiedemann or Russell-Silver syndrome. *S.T. South[1,2], H. Whitby[1], T. Maxwell[1], E. Aston[1], A.R. Brothman[1,2], J.C. Carey[1].* 1) Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT 84132-2117; 2) ARUP Laboratories 500 Chipeta Way Salt Lake City, UT 84108-1221.

Paternal duplications of chromosome region 11p15 can result in Beckwith-Weidemann syndrome (BWS), whereas maternal duplications of the same region on 11p15 can result in Russell-Silver syndrome (RSS). These two syndromes have numerous opposing phenotypes with BWS characterized by fetal gigantism, macrosomia, asymmetry due to hemihyperplasia, and increased risk for embryonal tumors; whereas, RSS is characterized by prenatal and postnatal growth retardation with a relatively normal head circumference and asymmetry due to hemihypotrophy. The differences in the phenotype are proposed to be due to altered dosage of imprinted genes that control growth within this region of 11p15. Wolf-Hirschhorn syndrome (WHS) is due to deletions of a region in 4p16.3 and is characterized by prenatal and postnatal growth delay, microcephaly, mental retardation/developmental delay, characteristic facial features and seizures. There is no known parent-of-origin effect for deletions of the WHS critical region and no genes are known to be imprinted in this region. We present 3 individuals with apparently identical unbalanced translocations resulting in a derivative chromosome 4 with both a deletion of 4p16.3 and a duplication of 11p15. Two of these individuals are family members with one inheriting the derivative 4 from her balanced mother and the other inheriting the derivative 4 from his balanced father. The third individual is unrelated and inherited his derivative 4 from his balanced father. While the findings of these individuals included some features of WHS and RSS or BWS, the phenotypes as an aggregate are distinct from these syndromes. The genomic and phenotypic characterization of these three individuals will be presented and will demonstrate how unbalanced translocations can result in the modification of chromosome duplication and deletion syndromes.

595/F

Independent NF1 and PTPN11 mutations in a family with Neurofibromatosis-Noonan syndrome. *C.T. Thiel[1], M. Wilken[2], M. Zenker[1], R. Fahsold[3], A. Rauch[1].* 1) Institute of Human Genetics, University Hospital Erlangen, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Private pediatric clinic, Eppenreutherstr. 28, Hof, Germany; 3) Private clinic Prager & Junge, Dresden, Germany.

Neurofibromatosis-Noonan syndrome (NFNS), an entity which combines both, features of Noonan syndrome (NS) and Neurofibromatosis type 1 (NF1), was etiologically unresolved until recent reports demonstrating NF1 mutations in the majority of patients with NFNS. The phenotypic overlap was explained by the involvement of the RAS pathway in both disorders. 95% of patients with NF1 show loss of function mutations of the NF1 gene, encoding a GTPase-activating protein which terminates Ras-GTP signalling, whereas NS is genetically heterogeneous with activating mutations in genes of the RAS pathway, in particular PTPN11. We report on an 18 month old girl with developmental delay, mild pulmonary stenosis and craniofacial anomalies suggestive of NS. In addition she had 11 café-au-lait spots, compatible with a diagnosis of LEOPARD syndrome. The development of bilateral optical gliomas at 19 month, though, suggested NF1. An otherwise healthy brother had 5 café-au-lait spots and mild craniofacial anomalies (broad nasal bridge) while the mother had about 10 café-au-lait spots without any further signs of neurofibromatosis. Mutational analysis of the NF1 and PTPN11 genes in the proband revealed a maternally inherited heterozygous splice-site mutation c.4661+1G>C in intron 27a of the NF1 gene and a de-novo PTPN11 missense mutation c.5C>T (p.T2I). The NF1 mutation was also present in the brother and the mother and interestingly was distinct from the recently described recurrent 3-bp inframe deletion in exon 17 described as the cause of absence of neurofibromas in Neurofibromatosis. In contrast to published cases, in the proband's mother and brother the NF1 mutation resulted in only a mild NF1 phenotype without major signs of Noonan syndrome, despite it's location within the GAP domain forming region. However, the unexpected additional PTPN11 mutation explains the index patient's craniofacial NS features.

596/F

Diaphragmatic hernia, renal cysts and cardiac abnormalities: A new X-linked condition? *M. Thompson[1,4], S. Keating[1,4], P. Shannon[1,4], G. Seaward[2,4], J. Pierre-Louis[3,4], H. Sroka[3,4], D. Shaw[1,4], A. Wolff[1,4], D. Chitayat[3,4].* 1) Dept Pathology & Lab Medicine, Mount Sinai Hosp, Toronto, ON, Canada; 2) Dept of Obstetrics and Gynecology, Mount Sinai Hosp, Toronto, ON; 3) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hosp, Toronto, ON; 4) University of Toronto, Toronto, ON.

Congenital diaphragmatic hernia (CDH) occurs in 1:4,000 live births. In most cases, it is isolated and has a multifactorial mode of inheritance. Familial CDH makes up 0.9-2% of all cases, and of these, 8-10% have bilateral CDH. We report a hitherto new familial condition with CDH, cardiac, renal and other abnormalities with an apparent X-linked mode of inheritance. The parents of two affected siblings were Caucasian and non-consanguineous. The mother's brother died of diaphragmatic hernia soon after birth, further information unavailable. Case 1: A male infant with karyotype 46, XY, was born at 39.6 weeks gestation with coarse facial features, left CDH, tapering fingers, hypoplastic lungs, a small heart with dysplasia of all 4 valves, dextrocardia, dilatation of ascending aorta, tubular hypoplasia of the isthmus, fenestrated foramen ovale, elongated main PA, large kidneys, a small placenta and a 2 vessel cord. The infant died at 3 hours. Case 2: A stillborn male, brother of case 1, with karyotype 46, XY, was born at 36 weeks gestation with coarse facial features, bilateral CDH, tapering fingers, hypoplastic lungs, cardiovascular anomalies including dysplasia/thickening of all 4 valves, aneurysmal dilatation of ascending aorta, persistent left SVC, elongated ductus arteriosus; malrotated bowel, hepatomegaly, enlarged cystic kidneys, thymus and spleen, large placenta and rare CNS white matter calcifications. The long bones showed long standing growth disturbance and the metacarpals were short. To the best of our knowledge, only 4 X-linked conditions have been reported with CDH: Simpson-Golabi-Behmel, thoracoabdominal, craniofrontonasal and MIDAS syndromes. The clinical manifestations in our cases are not consistent with any of these conditions and point toward a new, likely X-linked condition.

597/F

High prevalence of Food Allergies in Patients with Ehlers-Danlos Syndromes. *H. Zhang[1], B.F. Griswold[1], L. Sloper[1], M. Lavallee[3], C.A. Francomano[2], N.B. McDonnell[2], A. Gustafson[1].* 1) LCI, NIA/NIH, Baltimore, MD; 2) GBMC, Baltimore, MD; 3) IUSM, South Bend, IN.

Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue that are characterized by joint, skin, and vascular abnormalities. Complete physicals and medical histories were obtained from 95 patients with hypermobile, classical, and vascular EDS enrolled in the National Institutes on Aging Protocol 2003-086, "Clinical and Molecular Manifestations of Heredity Disorders of Connective Tissue." We found a high prevalence of food allergies in patients with EDS (14%) when compared with the general population (P<.0001). We also found a significantly higher incidence of gastrointestinal manifestations in our cohort when compared with the general population (P<.0001). The presence of food allergies also seems to correlate with gastrointestinal dysfunction in some patients. Of the patients who reported constipation, irritable bowel syndrome, gastroesophageal reflux disease, and/or chronic abdominal pain, many also reported having a food allergy (40%, 42%, 17%, and 20%, respectively). Collagen abnormalities may cause mucosal lesions, altering tissue integrity and increasing the chance of larger proteins crossing the mucosal barrier and creating an immunogenic response. Multiple studies have correlated eosinophillic gastrointestinal disorders, allergic responses that fall in between IgE and TH2-type responses that are mediated by IL-5 and other eotaxins, with classic mast cell tissue degranulation, producing gastrointestinal disorders similar to those seen in our patients. Understanding the mechanisms associated with food allergies in patients with EDS may aid in development of effective treatments.

598/F

ARC syndrome in three male siblings with classic and new findings. *K. Goodin[1], P. Gissen[2], A.S. Knisely[3], N. Ambalavanan[2], A. Theos[4], D. Kelly[5,6], S.L. Rutledge[1,2,3].* 1) Genetics, University of Alabama at Birmingham, Birmingham, AL; 2) Pediatrics, University of Alabama at Birmingham, Birmingham, AL; 3) Neurology, University of Alabama at Birmingham, Birmingham, AL; 4) Dermatology, University of Alabama at Birmingham, Birmingham, AL; 5) Pathology, University of Alabama at Birmingham, Birmingham, AL; 6) Department of Pathology and Laboratory Services, The Children's Hospital of Alabama, Birmingham, AL; 7) Section of Medical and Molecular Genetics, University of Birmingham, Birmingham, UK; 8) Institute of Liver Studies, King's College Hospital, London, UK.

Arthrogryposis-Renal tubular dysfunction-Cholestasis (ARC) syndrome presents with the findings noted in the syndrome title. ARC is also associated with failure to thrive, recurrent infections, and ichthyosis. Dysmorphic features include low set ears, sloping forehead, and hirsutism. ARC is an autosomal recessive disorder associated with germline *VPS33B* mutations which may alter vesicular transport. We report three Guatemalan male siblings who presented in the neonatal period with cholestatic jaundice, failure to thrive, recurrent infections, and dysmorphic features previously associated with ARC. The second child had ichthyosis on skin biopsy. In addition, the children had wide-spaced nipples and elevated cerebrospinal fluid (CSF) protein levels; neither feature has so far been reported in ARC. CSF protein concentrations were prominently elevated, (~200-600 [mg/dL, expected 15-45 mg/dL]). The first two infants died before definitive diagnosis. The third child was homozygous for a *VPS33B* mutation previously associated with ARC. Liver biopsy demonstrated findings associated with ARC and consistent with abnormal intracellular protein transport. Due to the clinical similarity, it is presumed that all three siblings had ARC. Our observations further support the association of ARC with findings previously reported. They also identify a novel association of ARC syndrome with wide-spaced nipples and elevated CSF protein levels; the latter could be secondary to a protein trafficking defect in neural tissues. These observations require confirmation in a larger set of patients.

599/F

Ultrastructural changes and genetic study in Associated congenital glaucoma case with Sturge-Weber syndrome. *D. Pathak[1], M. Tanwar[1], R. Dada[1], R. Sihota[2], V. Gupta[2], T. Das[1], T. Dada[2].* 1) Anatomy, All India Institute of Medical Sciences. New Delhi, India; 2) Dr. R.P.Centre for Ophthalmic Sciences, AIIMS New Delhi, India.

Congenital glaucoma (PCG) is a genetic disease which manifests at birth or in infancy. Congenital glaucoma may be of primary, secondary or associated. Primary congenital glaucoma (PCG) is characterized by buphthalmos, high intraocular pressure (IOP), corneal edema and photophobia. In secondary congenital glaucoma PCG is associated with other ocular syndrome and in associated congenital glaucoma it is associated with other extra-ocular syndrome. In the present study of 51 cases of congenital glaucoma were enrolled, of these 2 cases had Sturge-Weber syndrome and one case had PCG with Down syndrome and Axenfeld-Rieger syndrome. Clinical diagnosis of Sturge-Weber syndrome was made on the bases of presence of facial port-wine stain on the right side of face. Method: Fifty cases of PCG were enrolled in this genetic and ultrastructure study (Scanning electron-microscopy). For cytogenetic analysis, lymphocyte cultures were set and chromosomes were analysed with GTG banding. CYP1B1 gene was screened for six mutations (Termination at 223, Gly61Glu, Pro193Leu, Glu229Lys, Arg368His and Arg390Cys)) by PCR-RFLP. For ultrastructure study, after informed consent surgical trabeculectomy tissues were collected and sent for scanning electron-microscopy. Results: Cytogenetically all cases of congenital glaucoma and both cases of associated congenital glaucoma were normal. Both sample were negative for all six mutations On ultrastructure analysis all tissues showed trabeculardysgenesis. In both cases of Sturge-Weber syndrome the juxta cannalicular connective tissue had nodular thickenings and deposits of amorphus extra-cellular substance. The trabecular meshwork (TM) had compressed sheets of tissues. In certain areas several layers of TM appeared adherent. Conclusion: Trabecular dysgenesis and the presence of excessive connective tissue, nodular thickenings and adherence of several layers of TM may lead to obstruction of aqueous outflow in associated congenital glaucoma cases causing congenital glaucoma.

600/F

Follow-up of italian families who received a prenatal diagnosis of triple X. *V. Viassolo[1], F. Forzano[1], S. Gattone[1], F. Faravelli[1], E. Grosso[2], U. Cavallari[3], E. Folliero[4], D. Quagliarini[4], F. Lalatta[3].* 1) Clinical Genetics Unit, Galliera Hospital, Genova, GE, Italy; 2) Genetica Medica, ASO S.Giovanni Battista, Torino, Italy; 3) Medical Genetics Unit, Department D.B.N. Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milan, Italy; 4) Prenatal Diagnosis Unit, Department D.B.N. Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milan, Italy.

Sex chromosome abnormalities (SCA) are the most frequently occurring chromosomal abnormalities both at prenatal diagnosis and at birth. Approximately 1/400 newborns has SCA and incidence at prenatal diagnosis is even greater (1/250-1/300). Among SCA, 47,XXX, which is expected to have few clinical consequences to the affected individual, requires complex and challenging genetic counselling and outcome is often not fully documented. We have identified 61 couples who required genetic counselling after prenatal diagnosis of 47,XXX in the first or second trimester of pregnancy (period 1998-2005). Among these, 32 accepted to be included in our study. Average maternal age was 38 years. Eight couples performed fetal karyotype for reasons other than maternal age. The protocol included clinical genetic evaluation of the carrier girls comprehensive of auxological measurements and detailed personal history. A questionnaire including an assessment of motor, language, behavioural and cognitive skills was then administered. Preliminary results did not show any serious clinical consequence, with the exception of one individual with global developmental delay and microcephaly. Anthropometric parameters evaluated at birth and at the follow-up age were within the normal range. In six cases we identified a congenital anomaly (club foot, flat foot, lymphangioma, preauricular tag, thyroid agenesis, hip dysplasia). In three cases language delay was identified. The present study might contribute to better understanding the 47,XXX phenotype and to provide a better counselling in prenatal diagnosis settings.

601/F

A New Case of Prenatally Diagnosed Trisomy 12 Mosaicism: Physical and Developmental Follow-up. *Y. Watanabe[1], Y. Kudo[1], K. Egami[1], Y. Koteda[1], K. Suda[1], S. Yano[2], M. Yoshino[1], T. Matsuishi[1].* 1) Pediatrics and Child Health, Kurume University School of Medicine, Kurume , Fukuoka, Japan; 2) Genetics Division, Pediatrics LAC+ USC Medical Center, University of Southern California Los Angeles, CA.

Prenatal diagnosis of trisomy 12 mosaicism poses serious counseling problems since it is a rare condition and outcome of the pregnancy is reportedly quite variable. At least 27 cases have been reported. Approximately half of the cases underwent termination. Five out of 27 cases were reportedly associated with major anomalies including dysmorphic facies, congenital heart defects, and pigmentary dysplasia of the skin. Seven out of 27 cases were reportedly grossly normal at birth. There have been only four reported liveborn cases who were physically and developmentally normal up to age 5 months to 7 years. We report another liveborn case prenatally diagnosed with this condition, showed multiple anomalies and developmental delay at age 4 months. Case report: Patient is a 2 9/12 year-old female born to healthy nonconsanguineous parents. The mother was a 25 year-old G2P1-2Ab0 female who was in good health. The mother was diagnosed with polyhydramnios. The patient was a 37week, 2989g infant born by NSVD. The mother had prenatal studies including amniocentesis which revealed trisomy 12 mosaicism (46, XX[23]/ 47, XX+12 [21]). Chromosome studies were repeated with cord blood and peripheral blood at birth, which revealed normal results. The patient was noted to have congenital nystagmus and ASD at birth. Global developmental delay was noted at 4 months. PE at age 6 months revealed abnormal skin pigmentation covering the bilateral ankle area to the buttock area following the Blaschko's line, a high arched palate, and dysmorphic facies with hypertelorism. Chromosome studies with skin fibroblasts revealed mosaic trisomy 12 (46, XX[58]/ 47, XX+12 [2]).DQ at age 19 months was 49. Conclusion: Reporting postnatal outcome of infants who are prenatally diagnosed with trisomy 12 mosaicism is important to provide more information for better genetic counseling.

602/F

A de novo case of the 17q11.2 microdeletion syndrome presenting with multiple congenital anomalies and brain abnormalities to a consanguinous family: A challenging example in genetic counseling. *R.E. Falk, R. Conway.* Medical Genetics Institute, Cedars-Sinai Medical Ctr., Los Angeles, CA.

We present a 3 year old girl, born to consanguineous Sephardic Jewish parents, who are known carriers of Sandhoff disease, for which prenatal diagnosis was performed with normal results. The child presented initially in the newborn period with dysmorphic facies and multiple congenital anomalies, including complex congenital heart disease, imperforate anus, and unilateral dysplastic kidney likely secondary to vesicoureteral reflux. Her brain MRI was abnormal with frontal lobe hypoplasia and decreased sulcation and the neurologic picture was significant for microcephaly, hypotonia, and seizures. The newborn exam was also significant for three cafe-au-lait macules. The diagnosis of the 17q11.2 deletion syndrome encompassing the NF1 locus was confirmed by comparative genomic hybdridization after routine chromosome analysis was found to be unremarkable. The child remains severely globally developmentally delayed, without any regression. With time additional cafe-au-lait macules and early intertriginous freckling have developed, providing a clinical diagnosis of neurofibromatosis, type 1. Neither parent showed the deletion by confirmatory FISH of the 17q11.2 locus. This case was also significant for a sister of the proband who died in the newborn period of fetal hydrops and congenital heart disease. FISH on liver tissue obtained from autopsy of the sister did not show the 17q11.2 deletion, excluding the possibility of gonadal mosaicism as an explanation for her findings. Our patient has a more severe neurodevelopmental phenotype than is typical for the 17q11.2 deletion syndrome. Moreover, while congenital heart disease is reported as a common feature in other patients with this microdeletion, imperforate anus and urinary tract anomalies are not reported frequently and we believe they may represent new features of the 17q11.2 deletion syndrome. While this case may expand the phenotype of this particular microdeletion, the consanguinity in this case and the family history raises the possibility of a secondary recessive diagnosis and complicates genetic counseling for the family.

603/F

Association of *BDNF* haploinsufficiency with childhood overweight in WAGR Syndrome. *J.C. Han[1], C.M. Menzie[1], E.L. Sanford[1], D.C. Adler-Wailes[1], M.J. Raygada[1], M. Jones[2], F.L. Lacbawan[2], O.M. Rennert[1], J.A. Yanovski[1].* 1) National Institute of Child Health and Human Development, Bethesda MD; 2) National Human Genome Research Institute, Bethesda, MD.

Background: WAGR Syndrome (Wilms tumor, aniridia, genitourinary anomalies, mental retardation) is caused by contiguous gene deletions at 11p13. Haploinsufficiency of *WT1* and *PAX6* accounts for the main features, but deletion of other genes may cause additional features, such as hyperphagia and obesity, which are observed in a subset of patients. Brain-derived neurotrophic factor (*BDNF*), located 4 Mb telomeric to *PAX6*, has been shown in animals to be important in energy homeostasis. We hypothesized that the obesity sub-phenotype in WAGR is attributable to *BDNF* haploinsufficiency. **Methods**: 28 patients with WAGR (age 11.8±7.4y) had deletion mapping by microarray oligonucleotide CGH, with 57k probes spanning 11p (average resolution 400 bp) and 43k probes genome-wide. Confirmatory genotyping used 30 microsatellite markers spanning 11p12-14. Fasting serum BDNF concentration was measured by ELISA. **Results**: 11p deletions were 1.0 to 26.5 Mb in size, and 61% had a deletion involving *BDNF* (*BDNF+/-*). *BDNF+/-* had significantly higher BMI Z-scores at age 5y (1.97±1.10 vs. 0.11±1.82, p=0.008) and 10y (2.18±0.33 vs. 0.87±1.20, p=0.043) compared to those without *BDNF* deletion (*BDNF+/+*). These differences remained significant after adjusting for parental BMI (5y: p=0.002, 10y: p=0.007). Childhood overweight (BMI>95th%ile by 10y) occurred in all *BDNF+/-* subjects, but in only 1 *BDNF+/+* subject (p<0.0001). Consistent with haploinsufficiency, *BDNF+/-* had approximately 50% lower serum BDNF concentration compared to *BDNF+/+* (13.9±5.4 vs. 29.3±14.5 ng/mL, p=0.006). **Conclusions:** *BDNF* haploinsufficiency was associated with lower serum BDNF and higher BMI Z-score in WAGR patients, suggesting that BDNF is important in human energy homeostasis and that WAGR patients with deletions involving *BDNF* will develop the obesity sub-phenotype. Further studies are needed to establish other genotype-phenotype correlations in WAGR Syndrome, and high-resolution CGH may be beneficial in defining molecular breakpoints to guide clinical care.

604/F

Decreased hospitalization and recurrent infections in a child with 22q11 microdeletion syndrome after start of triweekly intravenous gamma-globulin therapy. *A. Khan[1], P. Dent[2].* 1) Dept Medical Genetics and Pediatrics, Alberta Children's Hospital, University of Calgary, Calgary, AB, Canada; 2) Dept Pediatrics, McMaster Children's Hospital, McMaster University, Hamilton, ON, Canada.

Humoral immunodeficiency may contribute to frequent infections in 22q11 microdeletion syndrome. We describe a girl with 22q11 microdeletion syndrome who, after repair of her truncus arteriosus, had prolonged admissions with bacterial infections and a persistent state of poor health. With the repetitive use of antibiotics, multi-drug resistant organisms developed. Because her immunoglobulin levels were consistently in the low normal range despite her frequent infections, we started intravenous gammaglobulin (IVIG) infusions at 9 months of age. Infusions were initially given every 4 weeks at a dose of 900 mg/kg. To achieve pre-infusion IgG levels of >6 g/L at 14 months the frequency was increased to triweekly and the dose to 1800 mg/kg. Prior to the start of triweekly IVIG infusions, she had a total of 339 inpatient hospital days at 821 days of age. She had 8 respiratory, 2 gastrointestinal and 2 gastrostomy site infections. After the start of triweekly IVIG, she had 3 respiratory infections over 302 days and was hospitalized 2 days for pneumonia. After starting IVIG therapy, she made developmental gains and had improved growth commensurate with decreased hospitalization. She had a modest reduction in T cell numbers as is seen in most 22q11 patients. We did not find a correlation between the frequency of infections or hospitalizations with reference to white blood cell populations: total leukocyte count, absolute neutrophils, absolute lymphocytes, and T-cell subsets. The need for IVIG replacement therapy at these doses suggests hypercatabolism of IgG may have contributed to her susceptibility to infection. We conclude that defects in humoral immunity in 22q11 microdeletion syndrome can exist despite normal immunoglobulin levels. IVIG infusions can be one option in cases with difficult to control infections, however more detailed studies are required to determine whether there are any benefits overall in 22q11 microdeletion.

605/F

Novel Chromosome 20p12.3 Deletion Associated with Learning Difficulties and Dysmorphic Features in a Mother and Son. *J.V. Thakuria[1], S. Waisbren[2], G.F. Cox[1, 3].* 1) Div of Genetics, Children's Hospital, Boston, MA; 2) Dept of Psychiatry, Children's Hospital, Boston, MA; 3) Genzyme Corp, Cambridge, MA.

A 5 year old boy presented with a history of developmental delay, mild dysmorphic features, Wolff-Parkinson-White (WPW) syndrome, and hypoglycemic episodes. He was a former 8 lb term boy born by vaginal delivery to a 34 year old G4P2SAB2 mother following an uncomplicated pregnancy, labor, and delivery. Four hours after birth, he developed supraventricular tachycardia and was diagnosed with WPW, now controlled with sotalol. He has had 3 episodes of fasting-induced hypoglycemia, one associated with a seizure. EEG and a metabolic evaluation have been unrevealing. Brain MRI showed ventriculomegaly with benign external hydrocephalus. Dysmorphic features included macrocephaly, frontal upsweep, downslanting palpebral fissures, epicanthal folds, hypertelorism, long philtrum, microstomia, small ears with thick helices, and broad thumbs and toes with persistent fetal pads, features reminiscent of FG syndrome. He has moderate cognitive, gross motor, and speech delays as well as hyperactivity and sensory issues. Genetic evaluation included an apparently normal 46,XY male, fragile X testing, and PTEN mutation analysis. Chromosomal microarray testing (Baylor Version 6.1) revealed a microdeletion at 20p12.3, and subsequent high resolution karyotype and fine mapping of the breakpoints revealed a 2.33 Mb deletion. His mother carries the same microdeletion, but not his father or maternal grandparents. She also has a history of learning difficulties, similar facial features, and heart palpitations but without WPW by ECG and Holter monitoring. Further evaluations of WPW by stress testing and neuropsychological testing are being considered. In summary, we present a two-generation family with a de novo and apparently novel microdeletion that appears to be associated with learning difficulties, dysmorphic facies, and possibly WPW and hypoglycemic episodes. Recently a second case of WPW associated with a smaller deletion in the same region has been identified (Lalani, et al, this meeting). Further molecular analysis of this region may yield one or more genes responsible for these findings.

606/F

Turner syndrome and trisomy 14 chromosomal mosaicism in a patient: First reported case. *M. Díaz-Rodríguez[1,2], L.E. Becerra-Solano[1,2], L.I. Arnaud-López[1,2], J.M. Mantilla-Capacho[1,2], M. Ortíz-Aranda[1,2], A.I. Vasquez[1], J.A. Nastasi-Catanese[1,2,3], L.E. Figuera[1].* 1) División de Genética, CIBO-IMSS, Guadalajara, Jalisco, México; 2) Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México; 3) Universidad de Oriente, Núcleo Bolívar, Unidad de Genética, Ciudad Bolívar, Bolívar, Venezuela.

Turner syndrome (TS) has an occurrence about 1:2500 to 1:3000 in live-born girls. Around 50% of them have a X monosomy (45,X), and one third have a mosaicism for 45,X. On the other hand, Trisomy 14 is a rare aneuploidy characterized by growth and psychomotor retardation, microphthalmia, broad nasal bridge, wide mouth, asymmetries in face and limbs, and cutaneous pigmentary abnormalities. It has been proposed, for both aneuploidies, that pure lines are lethal. We present a 26 year-old female with a karyotype mos45,X[45]/47XX,+14[5]. Her clinical features were: short stature, small and deep eyes with convergent strabismus, short downward slanting palpebral fissures, broad nasal bridge, mouth with turned down corners, short and wide neck, multiple nevi, swirly hyperpigmentation of the skin, wide thorax, mammary tanner stage II-III, cubitus valgus, limitation on elbows movements, hypertrichosis on forearms, bilateral short fifth metacarpal, shortening in the fourth metatarsus, edema in feet, and body asymmetry (right hemihyperplasia). The patient showed spontaneous menarche at 15 year-old and secondary sexual development. X-ray studies: diminished bone density, lumbar-sacral scoliosis and lordosis, asymmetry and shortness in the lateral segment of vertebral body in vertebras T5, T7 and L2, asymmetric hip, dysplasic right femoral head, dislocation of patella and lateral deviation in the fourth metatarsal. Hormonal profiles (FSH, LH, prolactin and thyroid hormones) were normal. Because there was a overlapping in clinical manifestations of TS and trisomy 14, the determination of the cell line that had the most influence on the phenotype was not possible. We propose for this mosaicism, resulting from a double event, each cell line is rescuing the other one.

607/F

Genetic and ultrastructural study in congenital glaucoma case with Down syndrome and Axenfeld-Rieger syndrome. *M. Tanwar[1], D. Pathak[1], R. Sihota[2], T. Das[1], T. Dada[2], V. Gupta[2], R. Dada[1].* 1) Anatomy, All India Institue of Medical Sciences, New delhi, India 110029; 2) Dr R.P. Centre for Ophthalmic Sciences, New delhi, India- 110029.

Down syndrome(DS) is a constellation of clinical findings characterized by mental and motor retardation , simian crease, hyperflexiblity, oblique palpebral fissures and enlargement of tongue. Ocular and adnexal findings are quiet common in these cases. In the present study 25 cases of congenital glaucoma were included. One of these cases had Axenfeld-Rieger syndrome(ARS) along with congenital glaucoma and Down syndrome. Materials: 25 cases of congenital glaucoma were enrolled in this study. One case of congenital glaucoma had Down syndrome and ARS. Method: To identify any karyotypic abnormalities, lymphocyte culture were set and 25 well spread G-banded metaphases were analyzed. CYP1B1 gene was screened for six mutations (Termination at 223, Gly61Glu, Pro193Leu, Glu229Lys, Arg368His and Arg390Cys)) by PCR-RFLP. After informed consent surgical trabeculectomy tissues were collected and sent for scanning electron microscopy to identify structural changes in trabecular meshwork.For EM study specimens were fixed in glutraldehyde fixative for 12 hrs and then post fixed in the osmium tetra oxide at 1% for 2 hrs.and processed for Scanning electron microscopy. Result: On cytogenetic analysis 47,XX+21 chromosomal complement was confirmed. This sample was negative for all six CYP1B1 mutations. Ultrastructural study revealed a very compact trabecular meshwork with marked narrowing of intratrabecular spaces. Also the intratrabecular spaces and Schlemn's canal were obliterated by endothelial cells and connective tissue.Conclusion: ARS also known as anterior chamber cleavage syndrome and characterized by mesodermal dysgenesis of cornea and iris. Obliteration of highly compact trabecular spaces and Schlemm's canal by endothelial cells may obstruct the normal aqueous out flow for glaucoma in this case with ARS. Several ocular anomalies are associated with Down syndrome but its association with PCG and ARS has not been documented. Thus it is important to analyze more cases of associated congenital glaucoma.

608/F

Changes in Phenotype of Bloom's Syndrome with New Manifestations in Adult Individuals. *E. Passarge, H. Löser.* Human Genetics, Inst Humangen, Univ Essen, Essen, Germany.

Bloom's syndrome results from autosomal recessive mutations in the BLM gene located on human chromosome 15 at 15q26.1, encoding a DNA helicase with homology to RecQ in E. coli (MIM 210900). Its phenotype includes (i) pre- and postnatal growth retardation, (ii) facial features with dolichocephaly and a narrow face, (iii) light-sensitive facial telangiectasia in most patients, (iv) manifestations of genomic instability as revealed by a 10-fold increase of spontaneous sister chromatid exchanges, breaks and homologous exchanges between chromosomes, and an increased rate of somatic mutations. Affected individuals develop similar types of cancer as in the population, but at a much younger age (about 1 in 4).

We report data of a longterm study of the natural history of 15 individuals with Bloom's syndrome observed during the past 38 years in Germany. We found that the phenotype in adult individuals becomes less distinctive with age than it is in children. In spite of persistent feeding difficulties, such as lack of appetite or regurgitation, adult individuals tend to gain weight. A new finding is development of diabetes mellitus type 1 or type 2. This has been observed in 27 of 117 patients (23%) of individuals in the Bloom's Syndrome Registry (J. German, M. Sanz, E. Passarge, unpublished data). The skin manifestations tend to improve with age. We diagnosed Bloom's syndrome prenatally in a family known to be at risk. When the parents were informed about this diagnosis they changed their mind and decided to carry the pregnancy to term. Retarded growth was evident during all stage of the pregnancy and the affected infant weighed only 2000 g at birth at 40 weeks of gestation. However, he lacked the typical appearance of Bloom's syndrome. We conclude that the phenotype of Bloom's syndrome is wider than recorded previously. It remains to be seen whether the molecular type of mutation present in an individual influences the phenotype.

Acknowledgement: We thank J. German, M. Sanz, and N.A. Ellis, New York, for cooperation and mutational analysis.

609/F

Three-year-old girl with Juvenile Huntington Disease. *S. Sakazume[1,3], H. Ohashi[1], S. Yoshinari[2], T. Ishii[4].* 1) Division of Clinical genetics, Saitama childrens medical center, Saitama, Saitama, Japan; 2) Division of Neurology, Saitama childrens medical center, Japan; 3) Division of Clinical genetics, Gunma childrens medical center, Shibukawa, Gunma, Japan; 4) Division of Clinical genetics, Chiba university, Chiba, Japan.

Huntington disease is neuron degenerative disease showing involuntary movement and change in character, commonly-noted in the scene of genetic counseling in adult. The causative mutation is 5' CAG repeat expansions of HD gene, also paternal anticipations are observed in some cases. Here we describes relatively rare early childhood onset HD. The patient is a girl born with term uneventful delivery. During infancy, her motor and mental development was normal. On her third year of life, she could walk stably and run, and speak meaningful words and many short sentences. Also her growth was normal. At the age of 2 years and 11 months, her care giver noticed ataxic gait and difficulty in speech. At the age of 3 years and 6months, she could not speak any meaningful words. Around the same time, convulsive seizure started frequently and it was diagnosed as epilepsy by EEG. The convulsion was controlled by Carbamazepine. Brain MRI showed no abnormal findings at the time. Her CAG repeat of HD gene was remarkably expanded until 160 repeat. Her mother was also diagnosed as a patient of HD just after her delivery. The mothers CAG expansion was about 60 repeat. In this family, patients were identified at least in four generations. This patient is a one of the youngest patient ever diagnosed and maternal CAG expansion is characteristic in this case.

610/F

Familial supernumerary teeth - clinical variability and genetic heterogeneity. *C. Albu, R. Purcarea, D.F. Albu, E. Severin.* Dept of Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania.

Background: Supernumerary teeth are a developmental anomaly characterized by teeth in excess of the normal number. Extra teeth are relatively common in the permanent dentition. Heredity is often involved, but specific genes are not yet known. Clinical variation included the number, location, direction of eruption and morphologically type of the supernumerary teeth. Objectives: To analyze the inheritance pattern of familial supernumerary teeth; to describe the clinical phenotype and supernumerary teeth pattern in families; to identify the genetic cause of clinical variability of anterior maxillary supernumerary teeth as observed in our cases. Patients and Methods: A group of 26 Caucasian patients (15 males and 11 females) with isolated supernumerary teeth located in premaxilla were investigated; the diagnosis of supernumerary teeth has been made by oral and radiographic examinations; information about families' medical history of supernumerary teeth was collected. Results: The upper incisor region was the common site for supernumerary teeth. The most frequent permanent extra tooth was mesiodens followed by the supplemental upper lateral incisor. Familial inheritance occurred and involved two or three generations. The inheritance pattern of supernumerary teeth was different and no simple mode of transmission was found.The affected members within the same family often exhibited variability in clinical presentation. Familial predisposition to increase the number of teeth in the relatives of those affected was noted. Conclusions: Supernumerary teeth are an inherited developmental anomaly in families. Various clinical phenotypes of an isolated extra tooth are determined by mutations in different genes. Early diagnosis of supernumerary teeth prevents the clinical complications.

611/F

Maternal fever and congenital heart defects: findings from the National Birth Defects Prevention Study. *L.D. Botto[1], M. Bishop Stone[2], E. Lammer[3], M.L. Browne[4], M.L. Feldkamp[1], G.M. Shaw[5], the National Birth Defects Prevention Study.* 1) Pediatrics/Medical Genetics, University of Utah, Salt Lake City, UT; 2) Utah Birth Defect Network and University of Utah, Salt Lake City, UT; 3) Children's Hospital, Oakland, CA; 4) New York State Department of Health, Troy, NY; 5) California Birth Defects Monitoring Program, Berkeley, CA.

Maternal fever in early pregnancy has been associated with an increased risk for heart defects in some studies. However, it is unclear whether such risk varies by type of defect and febrile illness. To examine these aspects we used data from the National Birth Defects Prevention Study (NBDPS). NBDPS is an ongoing population-based study of birth defects in the United States. Cases are ascertained through population-based registries, and controls are selected randomly from births in the same areas and birth years. Detailed information on fever is obtained through structured maternal interviews. Pediatric cardiologists classified cardiac phenotypes. The study includes 5,446 case-infants with major heart defects and 5,008 unaffected controls with birth years from 1997 through 2003. To help disentangle fever from infection, we defined three main first-trimester exposure groups: mothers with a reported fever, mothers with a reported infection but no fever, and mothers with neither (reference group). We excluded mothers with diabetes, and we adjusted analyses for maternal demographics, lifestyle factors, vitamin use, and chronic illness. Febrile illness, rather than illness alone, was associated with a moderately increased risk for selected defects, with some variation by source of fever. With fever from respiratory illness, the estimated relative risk for heterotaxy was 1.9 (95% confidence interval 1.1 to 3.2) and for aortic stenosis was 1.8 (1.0 to 3.3); with fever due to urinary tract and pelvic infections, the risk for right-sided obstructive defects was 4.1 (1.8 to 9.5); with fever from other sources, the risk for hypoplastic left heart syndrome was 5.0 (1.4 to 18.0) and for conotruncal defects was 3.4 (1.4 to 8.1). If such associations are causal, febrile illness may be a cardiac teratogen with some specificity by underlying infection.

612/F

Van Den Ende-Gupta syndrome: Expansion of the phenotype and confirmation of autosomal recessive inheritance. *C.W. Carr[1], J. Zhang[2], J.D. Carron[3], R.S. Lachman[4], J.M. Graham[5], N.A. Kramer[5], O.A. Abdul-Rahman[1].* 1) Preventive Medicine, University of Mississippi Medical Center, Jackson, MS; 2) Department of Neurosurgery, University of Mississippi Medical Center, Jackson, MS; 3) Department of Otolaryngology and Communicative Sciences, University of Mississippi Medical Center, Jackson, MS; 4) International Skeletal Dysplasia Registry, Cedars-Sinai Medical Center, Los Angeles, CA; 5) Department of Medical Genetics, Cedars-Sinai Medical Center, University of California, Los Angeles, CA.

Van den Ende-Gupta syndrome (VDEGS) is a multiple congenital anomaly syndrome characterized by blepharophimosis, arachnodactyly, and congenital contractures in the absence of psychomotor retardation. We report two African-American sisters born to non-consanguineous parents who have been diagnosed with VDEGS based on the presence of blepharophimosis, crumpled ears, arachnodactyly, and congenital contractures. The older sibling had an unusual laryngeal malformation characterized by globular cuneiform cartilages, short aryepiglottic folds, a tightly coiled epiglottis, and laryngomalacia. The second child born to this couple was examined in the newborn period and represents the first neonatal diagnosis of VDEGS, suggesting that VDEGS is recognizable at birth. The younger sibling is also experiencing upper airway obstruction and an otolaryngological evaluation is underway. This family provides evidence for expanding the phenotype to include laryngeal anomalies and support the concept of autosomal recessive inheritance. We are currently working to identify the genetic basis for this disorder using RNA expression profiling of skin fibroblasts and linkage studies. We suspect that based on a phenotype overlapping that of Beals syndrome, the gene involved likely plays a role in the fibrillin pathway. Additionally, the recessive nature of VDEGS suggests that the gene may function as an enzyme involved in the modification of fibrillin or other connective tissue matrix proteins.

613/F

Steroid 21-hydroxylase gene analysis in a cohort of Indian patients with classical Congenital Adrenal Hyperplasia. *S. Dubey[1], S. Rao[2], C. Saravanan[1], A. Maitra[1].* 1) National Institute for Research in Reproductive Health, JM Street, Parel Mumbai, India; 2) Bai Jerbai Wadia Children's Hodpital, Parel, Mumbai, India.

Steroid 21-hydroxylase enzyme deficiency is the most common cause of congenital adrenal hyperplasia. It is an autosomal recessive disorder with a wide range of clinical manifestations ranging from severe to mild form. The disease is attributed to mutations in the 21-hydroxylase gene (CYP21), encoding 21-hydroxylase enzyme. A total of 14 common mutations and 29 polymorphisms have been identified in CAH patients, apart from about 100 rare mutations specific to different populations. Data in Indian population is however sparse. Present study has been undertaken with the specific objective to identify mutations in the 21-hydroxylase gene in Indian cases with classical CAH. The approach involves the selective amplification of active CYP21 gene followed by multi-step sequencing and identification of the variant by automated analysis against the reference sequence. Forty-five index cases along with their family members were enrolled with the aim of determining frequency of different mutations in a cohort of Indian patients. Seven kinds of mutations were found in the 45 index cases analyzed. These were; intron-2 splice (28.8%), Q318X (20%), gene deletion (17.7%), I172N (11%), R356W (4.4%), cluster of mutations (N235E, I236N, V237E, M239) in exon 6 (4.4%) and 306insT (4.4%). More than 15% of patients were compound heterozygous. Two novel mutations, F305V (GeneBank Accession No. EF563986) and an insertion of 9 bases in exon 2 (codon 70) were found in two of these cases. Good genotype phenotype correlation was also observed in our study. This is the first report of screening of CYP21 gene by sequencing in the Indian population. Intron-2 Splice and Q318X are the most frequent mutations found in our CAH patients.

614/F

An unusual duplication 3q syndrome phenotype in a patient with der(22)t(3;22)(q26.3;p13). *R. Habibian[1], A. Hajianpour[1], L. Dong[1], R. Tefft[2], L.M. Randolph[3], L. Drugan[1], M.J. Hajianpour[4].* 1) Cytogenetics Laboratory, Genzyme Genetics, Monrovia, CA; 2) White Memorial Hospital, LA, CA; 3) Children Hospital of Los Angeles, CA; 4) Gene Trek Genetics, Burbank, CA.

We present a newborn baby boy with dup(3q) syndrome and phenotypic features similar to Cornelia de Lange syndrome (CdLS). He was born at 36 weeks of gestation to a 23-year-old, G2, P1 mother by C-section delivery due to fetal distress. Gestational history is remarkable for polyhydramnios and IUGR. Clinical findings included microcephaly, dysplastic right ear, with left anotia and atresia of left ear canal, bushy eyebrows, prominent maxilla with flat face, long philtrum, and depressed nasal bridge with anteverted nares, thin vermilion border of upper lip, downturned corners of mouth, micrognathia and bifid uvula. He had hypertrichosis, VSD, ASD, septal hypertrophy, mild hepatomegaly, micropenis with hypospadias and cryptorchidism, and clinodactyly of fifth fingers. His thumbs and great toes were broadened at the tips, and his nails were narrow and hyperconvex. He later developed hypoglycemia, hepatosplenomegaly, ascites and thrombocytopenia. These findings are also seen in neonatal hemochromatosis, mannosidosis and transaldolase deficiency. The latter may present with facial features similar to CdLS. An extensive metabolic workup was performed. Mannosidosis was ruled out. His ferritin level was 3300 (normal 22-322) and his iron level was 164 (normal 40-100). Pseudomonas grew from his endotracheal tube later, and he had Klebsiella sepsis. Gastrografin barium enema showed a possible microcolon. The baby died before completion of work-up for the above mentioned conditions. The chromosome analysis revealed 46,XY,der(22)t(3;22)(q26.3;p13) indicating a partial trisomy for 3q26.3 to 3qter. Duplication 3q syndrome shows phenotypic overlap with Cornelia de Lange syndrome (CdLS), which has been mapped to 5p13.1. However, hepatosplenomegaly, ascites, hypoglycemia and thrombocytopenia are not reported in duplication 3q syndrome. His high ferritin and iron studies indicated possible neonatal hemochromatosis, but liver biopsy could not be completed due to his precarious condition and then death.

615/F

Autosomal Dominant Duodenal Atresia - Report of two families. *J. Jessen[1], K. Chong[1,2], D. Chitayat[1,2].* 1) Mount Sinai Hospital, Dept. Prenatal Diagnosis and Medical Genetics; 2) Hospital for Sick Children, Division of Clinical and Metabolic Genetics, Toronto, Ontario, Canada.

Duodenal atresia (DA) is the result of a lack of epithelial apoptosis and recanalization of the duodenum at 8-10 weeks' gestation. Recent mouse model research suggests that some forms of atresia may be hereditary and result from deregulation of proliferation and apoptosis of the developing intestine through the fibroblast growth factor pathways indicating a critical role for the Fgf10 -Fgfr2b signaling pathway (Fairbanks, 2006). We report on two families with parent and child affected with duodenal atresia/stenosis (DS). Case 1: The couple presented with fetal ultrasound findings of "double bubble" at 27 weeks gestation, suggestive of DA. The mother was of Polish/Ukrainian and the father of Scottish/English descent. The couple was non-consanguineous. The father was born with duodenal stenosis (DS), which was confirmed during surgery shortly after birth. The pregnancy was conceived via IVF and was otherwise uncomplicated. Amniocentesis was done at 36 weeks gestation for polyhydramnios management and the karyotype was 47, XXY. The baby was born at 41 weeks gestation and the diagnosis of DA was confirmed at the corrective surgery. Case 2: The couple was seen regarding their first pregnancy which was complicated with fetal ultrasound findings of "double bubble". Amniocentesis was declined. The pregnacy was uncomplicated and the diagnosis of DA was confirmed at corrective surgery shortly after birth. The mother was of Italian/Polish/Ukrainian and the father of Scottish/English descent. The couple was non-consanguineous. The mother was born with DA corrected surgically and is otherwise healthy. Their second pregnancy was uncomplicated and the newborn is well. DA is a rare condition and has an incidence of 1:5,000-10,000 live births. Most cases are sporadic and 1/3 of the cases are associated with trisomy 21. Investigations of familial cases of duodenal atresia suggest an autosomal recessive inheritance in these individuals (Fonkalsrud, 1969; Mishalany, 1971). To the best of our knowledge these are the first two families suggestive of autosomal dominant inheritance.

616/F

Cerebro-facio-renal-digital-glandular syndrome (CFRDG), a "new" multiple malformation syndrome. *R. Lebel[1], C. Nichols[2], B. DuPont[1], T. Wood[1], J. Avery[1], P. Broome[1], M. Hutchinson[2], C. Shipley[2].* 1) Greenwood Genetic Ctr, Greenwood, SC; 2) Palmetto Richland Hospital, Columbia, SC.

The 37 week infant was born vaginally to a 28 year old G2P1 Rh+ Caucasian without reported exposure to teratogens, no consanguinity. Ultrasound showed hyperechoic cystic kidneys, suspected limb anomalies, Dandy-Walker malformation; oligohydramnios rendering examination incomplete. Prenatal impression favored Meckel-Gruber syndrome as most likely. Amniocentesis revealed karyotype 46,XX (normal for a female). Family history was negative for similar anomalies. Demise followed rapidly after delivery. Anthropometric measurements revealed macrocephaly, telecanthus, small hands and feet. Radiographs revealed hypoplastic facial skeleton, short upper limb tubular bones, normal length femora. Fontanels were large, ears low-set, face flattened. There was a high-arched palate and two large tongue polyps (microscopically: salivary glands). Clitoris was hypoplastic. There was bilateral equinovarus and postaxial polydactyly of all four limbs; both feet had syndactyly 5-6. Dandy-Walker malformation, hypoplastic vermis cerebelli and encephalomegaly were noted. A left-anterior nuchal mass was found (microscopically: mixed thyroid and thymus). Lungs were hypoplastic; there was cardiomegaly, hepatomegaly, and thymomegaly. Kidneys and ovaries had multiple cysts; there was medullary sponge kidney. Fibroblasts confirmed amniocytes: 46,XX. Levels of eight lysosomal enzymes were in the normal ranges (no apparent storage disease). The dysmorphology literature brought us to consider the hydrolethalus syndrome, the Mohr-Majewski syndrome, the Beemer-Langer syndrome, Oral-facial-digital syndrome type II, the Meckel-Gruber syndrome, and the visceroskeletal syndrome of Moerman et al (1985). All but the last (which has very few cases reported) are well established autosomal recessives. All had major overlap of important features, but also significant lack of concordance, so that none appears to accommodate this case. We propose it as a "new" entity: the cerebro-facio-renal-digital-glandular syndrome (CFRDG).

617/F

The Einstein/Montefiore Spina Bifida Clinic at Blythedale: A 20 Year Perspective. *R. Marion[1,2], L. Schendel[2], L. Seimon[1,2], J. Goodrich[1,2], S. Kogan[1,2], R. Borkow[2].* 1) Dept Pediatrics, Childrens' Hosp Montefiore, Bronx, NY; 2) Blythedale Children's Hospital, Valhalla, NY.

In February 1987, the Einstein/Montefiore Spina Bifida Clinic moved from the Bronx to Blythedale Children's Hospital in Valhalla, NY. Since that time, the clinic has provided care for 238 patients (pts)with myelomeningocele and related congenital anomalies of the spine. Recently, after 20 years of continuous service, we analyzed data from our population, evaluating: (1) number of pts entering the clinic each year; (2) frequency and causes of mortality; (3) frequency of allergy to Latex; (4) incidence of secondary medical complications, as well as other paramenters. Analysis of these data revealed that (1) since 1998, when enriched of food with Folic Acid began, the number of pts entering the clinic dropped from an average of 9.3/yr to 2.4/yr; (2) over 20 years, 12 pts. died (5.0 percent), significantly below national figures published in the 1980s; although the majority of deaths were due to complications related directly to the underlying disease, pts died from other causes, including child abuse, anaphylaxis due to Latex exposure and unrelated infections; (3) incidence of Latex allergy rose from less than 10 percent in 1987 to virtually 100 percent in 2007; and (4) as our population ages, the 3 most common secondary complications include obesity, chronic decubitus ulcers, and depression.

Over the 20 years that our clinic has functioned at Blythedale, management of pts with Spina Bifida has changed. Folic acid fortication has decreased the number of infants entering our clinic by 75 percent; coupled with the reduction in mortality, this has led to an increase in the average age of pts, making us a center that cares for older pts; universal allergy to Latex has altered the way we approach and counsel pts., as well as the way we provide equipment; and the presence of secondary complications noted above requires that some members of the multidisciplinary team, specifically nutritionist and psychiatrist, must play more of an active role in the care of older pts.

618/F

Lipodystrophy and multiple congenital anomalies : a new syndrome? *P. Sarda[1], J. Puechberty[1], L. Pinson[1], C. Coubes[1], G. Lefort[1], P. Blanchet[1], L. Van Maldergem[2].* 1) Dept Medical Genetics, Arnaud de Villeneuve Hosp, Montpellier Herault, France; 2) Génétique, Hôpital Henri Mondor Creteil, France.

Lipodystrophies represent a group of diseases characterized by abnormal body fat. Berardinelli-Seip congenital lipodystrophy is a very rare disorder in which congenital generalized lipoatrophy is associated with hepatomegaly, hypertriglyceridemia and acromegaloid features. This disorder follows autosomal recessive inheritance. Two loci have been identified : BSCL1 in 9q34 and BSCL2 in 11q13. At least a third locus must exist. We present what is probably a new syndrome with generalized lipodystrophy in a 14-year-old girl. She was the product of a term pregnancy complicated by intra uterin growth retardation. She presented a small omphalocele (surgically corrected at 6 days), generalized lipoatrophy and dysmorphic traits. Psychomotor development was normal. No cerebral, cardiac, abdominal or skeletal anomalies were noted. At age 6, she underwent surgery for volvulus of the small intestine. Puberty occurred normally. At age 14, the child presented normal weight and height but OFC was at -2.5 SD. Clinically there was severe generalized lipoatrophy. Cardiac examination revealed hyperlaxity of atrio-ventricular valves with mild mitral and pulmonary insufficiency. Skeletal anomalies included arachnodactyly and joint mobility restriction. The child also presented fine skin and cutaneous syndactylies of the fingers. She had dysmorphic traits with brachycephaly, acromegaloid face, dysplastic low-set ears, short philtrum. Intellectual level was normal. Triglyceride serum concentrations were variable. Thoraco-abdomino-pelvic MRI was performed to evaluate fat residue in the event of facial plastic surgery. Images revealed no cutaneous fat in the anterior and lateral planes of the body but a small layer of dorsal fat. Abdominal perivisceral fat was normal. No mutation was found for AGAPT2, BSCL2 and Cav1 genes. Our patient presents a particular lipoatrophic MCA syndrome without mental retardation which is probably a new lipodystrophic syndrome possibly due to a chromosome microanomaly.

619/F

Congenital absence of teeth in families. *E. Severin, C. Albu, D.F. Albu, R. Purcarea.* Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania.

Background - Congenital absence of permanent teeth is a genetic condition which tends to run in families because relatives share genetic material. Objectives - to analyze the pattern of familial hypodontia and to find evidence that mutation of PAX9 gene may cause some family members to be at risk for hypodontia. Setting and sample population - the study was conducted in two families with non-syndromic hypodontia in successive generations. Methods - The diagnosis of hypodontia has been made by clinical and radiographic examinations. A pedigree analysis was performed to determine the pattern of inheritance of the hypodontia phenotype. For DNA analysis, blood samples were collected and isolated DNA was analyzed by PCR. Results - The parents and their siblings did not share similar pattern of hypodontia with regard to the tooth class, region, symmetry and number of teeth involved. In the families, hypodontia followed a similar pattern of inheritance: autosomal-dominant with variable expression and reduce penetrance. Our study failed to confirm the association between the presence of a certain mutation in PAX9 and the resulting pattern of hypodontia as previously suggested some studies. Conclusions - The individual analyses of cases showed great diversities in the hypodontia pattern and degree of severity. Parents, sibs and other family members showed different clinical features of congenital absence of teeth. The cause of severe hypodontia cases - missing both frontal and back teeth - is not explained by PAX9 mutations.

620/F

A newly recognized overgrowth syndrome distinct from Proteus syndrome. *J.C. Sapp[1], R.D. Clark[2], J.T. Turner[1], J. van de Kamp[3], F. van Dijk[3], R.B. Lowry[4], L.G. Biesecker[1].* 1) National Human Genome Research Institute, Bethesda, MD; 2) Loma Linda University Medical Center, Loma Linda, CA; 3) VU Medisch Centrum, Amsterdam, Netherlands; 4) Alberta Children's Hospital, Calgary, Canada.

Syndromes with overgrowth as a major manifestation are clinically and phenotypically heterogeneous and incompletely defined. Proper clinical delineation of these syndromes is important both for research and for clinical care. We present here a series of eight patients who were previously diagnosed with Proteus syndrome but who do not meet published diagnostic criteria for this disorder and whose natural history is distinct. This newly delineated phenotype comprises progressive, complex, and mixed truncal vascular malformations, dysregulated adipose tissue, varying degrees of scoliosis, and enlarged, yet not distorted, bony structures without progressive bony overgrowth. Similarities between these patients' phenotype and that of Proteus syndrome include vascular malformations (low flow blood vessels and lymphatics), linear pigmented nevi, and excess fat deposition or lipomas. Differences between this newly described entity and Proteus syndrome are that the former includes non-progressive, non-distorting overgrowth that is generally congenital and of the ballooning type, and a stereotypical distribution of lesions that includes complex truncal vascular malformations, bilateral foot overgrowth, and lack of cerebriform connective tissue nevi. We conclude that the patients presented here have a phenotype that is both recognizable and distinct from Proteus syndrome and other overgrowth conditions.

621/F

Klinefelter's Syndrome with a chromosomal aberration of 47 XXY. *I. Yoshiuchi[1,2].* 1) Medicine, Yoshiuchi Medical Diabetes Institute, Japan; 2) Medicine, Saiseikai Kanagawa Prefecture Hospital, Kanagawa, Japan.

Diabetes mellitus is a complex disease characterized by insulin resistance and a failure of the pancreatic beta-cell. Klinefelter's syndrome is the most common sex chromosomal aberration of human male infertility. The incidence of diabetes mellitus in Klinefelter's syndrome is generally high. Most cases of diabetes mellitus in this syndrome show insulin resistance state. A 30-year-old man with diabetes has a chromosomal aberration of 47 XXY and abnormal sex hormonal findings, and was diagnosed as Klinefelter's syndrome. His insulin secretion was well preserved in an oral glucose tolerance test and the data of urinary C-peptide, but he required 120 units of insulin per day. He showed severe insulin resistance, severe hyperlipidemia and mild obesity. We also examined the relationship between inflammation markers and glucose metabolism in him. We observed that inflammatory states could contribute to the glucose metabolism and insulin resistance in a case of Klinefelter's syndrome with 47 XXY.

622/F

Homozygous CHRNG mutation in multiple pterygium syndrome, Escobar variant with CNS malformations. *A.I. Dagli[1], A. Michalk[2], K. Hoffmann[2], R.T. Zori[1].* 1) Division of Genetics,-Department of Pediatrics, University of Florida, Gainesville; 2) Institute of Medical Genetics, Charité Berlin, Humboldt University 13353 Berlin, Germany.

Multiple pterygium syndrome, Escobar variant is a non lethal form characterized by webbing of the neck and joints and arthrogryposis multiplex. This syndrome is an autosomal recessive disorder caused by mutations in the CHRNG gene that codes for the γ subunit of the acetylcholine receptor. The γ subunit is present before the 33rd week of life and is important for neuromuscular development (Hoffman et al., Am J Hum Genet, 2006).

We report dizygotic twin boys with clinical features of Escobar syndrome. They were born to healthy, non-consanguineous parents. Multiple pterygia, webbing of the neck, hip dislocation, scoliosis, rocker bottom feet and camptodactyly were present. They had an expressionless face with ptosis, micrognathia, cleft palate and prominent nasolabial folds. Both had undescended testicles. In addition, MRI of the head in one twin showed type 1 schizencephaly on the right, open Sylvian fissure on the left and cortical abnormalities. The other twin had a unilateral open Sylvian fissure.

The twins were found to have a homozygous mutation, c.752delCT, in exon 7 of the CHRNG gene. Though other clinical features are consistent with described cases, central nervous system malformations have not been reported. These CNS malformations may represent an independent phenomenon or an added rare feature of CHRNG related Escobar syndrome.

623/F

Williams syndrome-like phenotype in a mother and daughter with normal FISH results for Williams syndrome. *M.J. Hajianpour[1], Y. Bruno[2].* 1) Gene Trek Genetics, Burbank, CA; 2) White Memorial Medical Center, Los Angeles, CA.

We present a 31-month-old female, born at 30 weeks of gestation to a 27-year-old G1, P0 mother. Her birth weight was three pounds. She had congenital heart defect probably an aortic valve stenosis or coarctation of the aorta (?) which required surgery after birth. She also has congenital scoliosis with mild kyphosis and developmental delay. A recent chromosome analysis and fluorescence in situ hybridization for Williams syndrome and velocardiofacial syndrome were normal. Her weight and height are over 90th percentile and her head size is at 85th percentile for age. Other facial features include epicanthic folds, right eye esotropia, hypertelorism, Broad nasal bridge, anteverted nares, thick prominent lips, wide internipple distance, dorso-lumbar scoliosis to the right, lower dorsal kyphosis, mild hypermobility of fingers, and hypoplastic distal interphalangeal crease of the fifth fingers with restricted movement of distal IP joint. Her mother is similarly affected with congenital valvular heart defects, scoliosis, mental deficiency and thick prominent lips, all of which have been reported in WS. Several of clinical features may be seen in fifth digit syndrome, but the patients do not show overall facial gestalt and other related features.

624/F

Thumb abnormalities in the form of triphalangeal thumbs, hypoplastic thumbs, and polydactylous thumbs in 9 cases of velocardiofacial (VCF) syndrome. A vastly under appreciated feature of VCF syndrome. *B.D. Hall[1, 2], G.A. Stapleton[2], R.C. Rogers[2].* 1) Department of Pediatrics, University of KY, Lexington, KY; 2) Greenwood Genetic Center, Greenwood, SC.

Limb abnormalities of the upper extremities, except for the frequently recognized thin/long appearing fingers, are uncommonly reported in velocardiofacial (VCF) syndrome. Frequencies have varied between 1 and 6 percent of VCF cases. Preaxial involvement has been noted by Ming et al. (1997), Ryan et al. (1997), Kasaprzak et al. (1998), DeSilva et al. (1995), Shalev et al. (1996), Florez et al. (2004), and McDonald-McGinn et al (2005) totaling approximately 17 cases out of the thousands of reported VCF syndrome cases. This does not include 3 cases (mother and 2 children) reported by Hall (Proc Greenwood Genetic Center 24:145) in 2005 with hypoplastic or triphalangeal thumbs. Hall and colleagues are adding an additional 6 cases of VCF who have bilateral thumb abnormalities in the form of triphalangeal thumbs, hypoplastic thumbs, polydactylous thumbs, or broad thumbs. In some instances the diagnosis of VCF syndrome had not been considered because of the presence of preaxial defects and in others it had been relegated to "possible" or rule/out diagnosis. Even those VCF cases without overt preaxial deficiencies or defects often had flexion crease aberrations of the thumb with normal thenar muscle mass suggesting that the majority of VCF cases have some preaxial abnormality. This may be an important additional clue in raising the suspicion of the VCF diagnosis.

625/F

Amniotic Bands, Cleft Lip and Palate, and Supernumerary Nipple: A Rare Phenotype? Literature Review and Discussion. *P.D.R.D. Nicola[1], F.R. Ferreira[1], C.A. Barbosa[2], L.R.J. Silva[1], D. Brunoni[1].* 1) Morphology, Universidade Federal de São Paulo, São Paulo; 2) Pediatrics, Hospital Geral Vila Nova Cachoeirinha, São Paulo.

The amniotic band sequence (ABS) is a condition where the normal fetus is under influence of destructive mechanisms causing a variety of congenital anomalies (syndactyly, limb and digital amputation, constriction rings, craniofacial clefts, and limb-body wall complex). Here we report a 1 year old Brazilian girl, only child born to a non consanguineous young parents, with typical amniotic band sequence (ABS), with limb defects and constriction bands. At the neonatal period this patient had a complete left cleft lip and palate, a supernumerary nipple on the left, and two skin papillae on the proximal right arm and on the lumbosacral region. The neuropsicomotor development and the behavior were normal. In 2000, Guion-Almeida e Richieri-Costa (Clinical Dysmorphology) reported a 14 years boy, born to consanguineous parents, presenting ABS, bilateral cleft lip and palate, preaxial polydactyly and supernumerary nipple. In 2005, Robin et al (American Journal of Medical Genetics) described a girl, born to non consanguineous parents, with ABS anomalies, cleft lip and palate, preaxial polydactyly, supernumerary nipple and skin papilla. Now, we report another child with nearly identical phenotype to these two cases previously described.

626/F

Dentinogenesis Imperfecta type II: Report of a Mexican Family. *M. PADILLA-ROSAS[1,2,3,4,5], J.A. VELAZQUEZ-RODRIGUEZ[3], O.A. AGUILAR-RAMIREZ[4], L.E. FIGUERA[1,6].* 1) CIBO-IMSS, Guadalajara, Jalisco, Mexico; 2) Doctorado en Genética Humana; 3) Escuela de Odontología, CUCS-UdeG; 4) Escuela de odontología Universidad Guadalajara LAMAR; 5) Division de Medicina Molecular; 6) Division de Genética.

Dentinogenesis Imperfecta type II (DGI-II) (OMIM 125490) is an autosomal dominant disorder in which both the primary and the permanent teeth are affected. It occurs with an incidence of 1:8,000 live birth. The teeth are amber and opalescent, the pulp chamber is obliterated by abnormal dentin. The enamel, although unaffected, tends to get fractured, it makes dentin undergo rapid attrition, leading to a marked shortening of the teeth. DGI-II has been linked to mutations in the dentin sialophosphoprotein (DSPP) gene (locus 4q21), the gene product is cleaved into two dentin-specific matrix proteins, dentin sialoprotein (DSP) and dentin phosphoprotein. Even though its incidence, there are only few reports on familial cases. Here we present a Mexican family with DGI-II, and special attention on clinical description is done. Along four generations have been reported teeth affectation, 5 males and 7 females with male-male inheritance; evaluated individuals show the characteristic changes in color, pulp chamber, fractured enamel and dentin attrition in occlusal and incisal surfaces. The objective this report is to aware on an early diagnosis and genetic counseling.

627/F

Fifth female patient with Myhre syndrome. A further delineation. *M.L. Ramirez-Duenas[1,2], L.E. Becerra-Solano[1,2], J.A. Nastasi-Catanese[1,2,3], J.J. Toscano-Flores[1,2], L.E. Figuera[1,2], E. Matute[4].* 1) Division de Genetica, Centro de Investigacion Biomedica de Occidente, Guadalajara, Jalisco, Mexico; 2) Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México; 3) Universidad de Oriente, Núcleo Bolívar, Unidad de Genética, Ciudad Bolívar, Bolívar, Venezuela; 4) Instituto de Neurociencias C:U.B:A. Universidad de Guadalajara, Guadalajara, Jalisco, México.

Introduction: Myhre syndrome(MS)(OMIM 139210) is characterized by short stature, conductive and sensorial deafness,"muscular hypertrophy", limited joint movement, cryptorchidism, and distinctive facial appearance. To date, 15 cases (11 males and 4 females)have been described. The present case is the 5th. female MS patient. Case report: The 13-year-old patient is the 2nd child born to a unrelated 42 and 43 year-old mother and father. She born after a full term pregnancy by cesarean section because of premature rupture of amnion; birthweight was 2150 g. spontaneous cry were referred. At birth, unilateral cleft lip and palate was noticed. Her developmental milestones were normal; she is attending to a regular school. She had menarche at 9 years of age, her menstrual periods have been regular. Physical examination At 11 years of age, her height was 129 cm (-3pct), weight of 29 kg (3-10 pct), and OFC of 49 cm (-3, -2.75 SD). Her physical appearance was squared, by heavy body habitus, short neck and short stature; microbrachycephaly, wide forehead, flat and wide facies, slant-up palpebral fissures, blepharophimosis, flat nasal bridge with hypoplastic left nare, flat maxilla, upper lip with a left surgical scare, overcrowded teeth, repaired clef palate, prognathism, bilateral microtia type I were observed; shortened upper limbs, limited elbow pronosupination, small hands with clinodactyly of the fifth fingers and a wide space between 4th and 5th finger. Her breast and pubic hair development were on III-IV Tanner stage, X-ray studies showed a thick skull vault, broad ribs, mild hypoplastic iliac bones, and broad and short femoral necks. Chromosomal analysis at 550-600 G-bands resolution was normal. The clinical spectrum in MS patients (males and females)is review.

628/F

Autosomal dominant disorder involving deafness, ear pits and hypertelorism: A novel syndrome? *S. Sampath[1], Y. Lacassie[2], B. Keats[1].* 1) Department of Genetics, LSU Health Sciences Center, New Orleans, LA; 2) Department of Pediatrics, LSUHSC, Children's Hospital, New Orleans, LA.

We present an apparent novel autosomal dominant disorder affecting at least three generations of an African-American family. The major features involve deafness, preauricular pits and hypertelorism. The proband is a one-month-old male referred for the evaluation of congenital hearing loss. On physical examination, preauricular pits, hypertelorism, penoscrotal inversion, punctal pits with lacrimal-duct obstruction, and abnormal palmar flexion creases were detected. The family history revealed the presence of multiple affected members; the deaf-mute father has similar features to the proband and even more striking hypertelorism. So far, we have performed detailed phenotypic evaluations and obtained DNA samples for 17 family members of whom 10 are affected. The large pedigree spans five generations and includes 14 other possible affected members from a total of 29. While the deafness is always bilateral, the preauricular pits are either unilateral or bilateral. Some affected members have deafness, preauricular pits and hypertelorism, while others manifest only hypertelorism. Abnormal palmar flexion creases and vertical creases in the 4th interdigital areas are present in two affected individuals; only the proband has punctual pits. Because the phenotype resembles BOR syndrome, the EYA1, SIX1 and SIX5 genes were screened for mutations. No mutations were found in either the coding sequences or in the intron-exon boundaries of these three genes, suggesting that this family may have a novel syndrome with hypertelorism being an important characteristic. We are currently undertaking a SNP-based whole-genome linkage scan to map the disease locus, and identify the gene associated with this disorder.

629/F

The Morphogenesis of Wormian Bones: A Study of Craniosynostosis and Purposeful Cranial Deformation. *P.A. Sanchez-Lara[1,2], J.M. Graham, Jr.[2], J. Lee[2], A.V. Hing[3], M. Cunningham[3].* 1) Dept of Medical Genetics, Children's Hospital Los Angeles, Los Angeles, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, David Geffen School of Medicine at UCLA, Los Angeles CA; 3) Division of Craniofacial Medicine, Dept. Pediatrics, University of Washington School of Medicine, Seattle WA.

Wormian bones (WBs) are accessory bones that occur within cranial suture lines. WBs occur more frequently in genetic disorders that reduce cranial ossification, possibly resulting from a more brachycephalic skull. The frequency and location of WBs are also known to vary with the type and severity of cranial deformation practiced by primitive cultures. We considered the hypothesis that the pathogenesis of WBs may be due to environmental variations in dural strain within open sutures and fontanelles, as well as with genetic variations in calvarial mineralization. In our study, we measured the cephalic index in 20 purposefully deformed pre-Columbian skulls and compared them to 20 anatomically-normal skulls used for medical school anatomy classes. There was no direct correlation between the cephalic index (CI) and the number of WBs in skulls. When the CI was grouped into three categories Normal (CI<81), brachycephalic (CI 81-93) and severely brachycephalic (CI >93), there was a trend in the number of WBs as the skull became more brachycephalic (p=0.039). We also tabulated the frequency and location of large WBs (> 1cm) in 3D-CT scans from 207 cases of craniosynostosis and compared these data with published data on 485 normal dry skulls from Parker (1905). There was a very significant difference between the two groups. Among cases of craniosynostosis, large WBs were more frequent (117 out of 207 3D CT scans) than in dry skulls (131 out of 485) (p<0001). We also found that midline synostosis, specifically metopic or sagittal synostosis has more WBs in the midline, whereas unilateral lambdoidal or coronal synostosis more often had WBs on the contralateral side. Taken together, these data suggest that WBs may arise as a consequence of mechanical factors that affect dural strain within sutures and fontanelles.

630/F

Johanson-Blizzard Syndrome: Report of a Molecularly Confirmed Mild Case Associated with Unilateral Postaxial Hexadactyly. *A.G. Shealy[1], B. Kaplan[2], C.A. Crowe[1].* 1) Cleveland Clinic Genomic Medicine Institute, Cleveland, OH; 2) Department of Pediatric Gastroenterology, Cleveland Clinic, Cleveland, OH.

Johanson-Blizzard syndrome (JBS) is a rare autosomal recessive condition that causes exocrine pancreatic insufficiency and distinctive hypoplastic nasal alae in all cases. Mental retardation, sensorineural hearing loss, short stature, scalp defects, dental problems and abnormal hair patterns are present in a majority of cases. Rarer features include hypothyroidism, imperforate anus and genitourinary anomalies. An estimated incidence is reported to be 1/250,000. There is an increased risk of death in childhood usually due to severe malabsorption. JBS is caused by mutations in the *UBR1* gene on chromosome 15q.

This 7 year-old female patient was referred to genetics by gastroenterology for evaluation of possible JBS. Pancreatic insufficiency (PI) was diagnosed at 18 months of age secondary to the presence of undigested food in foul-smelling stools. While her birthweight was normal, there was a history of poor weight gain, and prior to diagnosis of PI, her weight and height had dropped to slightly below the 3rd percentile. At diagnosis, she was placed on fat-soluble vitamins and pancreatic enzyme replacement. Her growth parameters improved and she is now in the 10-25th percentiles. She continues to receive pancreatic enzyme replacement but has low normal levels of vitamins, even after parents discontinued supplementation. A 2006 CT revealed complete fatty replacement of the pancreas. Additional features of JBS in this girl with normal intelligence, include hypoplastic nasal alae, small deciduous teeth, an upsweeping frontal hairline, multiple moles and hearing loss. Research testing for mutations in *UBR1* detected two mutations, (IVS1+4G>C and c.1978-1980delGTT), molecularly confirming the diagnosis of JBS. Parental carrier testing is pending. Interestingly, this patient was found to have postaxial hexadactyly of her left hand. Although fifth finger clinodactyly has been reported, to our knowledge no cases of JBS in conjunction with hexadactyly have been published. There was no one else in this non-consanguinous family with similar features.

631/F

Intestinal malrotation and Hedgehog signaling defects - an epidemiologic study based on the NorCAS database and London Dysmorphology Database. *S. Munir[1], A. Muneer[2].* 1) Institute of Human Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; 2) Department of Paediatric Surgery, Birmingham Children's Hospital NHS Trust, Birmingham, United Kingdom.

The Northern congenital abnormality survey (NorCAS) database, based in Newcastle upon Tyne,UK, was used to conduct an epidemiologic study of intestinal malrotation in the surrounding region. This constitutes only the second such study to be undertaken specifically to look at features associated with intestinal malrotation, and with 157 cases (live births and fetal deaths) over more than twenty years, constitutes the largest study of its kind to date. Unlike in the previous study, of cases in Hawaii (Forrester and Merz,2003), under half of case (70 cases - 44.6%) had associated features. In a third of such cases, termination of pregnancy was undertaken. Almost a third of children born with syndromic malrotation died within the first year of life, mainly as neonates. 27% of cases had cytogenetic abnormalities identified. Broadly, the commonest non-gastrointestinal features occuring significantly were cardiac anomalies, renal defects, vertebral anomalies, limb defects, and pulmonary lobar anomalies. Mutant mice with Hedgehog signaling defects exhibit intestinal malrotation and features of VACTERL . A qualitative study undertaken by us prior to the epidemiologic study, using the London Dysmorphology Database (LDDB), had also predicted features of VACTERL to be prominent in many disorders featuring malrotation. Therefore, disorders exhibiting a combination of intestinal malrotation and components of VACTERL may be good candidates to search for Hedgehog signaling defects. Using the LDDB, of the top ten candidate disorders identified as the most likely to involve Hedgehog signaling defects based on these criteria, 3 have been confirmed to involve Hedgehog signaling defects (Pallister-Hall, Smith-Lemli-Opitz and Simpson-Golabi-Behmel). We believe the basis for features of VACTERL being present in a significant proportion of children with intestinal malrotation is that many of them had Hedgehog signaling defects during development, rather than this phenotype representing a developmental field defect.

632/F

Generalized arterial calcification of infancy (GACI): two novel ENPP1 mutations in a stillborn fetus. *J. Martinovic[1], A. Bazin[1], O. Catanas[1], F. Briget[1], F. Duchatel[3], Y. Nitschke[2], F. Rutsch[2].* 1) Department of Pathology, Laboratory Pasteur-Cerba, Pontoise, France; 2) General Pediatrics, University Children's Hospital, Muenster, Germany; 3) Department of Gynecology and Obstetrics, Hospital Rene Dubos, Pontoise, France.

GACI is a rare disease not mentioned in current pathology textbooks. We present a male fetus born to a non-consanguineous couple at 39 gestational weeks. The mother was a healthy 28 year-old GI. The pregnancy was uneventful with unremarkable ultrasound scans performed at 13, 22, and 32 weeks of gestation. A stillborn macerated fetus was delivered spontaneously. TORCH, thrombophilia, and Kleihauer screening were negative. The birth weight was 3300g, the length 49cm. External examination was normal. Internal examination revealed pleural and pericardial effusions and moderate splenomegaly. Striking cardiomegaly with calcifications of the great vessels were noted. Radiological examination showed global and bilateral calcifications of the brachial and femoral arterial network. Histology confirmed generalized arterial calcifications (heart, kidneys, lungs, spleen, pancreas, thalami), particularly at the level of the internal elastic lamina. The association of these findings was highly suggestive of GACI (MIM 208000), a recessive disorder linked to the ENPP1 gene. ENPP1 encodes for ectonucleotide pyrophosphatase/phosphodiesterase-1, a cell surface enzyme that generates inorganic pyrophosphate. This solute serves as an essential inhibitor of calcification. Direct sequencing of ENPP1 revealed that the fetus was compound heterozygous for the two novel mutations c.826G/A (p.D276N) in exon 9 and c.1412A/G (p.Y470C) in exon 14. GACI has a recurrence risk of 25% among sibs. Correct diagnosis of the disease is essential for appropriate genetic counselling. Mutation analysis of ENPP1 in the index case is a prerequisite for prenatal diagnostic testing in a subsequent pregnancy.

633/F

Natural History and Molecular Genetics of Pediatric Bilateral Testicular Tumors. *M.T. Collins[6], R. Nandagopal[1], D.P. Merke[1], E.W. Leschek[2], T. Shawker[3], S.K. Libutti[4], J.A. Carney[5], C.A. Stratakis[1].* 1) NICHD, NIH; 2) NIDDK; 3) Dept. of Diagnostic Radiology, Warren Grant Magnuson Clinical Center, NIH; 4) Surgery Branch, Center for Cancer Research, NCI; 5) Dept. of Laboratory Medicine & Pathology, Mayo Clinic College of Medicine; 6) Craniofacial & Skeletal Diseases Branch, NIDCR, NIH.

Testicular tumors are rare in childhood, constituting about one percent of all pediatric solid tumors. Unlike malignant tumors (e.g. seminoma, embryonal carcinoma), most benign testicular tumors (BTTs) are part of identifiable genetic syndromes. We studied the largest series of patients (N=94) with BTTs to date; all were seen over the past 20 years at the National Institutes of Health. Each had one of the following conditions: Carney Complex (N=44), Peutz-Jeghers Syndrome (N=7), Congenital Adrenal Hyperplasia (N=11), McCune Albright Syndrome (N=31), or Familial Male Precocious Puberty (N=1). We analyzed retrospectively all males who presented to the NIH with BTTs from 1985-2006. Information gathered for each patient included age, gender, clinical characteristics, mutational analysis, management, and when present, histological findings and surgical outcomes. In total, 257 males were studied; 94 had testicular involvement, documented by abnormal ultrasound findings. All of these patients had bilateral tumors; none progressed to malignancy. Thirteen underwent an invasive procedure (excisional biopsy/orchiectomy), prompted by testicular enlargement, gynecomastia, or suspicious ultrasound features. None of the tumors had histological signs of malignancy, recurred locally, or metastasized. All of these patients had a mutation in one of the following five genes: *PRKAR1A*, *STK11/LKB1*, *CYP21A2*, *GNAS*, or the LH receptor gene (*LHCGR*). We conclude that childhood BTTs are usually benign and are associated with genetic syndromes that have been molecularly elucidated. In retrospect, few of our patients needed excisional biopsy or orchiectomy. Molecular testing for *PRKAR1A, STK11/LKB1, CYP21A2, GNAS*, or *LHCGR* should be considered in patients with BTTs to confirm the benign nature of the lesion and for clues to the presence of a molecularly-defined syndrome.

634/F

Genotyping for Graves' Ophthalmopathy. *S.W.Y. Chiang, K.K.L. Chong, P.O.S. Tam, C.P. Pang.* Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong.

Purpose: A high incidence of childhood Graves' disease (GD) has been documented in the Hong Kong Chinese population and the patients are commonly presented with ocular manifestations. Several genes, such as CTLA-4 and IL-13, have been identified to be associated with GD. However their effects on Graves' ophthalmopathy (GO) remain controversial. The aim of this study is to investigate the possible association of genetic polymorphisms in CTLA-4 and IL-13 with GD and its ocular manifestations in pediatric GD patients. Methods: 177 childhood GD patients (age range 5-23) and 151 healthy control subjects (age range 4-18) were recruited. Blood samples were collected for DNA extraction. We genotyped 2 SNPs in IL-13 (-1112C>T and 2044G>A), 2 SNPS in CTLA-4 (49A>G and 8358A>G) and the variant fragment length of the dinucleotide (AT)n repeats in the 3'UTR of CTLA-. The genotype results were then correlated with clinical phenotypes, biochemical parameters and ocular manifestations. Results: The 2 SNPs in CTLA-4 (49A>G and 8358A>G) are in the same haplotype block with the GG haplotype significantly associated with increase GD risk (p= 0.0072). The variant length of dinucleotide (AT)n repeats in CTLA-4 also associated with GD with the shortest allele (192 bp) conferred a protective effect (p=0.000047). On the other hand, IL-13 did not confer association to GD risk. IL-13 -1112C>T may associated with IgE elevation (p=0.044) and 2044G>A may be associated with increased risk of proptosis (p= 0.02). However, the difference is insignificant after bonferroni correction (pc=0.22 and 0.1 respectively). Association of these polymorphisms with GO cannot be established. Conclusions: Our study shows that CTLA-4 confers susceptibility to our childhood GD patients in Hong Kong Chinese while IL-13 confers no association to GD risk but may affects the biochemical and ocular features of the patients. No association between these 2 genes and clinically evident GO can be found.

635/F

The NIDDK Central Repository. Using legacy data & samples to address new questions. *P.C. Cooley[1], C.O Scheper[1], Y. Qin[1], C.F. Turner[1], S. Cantor[1], H. Ray[1], R. Rasooly[2].* 1) RTI International, Durham, NC. Research Computing Division, Bioinformatics; 2) NIH/NIDDK.

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) conducts and supports research on many of the most serious diseases affecting public health, including diabetes, liver and kidney diseases. In many cases, the study has collected genetic samples that can be linked to phenotypic data from the subjects; therefore, it is possible to match genotypes to phenotypes and perform many genetic analyses. As high-density genotyping becomes increasingly available, the ability to relate the genetics of patients to results of previous NIDDK-sponsored clinical trials becomes timely. Several NIDDK-supported studies have focused on diabetes and diabetic complications. If consistent phenotypes can be identified across studies and their observations pooled, it might be possible to assemble a dataset with sufficient power to support studies that are not possible from any one individual study. Entirely new studies could be conducted with no additional data collection. To help accomplish that goal and to preserve and distribute valuable resources, NIDDK has established a Central Repository to collect and distribute the data and samples collected by NIDDK-sponsored studies. The Repository co-locates multiple databases to make the data and the associated biological and genetic samples available to the scientific community. The repository also catalogues, retrieves and checks the integrity of study data, manages data requests, and answers researchers' questions. It currently houses publicly available data and samples from 12 studies, including four genetics-based diabetes studies, and is collecting data and samples from more than 40 other ongoing studies for future distribution. The NIDDK Central Repository is a powerful tool for performing in-depth secondary analysis of previously collected data. It will be key in increasing the impact of diabetes studies and, ultimately, will make it possible for researchers to conduct entirely new studies without having to collect data or additional biological samples.

636/F

Circadian rhythm abnormalities of melatonin in Smith-Magenis syndrome patients with *RAI1* point mutation. *D.X. Dang[1,4], D. Glaze[2,3,4], R.J. Reiter[5], D.X. Tan[5], J.R. Lupski[1,2,4], L. Potocki[1,4].* 1) Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Baylor Col Medicine, Houston, TX; 3) Neurology, Baylor Col Medicine, Houston, TX; 4) Texas Children's Hospital, Houston; 5) Cellular and Structural Biology,Univ Texas Health Science Ctr., San Antonio, TX.

Smith-Magenis Syndrome (SMS) is a multiple congenital anomalies and mental retardation disorder in which neurobehavioral abnormalities and sleep disturbances are major features. Most persons with SMS harbor a 3.7 Mb microdeletion within 17p11.2; however, several non-deletion patients have been reported who have heterozygous point mutations of the retinoic acid induced 1 gene (*RAI1*) within the SMS critical region. The majority of persons with del(17)(p11.2p11.2) and those with *RAI1* point mutations are reported to have subjective sleep disturbances. However, whereas circadian rhythm abnormalities of melatonin have been established in deletion patients, this finding has not been reported previously in *RAI1* mutation patients. Herein we report abnormal melatonin levels in two SMS individuals harboring *RAI1* point mutations. Both patients underwent a 24-hour sleep study during which urinary samples were collected for analysis. Subjective sleep disturbances were present in each patient. Objective abnormalities of sleep were noted in the younger of the two patients (age 11y 3m), including decreased total sleep time, multiple nocturnal awakenings, and abnormal sleep-stage distribution (increased percentage of REM sleep). The other patient (age 27 years) had a normal sleep study. Urinary excretion of 6-sulphatoxymelatonin (aMT6s), the major metabolite of melatonin, revealed an inversion of the circadian rhythm of melatonin in both individuals, as is typical in SMS microdeletion patients. We also find that aMT6s levels are lower in older patients with either point mutation or deletion as compared to younger patients. Our results further implicate the dosage effect of *RAI1* in the clinical phenotype of Smith-Magenis Syndrome.

637/F

Absence of dementia in Down syndrome. *E. Doran[1], T. Tirosh-Wagner[2], L. Dai[2], F. Ezgu[2], L.G. Shaffer[3], J.O. Korbel[4], A.E. Urban[4], M. Snyder[4], I.T. Lott[1], J.R. Korenberg[2].* 1) Pediatrics, University of California, Irvine, Orange, CA; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) Signature Genomic Laboratories, Spokane, WA; 4) Yale University, New Haven, CT.

Down syndrome (DS) is associated with an increased risk of Alzheimer-like dementia (AD). Studies have shown that up to 75% of people with DS at age 60 years have AD. Usually caused by trisomy 21, rare individuals with partial trisomy 21 and DS features provide opportunities to identify the genes whose variation in copy number is incompatible with normal human development. In order to understand the genetic contribution to the risk of AD in DS, we have characterized the clinical and molecular features of individuals with partial trisomy 21. We now report a 65 year-old male with 47XY,+del(21)(q11.2q22.1)[18]/46XY[2]. Relevant clinical history includes: hypertension, pulmonary stenosis, and an episode of paranoid ideation and auditory hallucinations. Family history was remarkable for dementia in the patient's mother and 2 maternal aunts. Physical examination: brachycephaly, flat occiput, round face, upslanted palpebral fissures, Brushfield spots, flat nasal bridge, anteverted nostrils, down-turned corners of the mouth, short philtrum, highly vaulted palate, furrowed tongue, and short and broad webbed neck. Cognitive testing: WAIS-III; full scale IQ 69, verbal IQ 71 and performance IQ 72. Neurological exam and standardized dementia assessments revealed no clinical signs of dementia. Brain MRI revealed mild central atrophy, consistent for age. Molecular analysis using 1887 BAC clones microarray, FISH and high resolution isothermal microarray (tiled at~1/100bp), reveled increase in copy number from 28.1Mb-qter of chromosome 21 including genes for GRIK1, SOD1, HUNK, DYRK1A, DSCAM, SNF1LK. These results suggest that in this individual increased copy number of these specific genes is not sufficient to increase risk for AD in DS and supports the hypothesis that increase copy number of the gene encoding APP and other genes in the region, may be responsible for the risk of AD in DS. The results support our proposal of APP being a principle target for prevention of dementia in DS.

638/F

Molecular karyotyping of terminal 4q deletions: first attempts towards a genotype-phenotype correlation. *A. Dufke[1], D. Wieczorek[2], G. Gillessen-Kaesbach[3], R. Voigt[3,4], B. Albrecht[3], S. Singer[1], S. Poths[1], O. Riess[1], M. Bonin[1].* 1) Institut für Humangenetik, Abteilung Medizinische Genetik, Universitätsklinikum Tübingen, Tübingen, Germany; 2) Institut für Humangenetik, Universitätsklinikum Essen, Universität Duisburg-Essen, Essen, Germany; 3) Institut für Humangenetik, Universität zu Lübeck, Lübeck, Germany; 4) Gemeinschaftspraxis für pränatale Diagnostik und Humangenetik, Hamburg, Germany.

Terminal deletions distal to 4q31 are associated with a specific phenotype including Robin sequence and pathognomonic hypoplastic terminal phalange of fifth finger with symphalangism and hooked nail. They are usually associated with a wide spectrum of less specific minor anomalies, growth retardation, congenital heart defect, and varying degree of mental retardation. Few reports described familial inheritance of microscopically visible terminal 4q deletions with mild clinical effects and normal development. We performed molecular karyotyping in four patients with terminal 4q deletions with breakpoints ranging from 4q33 to 4q35 and a wide clinical spectrum: The first female patient had a severe classical 4q- phenotype and a de novo deletion 4q33-qter. Two familial microscopically visible deletions with breakpoints in 4q34 and 4q35, respectively, were diagnosed in patients with minor anomalies and nearly normal development at least in the carrier mothers. A fourth patient with a familial deletion 4q35-qter had no apparent clinical features, but infertility. However, his father carrying the same aberration was not infertile. Using the Illumina HumanHap300-Duo Genotyping BeadChip we were able to characterize the chromosomal breakpoints and deletion sizes in all patients revealing substantial inconsistencies between the molecular and cytogenetic results in two of the four patients. We thus show that molecular karyotyping is essential for establishing a reliable genotype-phenotype correlation.

639/F

Delineation of the genes responsible for the clinical features of monosomy 1p36. *M. Gajecka, K.L. Mackay, L.G. Shaffer.* Health Research & Education Center, Washington State University, Spokane, WA.

Deletions of 1p36 are relatively common, occurring in approximately 1 in 5,000 new borns. To date, we have ascertained 145 cases with monosomy 1p36, representing four possible classes of rearrangements: pure terminal deletions, interstitial deletions, unbalanced translocations, and complex rearrangements. For each individual, the type of rearrangement, deletion size, and parental origin of the deletion was determined. The purpose of this study was to identify the genes involved in the various clinical features of monosomy 1p36. We chose to analyze pure terminal and interstitial deletions to narrow the regions that are essential for monosomy 1p36 clinical manifestations. Translocations and complex rearrangements were excluded. Clinical data was obtained through a comprehensive questionnaire completed by the subject's physician or genetic counselor. Clinical information was collected for 67 individuals presenting with pure terminal deletions and 12 individuals with interstitial deletions. An 11 Mb distal region of 1p36 was evaluated for the eight most commonly observed features: cardiomyopathy, structural congenital heart defects, seizures, speech delay, large anterior fontanel, hearing impairment, hypotonia, and strabismus. This analysis has led to narrowed critical regions for each feature. The smallest region identified is for cardiomyopathy, a 1 Mb region containing 10 candidate genes. Two features, hearing loss and seizures, resulted in two critical regions each, separating the mild forms from the more severe presentation of each feature. In general, it was possible to map a critical region for each clinical feature and identify candidate genes in these narrowed regions.

640/F

Genotype - Phenotype Correlation in Czech Osteogenesis Imperfecta Patients. *I.J. Mazura[1,2], I. Marik[3], F. Mazurova[3], V. Baresova[4], S. Mazurova[4], O. Hudakova[3], P. Novosad[5].* 1) Department of Antropology and Human Genetics, Charles Univ. The Faculty of Science, Vinicna 7,128 44 Prague, Czech Republic; 2) Institute of Computer Science, Academy of Sciences CR , Pod vodarenskou vezi 2, 187 02 Prague 8,Czech Republic; 3) Ambulant Centre for Locomotor System Diseases, Olsanska 7, 130 00 Prague 3, Czech Republic; 4) Charles University Prague, 1st Medical Faculty, Katerinska 32, 120 00 Prague 2, Czech Republic; 5) Mediekos Labor,Ltd.,Antoninova 4464, 760 01 Zlin, Czech Republic.

Osteogenesis imperfecta (OI) is an autosomal dominant or recessive connective tissue disease characterized by extremly high bone fragility (brittle bone disease). The incidence of this disease is 1:10-50 000 newborns. Heterogenous syndrome with variable phenotypic expression is defined by clinical findings (skeletal and soft tissue manifestation, eye symptoms, hearing loss, dental defects and cardiovascular and pulmonary system involvement). OI is dividend into four basic clinical types (I.-IV.). We have analyzed 37 czech osteogenesis imperfekta patients with basic molecular genetic techniques (e.g. DNA extraction from leukocytes, specific amplification methods, sequence analysis) in five selected exons of COL1A1 gene (collagen alpha 1 chain of the gene). The sequence analysis was done in 37 DNA patient symple (21 girls, 16 boys). Mutations were found in 18 patients (substitutions and deletions). Some of the patients had more than one mutation in collagen alpha 1 chain gene. Most of watched patients were clinically classified as type I of osteogenesis imperfekta.We verify that more mutations in one genetic area (in one patient DNA sample) are not in correlation with clinical severity of the disease. We haven't found out any characteristic marker between compared patients with the same mutation. The results were supported by grant no. LN 00B107 of Ministry of Education, Youth and Sport, Czech Republic.

641/F

Limb duplication and ipsilateral renal agenesis: human homolog of mouse Polypodia? *G.E. Tiller, E.G. Yokoyama.* Dept Genetics, Kaiser Permanente, Los Angeles, CA.

Complete limb duplication is an unusual human malformation which may be isolated or associated with renal, genital, and/or other defects. We report a 1,400gm 32-week gestation triplet girl, conceived with Clomid, who was born to a 25 year-old G3P1sAb1 mother. Physical exam revealed right popliteal pterygium as well as mirror-image duplication of the right foot. Radiographs revealed duplication of the right femur and fibula, and ultrasound exam revealed absence of the right kidney. The infant grew well and was discharged at one month of age. The other triplets were unaffected, and the only familial anomaly was the paternal great-grandfather who had a single kidney. The pattern of malformations in our patient appears similar to those seen in the Polypodia (Ppd) mouse (Lehoczky JA et al., Mamm. Genome 17:903, 2006). Ppd is an X-linked dominant disorder which has been bred from a single affected CD-1 male, with approximately 20% penetrance. Phenotypic features overlap with those of mice exposed to retinoic acid in utero, as well as the Disorganization (Ds) mouse, which is an autosomal dominant disorder with reduced penetrance. The broader spectrum of anomalies seen in Ds mice implies that the effect of Ppd may lie downstream in the process of pregastrulation body patterning.

642/F

Familial Chronic Intestinal Pseudo-Obstruction and Recurrent Pancreatitis in Patients Harboring the Mitochondrial DNA A3243G Mutation. *D. Bonneau[1], C. Verny[2], P. Bonneau-Amati[1], F. Letournel[3], N. Dib[4], C. Le Maréchal[5], C. Férec[5], P. Reynier[1].* 1) Department of Genetics and Biochemistry, INSERM U694 and CHU, Angers, France; 2) Department of Neurology, CHU, Angers, France; 3) UPRES EA 3859, IFR 132, CHU, Angers, France; 4) Department of Gastroenterolgy CHU, Angers, France; 5) Department of Genetics, INSERM U613 and CHU, Brest, France.

The A3243G point mutation in the MTTL1 gene is associated with a broad spectrum of clinical manifestations including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) and maternally inherited diabetes mellitus with deafness (MIDD). Although gastrointestinal symptoms are often reported in MELAS as well as in MIDD, they are not usually prominent features of these diseases. Patients and methods: We report a family in which five members carried the mitochondrial DNA (mtDNA) A3243G mutation and presented with chronic intestinal pseudo-obstruction (CIPO); three of these patients also suffered from recurrent pancreatitis. The mutation was quantified in several tissue samples from affected patients. Respiratory chain activity was studied on muscle biopsies and fibroblast cultures. In addition, the thymidine phosphorylase gene involved in MNGIE, and three genes involved in chronic pancreatitis (PRSS1, SPINK1 and CFTR) were sequenced in affected patients. Finally, MTTL1 was tested in 36 unrelated patients suffering from recurrent pancreatitis; the sequencing of the PRSS1 and the SPINK1 genes in these patients had shown no mutations. Results: Heteroplasmy for the mtDNA A3243G mutation was found in all tissue samples obtained from affected patients. No mutations were found in the genes coding for thymidine phosphorylase, PRSS1, SPINK1 and CFTR in the affected patients. None of the 36 unrelated patients with recurrent pancreatitis carried any MTTL1 mutations. Conclusion: The mtDNA A3243G mutation is responsible for the gastrointestinal manifestations observed in this family and must therefore be regarded as a cause of the CIPO and the unexplained recurrent pancreatitis. However, this mutation is weakly involved in cases of recurrent pancreatitis without associated signs of mitochondriopathy.

643/F

Chronological changes of serum creatine kinase (CK) levels in molecularly confirmed Duchenne muscular dystrophy cases and examination of the cases with deviated CK levels. *Y. Okizuka, Y. Takeshima, M. Yagi, Y. Oyazato, H. Awano, Z. Zhang, M. Matsuo.* Dept Pediatrics, Kobe Univ, Kobe, Hyougo, Japan.

<Objective> Elevation of serum creatine kinase (CK) level is a well known hallmark of Duchenne muscular dystrophy (DMD). However, changes of its level have not studied in DMD cases whose diagnosis is molecularly confirmed. The present study is aimed at establishing standard levels of serum CK according to age in molecularly confirmed DMD cases. Furthermore, cases with the deviated CK levels from the standard were examined for its molecular background. <Methods> 121 DMD cases diagnosed by both clinical and molecular findings were enrolled in this study. Their age varied from ages 2 to 18 years. CK levels were examined by the Oliver-Rosalki method at 316 points. Means and standard deviations of CK levels were calculated per age-class. All statistical analyses were done following a transformation of CK to Napierian logarithmic. Furthermore, 78 uncertain dystrophinopathy cases were examined for the validity of the standard. <Results and Discussions> Average CK levels were maintained at extremely high level from 2 to 6 years old. The average CK levels declined sharply after 7 years old, whereas the decline became slow after 13 years old. In the analyses of 78 uncertain dystrophinopathy cases, remarkably, two cases were found to have significantly low CK level (<-2.5SD). Clinicaly these two cases showed mild phenotype, but their mutations of in the dystrophin gene were nonsense mutation and 4bp deletion, respectively, compatible with severe DMD. Extensive molecular analyses disclosed that one case had the nonsense mutation in C-terminal region and the other had productions of in-frame dystrophin mRNA, compatible with mild phenotype. Chronological changes of serum CK in DMD cases diagnosed by both clinical and molecular analyses were first established. In two cases with deviated CK levels, molecular backgrounds were clarified, therefore, our results were useful in evaluation of dystrophinopathy.

644/F

A complex insertion event produced a chimeric dystrophin-IL1RAPL1 transcript in the dystrophin gene. *Z. Zhang, Y. Takeshima, M. Yagi, A. Nishiyama, Y. Okizuka, H. Awano, M. Matsuo.* Dept Pediatrics, Kobe Univ, Kobe, Japan.

Duplications of one or more exons in the dystrophin gene located at Xp21.3-p21.2 are the second common mutation in Duchenne and Becker muscular dystrophies (DMD and BMD) and have been considered as a simple insertion of genomic region. Here, we report a DMD case with a complex duplication in the dystrophin gene, creating a chimeric dystrophin-IL1RAPL1 transcript. Multiplex ligation dependent probe amplification (MLPA) analysis revealed the duplication of exons 56-62 of the dystrophin gene. However, the analysis of the dystrophin mRNA of the patient by RT-PCR resulted in the identification of an unexpected 621-nucleotide insertion between the repetition of duplicated exons. The inserted 621bp nucleotide sequence was found to be homologous to exons 3-5 of the IL1RAPL1 gene in Xp22.1-Xp21.3. Though duplication of these exons was confirmed in his genome, nomal IL1RAPL1 mRNA was also obtained. These results indicated double insertions of IL1RAPL1 exons and dystrophin exons between exons 62 and 63 of the dystrophin gene. This is the first report of the complicated duplication in the dystrophin gene and provides a clue to understand the mutational mechanism of insertion event.

645/F

Quality of Life Investigation of Tibial Dysplasia in NF1 Patients Shows Differences in Outcome and Provides a Framework for Clinical Trials. *J.C. Carey[1,2], D.A. Stevenson[1,2], D.H. Viskochil[1,2], J. Siebert[2], S. Geyer[2], M. Winn[2], J. Roach[2], J. D'Astous[2], C. Marra[3], L. Colley[3], J. Friedman[3], P. Birch[3], E. Schorry[4], TD Working Group.* 1) Dept Ped/Div Med Genetics, Univ Utah Medical Ctr, Salt Lake City, UT; 2) Shriner Hospital for Children, Intermountain, Salt Lake City, UT; 3) University of British Columbia, Vancouver, BC, Canada; 4) Cincinnati Children's Hospital, Cincinnati, OH.

Tibial dysplasia (TD) occurs in about 5% of persons with neurofibromatosis type 1 (NF1) and is one of the criterion for the diagnosis of NF1. TD comprises a continuum of anterolateral bowing to the serious problem of pseudarthrosis (PA). Treatment of PA is complex and often requires multiple surgical procedures with varying degrees of success. Recently medical therapies for TD/PA, including bisphosphonates and dietary modalities, have been proposed. Methods: We have established a 4-yr multicenter study to investigate the natural history of TD/PA and to determine outcome measures for TD in future trials. The study methods involve surveying patients and families with TD/PA and NF1 using standardized health-related quality of life (QOL) instruments. Results: We compared 24 children with NF1/ TD to 63 NF1 children without TD using the PODCI and the HUI instruments. Applying the Mann-Whitney U-Test, we demonstrated that the means of Basic Mobility (15.3) and Sports/Physical Function (13.68) were markedly different than controls (46.7, 42.3)($p < 0.001$). Notably the scores for Happiness were no different. Using the HUI, we compared the overall scores and individual domains in cases and controls, and these varied between the groups only for Ambulation. There were no group differences in age distribution or gender. Discussion: This study is the first QOL investigation of the orthopedic aspects of NF1. The overall purpose of the project is to obtain outcome data for design of future medical/surgical therapeutic trials in patients with TD. These data - proof of principle - demonstrate that these QOL measures can be utilized effectively in designing such trials for the treatment of TD in NF and show outcome differences in QOL in NF1/TD patients.

646/F

The genetic evaluation of macroglossia. *Y. Zarate, R. Hopkin.* Div Hum Genetics, Cincinnati Chlds Hosp Med Ctr, Cincinnati, OH.

Macroglossia is a relatively common reason for genetic evaluation.Several etiologies have been defined with POSSUM listing over 70 conditions that can cause it.Little research has been published on the evaluation or management of patients with this anomaly.

We performed a retrospective chart review of patients who were initially evaluated for macroglossia alone or in combination with other findings.To date records on 36 patients have been reviewed. At the time of evaluation average age was 92 days, gestational age at birth was 36 +/- 4.8 weeks and birth weight of 3100 grams +/- 1186. Some relevant historical findings were positive family history in first degree relative for macroglossia, twin gestation and gestational diabetes. Atleast 3 studies were recommended in 15/36 patients (41.7%).Abdominal and renal ultrasound was the most frequent in 24/35 patients (68.5%). Other studies included chromosomal analysis, alpha feto protein (AFP)levels, urine mucopolysaccharidosis (MPS) screen, Beckwith Wiedemann (BWS)molecular testing and thyroid function tests. Abnormalities in BWS molecular were seen in 2/7 (28.5%). A single abnormal chromosome result was found (1/16: 6.2%). Abnormal ultrasounds and AFP were common in BWS but not in other patients.

BWS was the most frequent diagnosis seen in 22/36 patients (61.1%). Macrosomia was not universal in BWS but twinning was an important predictor of BWS. An expanded search that included macroglossia as a physical exam finding at any point independently of the reason for the initial evaluation, revealed 36 additional patients. Several other conditions were identified in small numbers including 9q34 deletion syndrome, transient neonatal diabetes, neurofibromatosis type I, Cardio Facio Cutaneous syndrome, Klippel-Trenaunay Weber syndrome among others.

These findings indicate that while BWS is the most frequent diagnosis associated with macroglossia there are a number of other less common conditions that may present with this finding.The diagnostic approach to this population will vary depending on the associated findings.

647/F

Differential gene expression in peripheral blood of ALS patients associated with genetical variation. *C.G.J. Saris[1], S. Horvath[2], P.W.J. Vught[1], M.A. van Es[1], H.M. Blauw[1], T. Fuller[2], J. Veldink[1], L.H. van den Berg[1], R.A. Ophoff[3,4].* 1) Department of Neurology, University Medical Center Utrecht, The Netherlands; 2) Department of Human Genetics en Biostatistics, University of California Los Angeles, CA; 3) Semel Institute of Neuroscience and Human Behavioral, University of California Los Angeles, CA; 4) Department of Medical Genetics, University Medical Center Utrecht, The Netherlands.

Objective: The genetics of the sporadic form of **Amyotrophic Lateral Sclerosis** is still largely unknown. Combining the genetical variation with genome wide gene expression profiles of complete blood (genetical genomics) within one individual will give insight in genes associated with the disease.

Method: Of 116 ALS patients and 110 matched healthy controls whole blood gene expression profiling using Illumina HumanRef-8 Expression BeadChip was combined with genome wide genotyping using 300K Illumina Infinium BeadChip.

Using weighted gene co-expression network analysis (WGCNA) genes can be grouped into modules with similar expression patterns. In the first half of the dataset two out of the seven identified modules were differentially expressed in ALS patients and validated in the second part. Mean expression per module (principal component) was mapped on the genome treating it as a quantitative trait. SNPs associated with the differential expressed modules are significantly associated with ALS status.

Conclusion: we find differentially expressed genes in peripheral blood that can be validated. These genes can be used as a biomarker for ALS. Using genome-wide genetic marker data we provide evidence that at least one of these modules is causal for ALS.

648/F

Early interstitial lung disease in familial pulmonary fibrosis. *B.R. Gochuico[1,6], P. Ren[1], N.A. Avila[2], C.K. Chow[2], T.J. Franks[3], W.D. Travis[3], J.P. McCoy, Jr.[4], R.M. May[1], H.P. Wu[1], D.M. Nguyen[5], M. Arcos-Burgos[6], S.D. MacDonald[1], I.O. Rosas[1].* 1) Pulmonary-Critical Care Medicine Branch, NHLBI, NIH, Bethesda, MD; 2) Diagnostic Radiology Department, CC, NIH; 3) Department of Pulmonary and Mediastinal Pathology, AFIP, Washington, DC; 4) Flow Cytometry Core Facility, NHLBI, NIH; 5) Surgery Branch, NCI, NIH; 6) Medical Genetics Branch, NHGRI, NIH.

Purpose: Familial pulmonary fibrosis (FPF) is a rare, autosomal dominant disease with variable penetrance. Identification of early, asymptomatic interstitial lung disease (ILD) in populations at risk of developing FPF may improve the understanding of the natural history of idiopathic pulmonary fibrosis (IPF), a progressive ILD of unknown etiology with a poor prognosis. Methods: To characterize features of early, asymptomatic ILD in family members of patients with FPF, 164 subjects from 18 kindreds affected with FPF were evaluated for ILD at the NIH Clinical Center. Bronchoalveolar lavage (BAL) cells were analyzed using flow cytometry. Lung biopsies were performed in six subjects with early ILD. Results: High-resolution computed tomography (HRCT) findings of early ILD were identified in 31 (22%) of 143 asymptomatic subjects. Subjects with early ILD were significantly younger than subjects with known FPF ($p<0.001$) and significantly older than related control subjects without lung disease ($p<0.001$). A history of smoking was identified in 45% of subjects with early ILD and in 67% of subjects with FPF; these percentages were significantly higher than that of related control subjects (23%) ($p=0.02$ and $p<0.001$, respectively). Percentages of activated CD4+ lymphocytes were significantly higher in BAL cells from subjects with early ILD compared to related control subjects ($p<0.001$). Lung biopsies performed in subjects with early ILD revealed various histologic subtypes. Conclusions: Early, asymptomatic ILD in individuals at risk of developing FPF can be identified using HRCT scan of the chest, especially in active or former smokers. In this cohort with early lung disease, CD4+ BAL cells are activated, and lung biopsies demonstrate different histologic subtypes of ILD.

649/F

Body mass index (BMI) and height velocity by age and gender in children with achondroplasia. *J.E. Hoover-Fong[1], K.J. Schulze[2], J. McGready[2], C.I. Scott[3].* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Bloomberg School of Public Health, Johns Hopkins Univ, Baltimore, MD; 3) AI DuPont Hospital for Children, Wilmington, DE.

OBJECTIVE: To examine BMI in relation to indices of height (e.g. body segments, height velocity) and develop age-appropriate BMI charts for clinical use in children with achondroplasia. METHODS: An anthropometry database was created from single observer data extracted from clinical records of 334 individuals with achondroplasia. BMI (weight, kg/height, m2) percentiles (5, 50, 95th) were estimated from birth to 16 years of age by gender, using a one month window (+ 0.5 months) around each time point and smoothed by a quadratic smoothing algorithm. Growth velocity (cm/year) was calculated at the mid-point between every two consecutive height values if the interval was 2-18 months. Upper and lower segment ratios were also examined by age. RESULTS: Data from 241 and 236 subjects contributed 1935 BMI and 1846 height velocity datapoints, respectively. A BMI peak in infancy and nadir in childhood is not observed in achondroplasia as in average stature children. From 2-6 years of age, the entire achondroplasia BMI distribution (5th-95th percentile) is above the 95th percentile of average-stature peers. Thereafter to 16 years, the lower half of the achondroplasia BMI distribution overlaps the upper half of the average stature BMI distribution. Although birth length is comparable between achondroplasia and average stature, peak height velocity during infancy in achondroplasia is nearly half that of average stature infants. There is also no evidence of a pubertal growth spurt in achondroplasia. Upper segment measurements, but not lower, were associated with BMI ($p=0.05$) by regression analysis. CONCLUSIONS: BMI-for-age is higher in children with achondroplasia than average stature peers, necessitating specific BMI curves for clinical use in this population. In achondroplasia, overall height is compromised due to limb shortening, therefore body mass is centered about the trunk. Health consequences associated with BMI have yet to be determined in this population.

650/F

Allele frequency of the COL2A1 3' VNTR in patients with pectus excavatum. *M. Stacey[1], S. Neumann[2], V. Proud[3], A. Fecteau[4], A. Pastor[4], R. Kelly[5], D. Nuss[5].* 1) Ctr Pediatric Research, Eastern Virginia Medical Sch, Norfolk, VA; 2) Department of Psychiatry and Behavioral Sciences, Eastern Virginia Medical School, Norfolk, VA; 3) Dept. Pediatrics, Division Medical Genetics, Children's Hospital of the King's Daughters, Norfolk VA; 4) Dept Surgery, Hospital for Sick Children, Toronto, Canada; 5) Dept Surgery, Children's Hospital of the King's Daughters, Norfolk, VA.

Pectus excavatum (PE) is the most common congenital anomaly of the chest wall causing compromised cardiac and pulmonary function. In a practice with approximately 55% Caucasian and 45% African-American patients, 95% of PE patients are Caucasian, with 45% showing familial tendencies suggesting a genetic etiology. Gene variants are known to be inherited with specific disorders, and thus variants may be at different frequencies in a patient population compared to controls. COL2A1 is expressed in cartilage, is responsible for tissue strength and durability and thus play a role in the etiology of PE. We investigated the 3'VNTR of the COL2A1 gene to identify allele frequency variation of this gene in 110 patients with PE, 122 family members and 30 controls. The VNTR was amplified by PCR and the number of repeats verified by sequencing. A significant increase ($p>0.05$) in heterozygosity was observed in patients vs. controls and unaffected family members. The number of tandem repeats within the VNTR in PE families was 7-13, with a peak at 10-11 repeats. These values are surprisingly similar to published results of Asians (8-12 repeats) rather than European Caucasians (13-15). A skewed distribution in the inheritance of the COL2A1 gene was suggested in this preliminary study in patients with pectus excavatum.

651/F

"Circumpapillary dysgenesis of the pigment epithelium" is in fact NOT a Pigment Epithelial Disease. *K.M. Janisch, J. Tosi, C.L.C. Chou, J.M. Kasanuki, J.F. Flynn, S.H. Tsang.* Bernard & Shirlee Brown Glaucoma Laboratory, Edward S. Harkness Eye Institute and Dept. of Pathology, Columbia Univ. College of Physicans & Surgeon, New York, NY.

PURPOSE: Circumpapillary dysgenesis of the pigment epithelium is an autosomal dominant condition (OMIM108985). Lesions begin in the peripapillary area but the macula will be the eventual site of involvement causing blindness and loss of activities of daily living. This disease is also characterized by bilateral geographic, helicoid destruction of the choroid, retinal pigment epithelium (RPE), and photoreceptors. The cellular origin of the disease is unknown, but may arise from abnormal RPE and/or choroidal function. A 1261T-C transition mutation in TEAD1 has been identified in affected families but the location of TEAD1 expression in the eye is unknown. METHODS: To determine the anatomical basis of disease, members of a three-generation New Jersey family were ascertained. Electroretinography (ERG) and genetic screening were performed. Fundus photographs, fundus RPE autofluorescence and optical coherence tomography were reviewed. Autofluorescence images were obtained by illuminating the fundus with argon laser light (488 nm) and viewing the resultant fluorescence through a band pass filter with a short wavelength cut off at 495 nm. To detemine the location of TEAD1 expression, we analyzed dissected mouse RPE and choroid by immunoblotting with an anti-TEAD1 antibody. RESULTS: ERG findings rule out neurosensory retinal involvement even at late stages of the disease. The presence of relatively intact RPE and diseased choroid in a three-year old proband indicates that the RPE is not involved in early stages of circumpapillary dysgenesis. Furthermore, Tead1 expression was observed in the choroid and not the RPE layer of cells. CONCLUSIONS: The primary cellular cause of circumpapillary dysgenesis of the pigment epithelium may be the choroid and not the RPE. Studies of the downstream targets of TEAD1 will open new avenues of research in the development of treatments for choroidal diseases.

652/W

Hyperacusis in persons with Smith Magenis syndrome: Expanding the SMS phenotype. *A.C.M. Smith[1,2], J. Bentley[3], C. Zalewski[3], R. Morse[1], W. Introne[1], C. Brewer[3].* 1) NHGRI/NIH, Bethesda,MD; 2) Georgetown Univ., Washington,DC; 3) NIDCD/NIH, Bethesda,MD.

Otolaryngologic abnormalities are documented in as many as 94% of individuals with SMS. Oversensitivity to loud sounds is an expressed parental concern. This study seeks to quantify the occurrence and severity of hyperacusis in persons with SMS and document the types of responses, triggers and palliative techniques. Hyperacusis is an oversensitivity to sounds that are tolerable to listeners with normal hearing. A 2-page questionnaire used to evaluate the severity of hyperacusis in William syndrome (WS)(Cohen et al., 2006) was mailed to parents of children with SMS participating in our IRB-approved protocol at NIH (SMS-US) & Australian families at Camp-Breakaway (SMS-AUS). Healthy SMS siblings (n=20) serve as controls. Completed questionnaires (n=63) include 47 SMS-US (mean age 12y) and 16 SMS-AUS (mean age 12.7y). No significant differences were found between SMS-US and SMS-AUS with respect to sensitivity to loud sounds (74% vs 87%) or mean hyperacusis severity score (3.9 vs 4.1), respectively, permitting the data to be combined (SMS-GP). Sensitivity to loud sounds was present in 78% compared to 10% of controls. Mean SMS-GP severity score (3.96, SD 2.92) was significantly higher than controls (0.90, SD 1.52) ($p<0.001$). Intolerance for loud sounds remained unchanged over time for 59%, and 33% improved with time. No significant relationship to degree or type of hearing loss was observed. Major triggers for distress were tiredness (47%) and mood (29%); tiredness was the most cited reason for heightened reaction in 50%. Common behavioral responses to distressing sounds were covering the ears with hands (88%), becoming upset (58%), or displaying anxiety/tension (52%). Self-injurious behaviors were triggered by loud sounds in 28%. Palliative measures or techniques for sound reduction varied with less distress reported when prepared or warned of impending sound. Thus, hyperacusis is pervasive in SMS; however, the SMS mean severity rating is less than reported in WS (mean 5.78,SD2.6)(Cohen et al., 2006), which shares some commonalities in physical and behavioral features.

653/W

CSI-OMIM - Clinical Synopsis Search In OMIM. *R. Cohen[1], A. Gefen[1], O. Birk[1], A. Melkman[2].* 1) Developmental Genetics (The Morris Kahn Laboratory of Human Genetics), Ben-Gurion University, Beer-Sheva, Israel; 2) Department of Computer Sciences), Ben-Gurion University, Beer-Sheva, Israel.

Note: AG and RC contributed equally to this project.

The OMIM database is a tool used daily by geneticists. Each syndrome page includes a Clinical Synopsis section containing a list of known phenotypes comprising the syndrome. The phenotypes are in free text and many different phrases are often used to describe the same phenotype, the difference originating in different spelling variations or typing errors, varying sentence structures and use of a verbal phenotype description as well as medical name.

Using natural language processing, an information vector was constructed for each phrase using Miriam-Webster online dictionary and medical dictionary, Princeton's Wordnet dictionary and NIH's MESH. The vectors were used to cluster the similar phenotypes into synonymous groups. This was followed by manual curation for weeding out the false positives. The syndromes listed in OMIM were organized using a linear clustering technique (SPIN) based on the frequencies of Clinical Synopsis phrases described in the syndrome.

This allows a better search for matching syndromes by choosing exact search terms to focus the search on the best matching syndromes.

654/W

Neuroaxonal dystrophy associated with osteopetrosis and brain dysgenesis: Prenatal diagnosis and neuropathological findings. *D. Chitayat[1,4,7], P. Shannon[2,7], W. Halliday[3,7], G. Seaward[4,7], A. Toi[5,7], M. Thompson[2,7], S. Blaser[6,7].* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada; 2) Department of Pathology and Laboratory Medicine, MSH; 3) Division of Pathology, Hospital for Sick Children, Toronto; 4) Department of Obstetrics and Gynecology, MSH; 5) Department of Diagnostic Imaging, MSH; 6) Diagnostic Imaging and Neuroradiology, HSC; 7) University of Toronto, Toronto, Canada.

Neuroaxonal dystrophy (ND) is a progressive disease characterized by widespread swelling of axons and neuropathy. Infantile, late infantile and juvenile forms have been reported and genetic heterogeneity has been recognized. The association of ND with osteopetrosis and brain dysgenesis have been reported only twice before. We report the prenatal presentation of such a condition in a fetus born to a consanguineous couple.

The mother was 17 years old and her husband was 32 years old. They were of Afghani origin and consanguineous. The pregnancy was complicated with fetal ultrasound findings of a small cerebellum and scalp edema at 24 weeks gestation. Fetal MRI showed severe hypoplasia of the cerebellum, medulla and pons and delayed abnormal sulcation and agenesis of the corpus callosum.

The pregnancy was interrupted and the autopsy showed coarse facial features, microcephaly with lissencephaly, absent olfactory bulbs and tracts, absent corpus callosum, a small cerebellum with posterior fossa cyst, bilateral cataract and dramatic central and peripheral nervous system axonal spheroids. There was an increased density of all long bones with bone in bone appearance. The karyotype was 46, XX and FISH for 17p13.3 was normal.

The combination of ND with osteopetrosis and brain dysgenesis has been reported initially by Rees et al., (1995). To the best of our knowledge, no further cases have been reported and this is the first case diagnosed in utero.

655/W

MRI and MRA brain anomalies in 22q11 Deletion Syndrome. *E. Chow[1,2], D.J. Mikulis[3,4], A.S. Bassett[1,2].* 1) Department of Psychiatry, Univ Toronto, Toronto, ON; 2) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, ON; 3) Department of Medical Imaging, University of Toronto, ON; 4) Department of Medical Imaging, Toronto Western Hospital, Toronto, ON, Canada.

22q11 Deletion Syndrome (22qDS) is associated with congenital cardiac defects (CHD), cognitive dysfunction, and psychiatric conditions including schizophrenia (SZ). This study aims to systematically assess for anomalies in brain, skull, and head and neck vessels in a large group of adults with 22qDS. A neuroradiologist blind to the deletion and psychiatric status of subjects systematically reviewed multi-planar sequences MRI brain scans and MR angiography (MRA) through the circle of Willis and neck vessels of 65 adults with 22qDS (26 M, 39F; mean age = 26.4y, SD = 9.6y) and 20 adults without 22qDS (10 M, 10 F;mean age = 29.6y, SD = 7.4y) for visually detectable anomalies. The 22qDS sample comprised of 28 subjects with SZ (22qDS-SZ) and 37 with no history of psychosis (22qDS-NP), and the comparison sample comprised of 11 subjects with SZ and 9 with no history of psychosis. Rates of anomalies in cortical and subcortical structures, skull base, circle of Willis and neck blood vessels were compared between the 22qDS and non-22qDS subjects and their subgroups. The 22qDS subjects had significantly more anomalies than the non-22qDS subjects, especially in the skull base, the most common being an enlarged C1, present in 39%; of 22qDS subjects and only 5%; of comparison subjects (p=0.0045). Cerebellar atropy, but not cortical atrophy, was also more common in 22qDS subjects (17% vs 0%; in comparison subjects; p=0.047). On MRA, low bifurcation of the carotid arteries was more common in 22qDS subjects than in comparison subjects (32% vs 6%, p=0.049), even though both groups had similar rates of CHD. When comparing 22qDS-SZ subjects to 22qDS-NP subjects, the two subgroups had similar age, IQ and rates of CHD, but the 22qDS-SZ subgroup had more intracanial anomalies such as cavum anomalies, cortical atrophy, and bright foci. The results suggest that in 22qDS intracranial anomalies are more associated with a history of psychosis, and skull base and vascular anomalies with the genetic condition.

656/W

Siblings with static encephalopathy and a microdeletion at 14q12 that includes the FOXG1B gene. *K. Herman[1], J. Pinter[2], M. Lipson[3], A. Patel[4], A. Pursley[4], S. Yang[1].* 1) Medical Genetics, University of California at Davis Medical Center, M.I.N.D. Institute, Sacramento, CA; 2) Child Neurology, University of California at Davis Medical Center, M.I.N.D. Institute, Sacramento, CA; 3) Medical Genetics, Kaiser Permanente Medical Group, Sacramento, CA; 4) Kleberg Cytogenetics Laboratory, Baylor College of Medicine, Houston, TX.

We are reporting two siblings with severe to profound mental retardation, congenital microcephaly, and static encephalopathy that were found to have a 0.7Mb deletion at 14q12 by chromosomal microarray analysis (RP11-96617->RP11-260G13). The mother of these children has mild to moderate mental retardation, but has not yet been tested to confirm presence of this deletion.

This deletion includes the FOXG1B gene, which is a highly conserved DNA-binding domain with expression in the developing brain restricted to telencephalic neurons (OMIM #164874). To our knowledge there have been no previous clinical reports of individuals with mutations or deletions of this gene. These cases represent the first report of microcephaly and mental retardation due to deletion of this gene and demonstrate the need for further research into the clinical phenotype associated with the FOXG1B gene.

657/W

ARSACS in the Dutch population: A frequent cause of recessive cerebellar ataxia? *S. Vermeer[1], H.P.H. Kremer[2], R.P.P. Meijer[1], B.J. Pijl[3], J.R.M. Cruysberg[3], J. Timmermans[4], M.M. Bos[2], H.J. Schelhaas[2], B.P.C. van de Warrenburg[2], N.V. Knoers[1], H. Scheffer[1].* 1) Departments of Human Genetics; 2) Neurology; 3) Ophthalmology; 4) and Cardiology, Radboud University Nijmegen Medical Centre, The Netherlands.

Introduction: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS:MIM 270550) is a neurodegenerative disorder characterized by a core phenotype of progressive early-onset cerebellar ataxia with evolving spasticity of the lower limbs and peripheral neuropathy. The disorder was first described among French Canadians in the isolated Charlevoix-Saguenay region of Quebec, but by now the disease has been recognized to occur worldwide. Therefore we initiated a systematic mutation analysis by direct automated sequencing of the coding regions of the huge SACS gene in Dutch ataxia patients.

Methods: Mutation analysis was performed in 31 index patients. Patients were classified into 3 different groups (A to C) based on clinical characteristics. All patients in group A (n= 11) showed the ARSACS core phenotype. Group B (n=6) consisted of patients whom did not have all three features of the core phenotype. Group C (n=14) consisted of patients for whom routine SACS mutation analysis was requested, without the phenotype being precisely defined. All 5 coding exons of the SACS gene were PCR amplified and subsequently sequenced.

Results: We identified mutations in the SACS gene in 13 (42%) adult patients out of 31 index patients. All mutations are novel and most likely are loss of function mutations. Most ARSACS patients were identified in group A (9 out of 11). All the identified ARSACS patients of whom we were able to collect detailed clinical information (11) displayed the core phenotype.

Conclusion: Apparently, in Dutch patients the prevalence of ARSACS seems substantially higher than previously estimated. The phenotype of ARSACS patients seems rather uniform and recognizable as supported by the high mutation detection rate in group A (82%).

658/W

The Syndrome of Megalencephaly, Mega Corpus Callosum and Complete Lack of Motor Development: Case Report. *C.A. Williams, H.J. Stalker, A.I. Dagli.* Raymond C. Philips Research and Education Unit,Division of Genetics, Department of Pediatrics, University of Florida, Gainesville.

The syndrome of megalencephaly, mega corpus callosum and complete lack of motor development (OMIM 603387) is an apparently rare condition since only 3 cases have been reported [Gohlich-Ratmann et al., Am J Med Genet, 1998]. Affected infants have severe macrocephaly, muscular hypotonia and profound cognitive deficits. The cause for the MCC syndrome is unknown, no familial cases have yet been reported, and both autosomal recessive and spontaneous dominant genetic mechanisms are possibilities.

We describe an additional case diagnosed at 15 months of age. Prior to the syndrome diagnosis, extensive metabolic and genetic studies, including array-based comparative genomic hybridization, were normal. Neonatal and postnatal MRIs showed generalized, severe enlargement and thickening of the corpus callosum, bilateral megalencephaly, generalized cortical thickening and apparent pachygyria. The corpus callosum thickness, on a midsagittal T1 weighted image, was: genu 1.03 cm, body 0.86 cm and splenium 0.98 cm (normal average values for age 15 months are, 0.75 cm, 0.4 and 0.8 cm respectively [Iai et al., Acta Paediatr, 1994]). The MCC syndrome is thus a congenital macrocephaly condition associated with a distinct MRI phenotype.

.

659/W

Autosomal Recessive Bestrophinopathy (ARB): a novel phenotype associated with *BEST1* mutations. *R. Burgess[1], I. Millar[2], B. Leroy[3], J. Urquhart[1], I. Fearon[2], P. Brown[2], A. Webster[4], G. Holder[4], F. Manson[1], G. Black[1].* 1) Medical Genetics, University of Manchester, Manchester, UK; 2) Life Sciences, University of Manchester, Manchester, UK; 3) Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium; 4) Moorfields Eye Hospital, London, UK.

Autosomal dominant mutations in *BEST1* are associated with retinal phenotypic heterogeneity. Best disease is a macular dystrophy caused predominantly by missense mutations and the ocular developmental disorder, ADVIRC, is casued by splicing mutations.

We describe a novel recessive phenotype in 6 families caused by homozygous or compound heterozygous mutations in *BEST1*, which we term autosomal recessive bestrophinopathy (ARB). ARB is a panretinal disorder characterised by macular abnormalities, widespread punctate flecks and progressive photoreceptor dysfunction. Like Best disease, patients had a reduced EOG light rise but distinctly had reduced and delayed full-field ERG responses.

One family has a homozygous nonsense mutation in exon 5 which we predict would cause a null phenotype through nonsense mediated decay. This is the first report of a human null bestrophin, and given the phenotypic similarity between our families, we presume the other missense mutations associated with ARB also reflect a loss of protein function.

BEST1 encodes bestrophin-1, a chloride channel expressed exclusively in the retinal pigmented epithelium. We studied the Cl^-channel activity of wildtype and ARB mutated bestrophin-1 in transfected HEK293 cells using whole-cell patch-clamping. We found that ARB mutant bestrophin-1 had a reduced channel function compared with wildtype protein. Co-transfection with wildtype bestrophin-1 did not inhibit the wildtype channel activity, in contrast to experiments with Best disease mutant constructs. These data are consistent with the recessive nature of ARB. These findings have important implications for genetic testing and counseling, and helps in the understanding of the molecular mechanisms underlying the pathogenesis of *BEST1* associated disease.

660/W

Clinical phenotype of adult patients with X-linked α-thalassemia/ Mental Retardation (ATR-X) Syndrome. *T. Wada[1], Y. Fukushima[1], S. Saitoh[2].* 1) Dept Medical Genetics, Shinshu Univ Sch Medicine, Matsumoto, Japan; 2) Dept Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan.

[Introduction] The dysregulation of epigenetics is one of the most important causes of mental retardation. ATR-X syndrome (OMIM #301040) is among syndromic X-linked mental retardation (MR), and is characterized by male patients, severe MR, dysmorphic facies, presence of HbH inclusion, genital and skeletal abnormality and characteristic behavior. The mutations of the *ATRX* gene on Xq13 cause this syndrome, as well as non-specific MR in both male and female. The *ATRX* gene encodes ATRX protein, which is thought to be involved in chromatin remodeling. However, the pathogenesis of this syndrome remains to be elucidated. More than 160 patients, including 40 cases in Japan, have been diagnosed as ATR-X, but we have little information about the natural history of this syndrome. Here we report the clinical phenotype of the adult patients with this syndrome. [Subjects] Twelve adult ATR-X patients, (Age range when evaluated 18-35 Yr; mean 23.8Yr), whose mutations in the *ATRX* gene were confirmed, were clinically evaluated. [Results] All patients had severe MR without expression of any meaningful words. Most of all showed autistic-like behavior, including little interest in those around them, avoidance of eye contact, or repetitive stereotype movement. All but one were not able to stand or walk without aid, while the one was able to walk alone, communicate non-verbally with others, and do his minimal daily life by himself. Nobody had psychiatric problems to be needed for medication. [Discussion] Considering that in general not all patients with severe mental retardation show autistic behavior, ATR-X shows a distinctive phenotype with severe MR and autistic behavior. Interestingly, patients with other diseases caused by the disturbance of epigenetics, such as Rett syndrome or Angelman syndrome, also show MR and autistic behavior. This suggests that ATRX may have a role in neurobiological mechanisms for both MR and autism through epigenetic mechanism. It is important to establish the natural history of the syndrome not only for clinical practice but also for basic research.

661/W

Case report: A girl with 46, XX, der (18, 21) (q10, q10). *S.M. Seyedhassani[1,2], S.M. Kalantar[1], T. Akhavan Karbasi[1].* 1) Medical Genetics Dept, Res/Clinical Ctr Infertility,Yazd, Iran; 2) Medical Genetics Dept,National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

The case is a 7 years old girl that was referred to clinic of genetic with learning problem and ptosis. The suggested diagnosis was myasthenia. She was borne from the five degree familial marriage with inbreeding coefficient 1/32. There were ptosis, poor feeding and mouth breathing at the birth, so that, she was in intensive care unit for 8 days. Past history also showed delayed development, such as walking in month 17 and speaking in 3 years old. Intelligent quantity recently is done and was 50. In physical examination; ptosis, sparse/lateral hypoplasia of eyebrows, dental caries, high arch, low set ear and refractive disorder of the eyes are seen. Tensilon test was negative and biochemical muscular test and blood aminoacid had normal patterns. Cytogenetic study is reported as 46, XX, der (18, 21) (q10, q10). Parents chromosomal study was normal. This case is documented and illustrated.

662/W

A boy with 46,XY,dup(16)(q22.1q23.1) and the ATR-X phenotype. *T. Tokutomi[1, 2, 3], T. Wada[4], M. Sasaki[3], E. Nakagawa[1, 3], S. Saitoh[5], M. Mukaida[2], Y. Goto[1].* 1) Dept. Mental Retardation & Birth Defect Research, National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan; 2) Dept. Forensic Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan; 3) Dept. Child Neurology, Musashi Hospital, NCNP, Kodaira, Tokyo, Japan; 4) Dept. Medical Genetics, Shinshu Univ., Matsumoto, Nagano, Japan; 5) Dept. Pediatrics, Hokkaido Univ., Sapporo, Hokkaido, Japan.

INTRODUCTION: The combination of α-thalassemia and mental retardation (ATR) is recognized in two distinct syndromes; ATR-X (MIM #301040) and ATR-16 (MIM #141750). ATR-X results from mutations of a putative chromatin remodeling factor encoded by *ATRX* at Xq13.3. ATR-16 is caused by a contiguous gene deletion involving the α-globin genes at 16p13.3. The presence of β-globin tetramers (HbH inclusions) in peripheral blood was originally used to define ATR-X. An ATR-X phenotype without α-thalassemia, however, was reported to be associated with *XNP* mutation, splicing mutation in *ATRX*, or a segmental duplication spanning chromosome 16p13.11-p13.3.

CLINICAL REPORT: The patient, a 10-year-old boy, closely resembled the phenotype of ATR-X except α-thalassemia. He showed characteristic clinical features including severe mental retardation, hypotonia, short stature, and characteristic facies. He had no evidence of HbH inclusions.

CYTOGENETIC ANALYSIS: G-banding and high-resolution chromosome analysis in the boy demonstrated an abnormal chromosome 16 with q22.1-q23.1 duplication. His parents had normal karyotypes. FISH using an internal BAC clone RP11-485F09 at 16q22.2 confirmed the duplication. Subtelomeric FISH analyses for 16p and 16q were intact.

DISCUSSION: We report a potential association between ATR-X phenotype and dup(16)(q22.1q23.1) in a patient. ATR has not previously been associated with duplication of 16q. Further precise analysis of the genomic region spanning this segmental duplication may identify novel genes possibly involved in pathways regulated by ATRX.

663/W

The Molecular Etiology of Peters anomaly, Microcornea, and Cataracts, in Family R0023, A Newfoundland Family. *L. Doucette, J. Green, B. Fernandez, T.L. Young.* Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada.

Introduction A Newfoundland family R0023 exhibits an autosomal dominant form of anterior segment dysgenesis, a disorder of the anterior eye segment. In this family, one individual is afflicted with Peters anomaly, a rare disorder characterized by iris-lens, lens-cornea adhesions, and corneal opacities, and is considered one of the most severe phenotypes of anterior segment dysgenesis. Other affected individuals exhibit microcornea, or congenital cataracts, less severe phenotypes of this disorder. Due to the Mendelian pattern of autosomal dominant inheritance seen in this family, we believe that this varying phenotype in affected relatives is caused by mutation of a single gene involved in the early stages of anterior segment development. Both genomic and cDNA will be obtained from affected family members to directly sequence 5 genes associated with Peters anomaly and anterior segment dysgenesis (namely PAX6, PITX2, PITX3, FOXC1, and CYP1B1). If causative mutations are not found in these 5 functional candidate genes a genome wide scan will be performed in order to determine the molecular etiology of Peters anomaly with cataracts and microcornea in this Newfoundland family. To date, the coding and untranslated regions and their intron/exon boundaries of 4 of these 5 genes (PAX6, PITX2, PITX3, and FOXC1) have been directly sequenced in 7 selected individuals from this family (5 affected, 2 unaffected). A number of SNPs have been identified at this point, but no causative mutations have been found in these four genes.

664/W

Founder Mutation in the PEX2 (PXMP3) Gene in the Jewish Karaite Population in Israel. *A. Singer[1], R.J.A. Wanders[2], A. Zung[3], C. Vinkler[4].* 1) Genetics Institute, Barzilai Medical Ctr, Ashkelon, Israel; 2) Department of Pediatrics, Academic Medical Centre, Emma Children's Hospital, University of Amsterdam, Amsterdam, The Netherlands; 3) Pediatric Endocrine Unit, Kaplan Hospital, Rehovot 76100, Israel; 4) Institute of Clinical Genetics, E. Wolfson Medical Center, Holon, Israel.

Zellweger syndrome (ZS), is the most severe form of the peroxisome biogenesis disorders (PBD). Milder phenotypes in the Zellweger spectrum are neonatal adrenoleukodystrophy (NALD); and infantile Refsum disease (IRD), the least severe. The clinical presentation in the neonatal period include profound hypotonia, characteristic facies, seizures, inability to feed, liver cysts with hepatic dysfunction, and chondrodysplasia punctata. Infants with this condition are significantly impaired and usually die during the first year of life, having made no developmental progress. Death is secondary to progressive apnea or respiratory compromise from infection in most cases. Prevalence of PBD is estimated to be 1:50,000 in the western population. Zellweger syndrome is inherited in an autosomal recessive mode. More than ten genes, most belong to the PEX family, were associated with PBD. The Karaites are a Jewish sect which does not recognize the authority of the post-Biblical tradition incorporated in the Talmud and in the latter Rabbinic works. In Israel the estimated number of Karaite Jews is 30-40,000. For many years they kept a close community life with high consanguinity rates and hence were at risk for genetic diseases. During the last decade we diagnosed a number of infants born to Karaite parents with neonatal ZS. Mutation analysis following complementation tests in fibroblasts and identifying the candidate gene revealed point mutation in the PEX2 gene. In all the cases studied a 550delC mutation was found. The identification of the mutation allows proper genetic counseling and prenatal diagnosis of ZS in this community. Population screening will allow us to calculate carrier rates in this community.

665/W

Identification of a point mutation associated with SMA by direct sequencing of genomic DNA. *K. Segers, V. Mathias, S. Gaillez, V. Bours.* Dept Human Genetics, CHU Sart Tilman, Liege, Belgium.

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterised by degeneration of motor neurones of the anterior horn of the spinal cord. Spinal muscular atrophy is linked to locus 5q13 in more than 95% of patients. This region, containing the SMN1 gene (Survival Motor Neurone) associated with SMA, is inverted and duplicated. SMN2 is a highly homologous gene located in the centromeric duplicated region. Homozygous deletion of SMN1, located in the telomeric position, accounts for the disease in 98% of patients and has been reported in infantile, intermediate and adult onset disease. Some small intragenic SMN1 mutations have also been described. Sequencing of the SMN1 gene at the genomic level is complicated by the presence of the homologous SMN2 gene. Search for point mutation is usually performed from cloned cDNA of the SMN1 gene. Here we report the identification of a point mutation, p.Y272C, by direct sequencing of the SMN genes at the genomic level in DNA stored from a deceased baby affected by Werdnig Hoffmann syndrome. This analysis permit to confirm the clinical diagnostic and to identify SMA carrier in the family. This result shows the relevance of performing sequencing at genomic level for SMA diagnostic.

666/W

Behavioral features in patients with FG (Opizt-Kaveggia) syndrome and a recurrent mutation, p.R961W, in the MED12 gene. *J. Graham[1], J. Visootsak[2], E. Dykens[3], R. Clark[4], K. Jones[5], J. Moeschler[6], R. Rogers[7], C. Schwartz[7], M. Friez[7], R. Stevenson[7].* 1) Cedars-Sinai Medical Center, Los Angeles, CA; 2) Emory University Medical Center, Atlanta, GA; 3) Vanderbilt University Medical Center, Nashville, TN; 4) Loma Linda University Children's Hospital, Loma Linda, CA; 5) Department of Pediatrics, UCSD School of Medicine, San Diego, CA; 6) Dartmouth-Hitchcock Medical Center, Lebanon, NH; 7) Greenwood Genetic Center, Greenwood, SC.

Opitz and Kaveggia (1974) reported a family of males with X-linked mental retardation, macrocephaly, imperforate anus and hypotonia. Risheg et al. (2007) identified an identical nucleotide substitution in exon 21 of MED12 causing tryptophan to replace arginine at amino acid 961 (p.R961W) in 6 families with FG (Opitz-Kaveggia) syndrome, including a surviving affected male from the original family. The previously defined behavioral phenotype consists of hyperactivity, affability, and socially-oriented attention-seeking behaviors. We conducted behavioral assessments on 10 patients with FG syndrome caused by this recurrent mutation, and compared their characteristics with data from individuals with Down syndrome (DS), Prader-Willi syndrome (PWS), non-specific mental retardation (NSMR), and Williams syndrome (WS), using the Vineland Adaptive Behavior Scales, the Reiss Profile of Fundamental Goals and Motivation Sensitivities, and the Achenbach Child Behavior Checklist. In our previous studies using clinically diagnosed patients with FG Syndrome, FG boys were significantly less anxious and withdrawn, but had similar socially-oriented, attention-seeking behaviors, when compared with WS. FG boys were physically more energetic and more curious than WS boys, with more need for order, while FG boys appeared less sensitive to pain, somatic complaints, rejection, and slights from others than WS boys. FG boys demonstrated significant relative strengths in their socialization skills, consistent with their personality.

667/W

Machado-Joseph Disease enhances genetic fitness: a comparison between affected and unaffected women between MJD and the general population. *P.R. Prestes[1,3], M.L.S. Pereira[1,2], I. Silveira[3], J. Sequeiros[3], R. Giugliani[1,4], L.B. Jardim[1,5].* 1) Medical Genetics Service, Hospital de Clinicas, Porto Alegre, RS, Brazil; 2) Department of Biochemistry, UFRGS, Porto Alegre, RS, Brazil; 3) Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal; 4) Department of Genetics, UFRGS, Porto Alegre, RS, Brazil; 5) Departmento of Internal Medicine, UFRGS, Porto Alegre, RS, Brazil.

Background: Machado-Joseph disease (MJD-SCA3), a spinocerebellar ataxia related to an expansion of a CAG tract, has already been related to anticipation and meiotic drift. However, fitness of MJD carriers has been little studied. Objective: To analyze genetic fitness of MJD patients, comparing them to their unaffected relatives and to the general population (GP) of origin. Subjects and methods: 182 informants, belonging to 82 MJD families, agreed to participate in the study. Informants supplied data about 828 MJD patients. Number of children (NC), gender, age, school attainment, menarche and menopause were compared between general and emeritus (older than 45 years of age or deceased) groups. Results: Mean NC of the GP and of MJD patients were respectively 1.90 and 2.93± 2.3 ($p = 0.0037$). Comparisons within families also showed differences: the mean NC of unaffected and affected emeritus MJD women were respectively 2.68 and 3.89 ($p = 0.0037$). Affected MJD women had earlier mean ages at the delivery of their first child and menopauses ($p < 0.011$ and 0.07, respectively). Among affected women, those who did not have children had larger CAG tract than those who had children ($p<0.05$). Conclusion: MJD enhances the fitness of its carriers, and this phenomenon seems to have a biological basis.

668/W

Polymorphism at the Sp1-binding site in the collagen type I COLIA1 gene in women with pelvic organ prolapse. *S. Kim, H. Cho, M. Jeon, H. Jung, J. Choi, N. Cho, S. Bai.* OB/GYN, Col Med, Seodaemun Ku, Yonsei Univ, Seoul, Korea.

We examined the possible influence of polymorphism at the transcription factor Sp1-binding site in the gene encoding α-1 chain of type I collagen (COLIA1) on the risk of pelvic organ prolapse. From May 2006 through September 2006, 15 patients were treated for pelvic organ prolapse at Yonsei University Medical Center. Fifteen control subjects with benign gynecological condition were selected by matching with age, postmenopausal status, and body mass index. DNA was obtained from peripheral blood leukocytes. The fragment of the first intron of the COLIA1 gene of type I collagen containing the Sp1-binding site was amplified by real time polymerase chain reaction. The polymorphism was identified with LightCycler Technology with hybridization probes. The melting curve analysis represented detection and visual discrimination based on the melting temperatures of normal and mutant alleles. Sequencing reactions were performed on each template using primer. The groups were similar with respect to parity, medical history, surgical history, and smoking. The homozygous peak was noted on the melting temperature of 57°C curve analysis. Sequencing reactions confirmed the G/G alleles in the 30 specimens tested. We could not find any polymorphism at the Sp1-binding site in COLIA1 gene of type I collagen in patients with both pelvic organ prolapse and control group. Our results suggest that the polymorphism at the transcription factor Sp1-binding site in the gene encoding α-1 chain of type I collagen is unlikely to be of clinical value in identifying Korean women who are at risk of pelvic organ prolapse.

669/W

Genomic Investigation of AMD. *P.O.S. Tam[1], T.K. Ng[1], S.W.Y. Chiang[1], L.J. Chen[1,2], W.M. Chan[1], D.T.L. Liu[1], D.S.C. Lam[1], C.P. Pang[1,2].* 1) Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong; 2) SU/CUHK Joint Shantou International Eye Center, Shantou, China.

Purpose: To investigate the association of HTRA1 in the risk of exudative AMD and its interaction effect with smoking and CFH in the development of exudative AMD. Methods: The whole gene of HTRA1 was sequenced by direct sequencing to investigate the existence of genetic variants in HTRA1 contributing to the risk of exudative AMD. A total of 163 exudative AMD and 183 controls were screened in the study. Smoking and a SNP of CFH were used to investigate the gene-environment and gene-gene interaction on exudative AMD, respectively. Results: A total of 45 sequence variants were identified in HTRA1 promoter, exons and exon-intron boundaries. Among which 4 SNPs have violated HWE and were excluded for further association and haplotype analysis. For the remaining 41 SNPs, 15 variants were found only in one AMD case while 6 variants existed in only one control. This leaves 18 SNPs for further association analysis. After Bonferroni correction, four variants still remained significantly associated with exudative AMD. Carriers of two risk alleles were at substantially higher risk (about 4 times higher) to exudative AMD than are carriers with one risk allele. Three haplotype blocks were constructed with the first haplotype block that lies in the promoter and exon one region was significantly associated with exudative AMD ($p= 6.68E-14$). Results from logistic regression suggested that the joint effects of smoking and specific SNPs were best described by independent multiplicative effects, without significant dominance nor interacting effects. Estimates from this model demonstrated a 15.71 fold increased risk to exudative AMD in homozygote carriers of the HTRA1-risk allele who were ever-smokers. A joint disease odds ratio of 23.3 for individuals with homozygous risk alleles at both loci containing HTRA1 and CFH was observed when compared with the baseline wild-type (non-risk) genotype. Conclusions: The promoter and coding exons of HTRA1 possess SNPs significantly associated with exudative AMD. Smoking and variant at CFH exerted no interaction with HTRA1 to the development of exudative AMD.

670/W

Mutation detection rate and genotype-phenotype correlations in patients with mutations in Ush2A, the gene encoding for Usherin. *E. Tsilou[1], J. Schultz[2], M.R. Meltzer[1], R. Caruso[1], A. Griffith[2], A. Madeo[2], C. Brewer[2], C. Zalewski[2], T. Friedman[2].* 1) National Eye Institute, National Institutes of Health, Bethesda, MD; 2) National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD.

Usher syndrome type II (USH2) is characterized by moderate to severe high-frequency hearing impairment, intact vestibular responses and progressive visual loss due to retinitis pigmentosa. Four loci are known for USH2, and three genes have been identified. USH2A is the most frequent molecular subtype of Usher syndrome. The purpose of this study was to define the mutation detection rate of USH2A and the clinical characteristics of patients with mutations in this gene. Mutation analysis of USH2A was performed in 19 patients with clinical characteristics of Usher syndrome type II (age range: 11 to 65 years, mean age of 40 years). All patients underwent audiologic, vestibular and ophthalmic evaluation. From the 19 patients, 16 total patients (84%) were found to have mutations in USH2A. 7 patients (37%) had two USH2A mutations identified, while 9 (47%) patients had only one USH2A mutation that we could find. 9 of these mutations were novel. All 16 patients had audiologic and vestibular responses consistent with Usher type II. Two patients were found to have vertical nystagmus. Both patients had normal brain imaging studies. Mean age of perceived night blindness was 18.7 years and mean age of retinitis pigmentosa diagnosis was 23 years. Visual acuity ranged from 20/16 to light perception and mean LogMar visual acuity for patients with measurable visual acuity was 0.39 (Snellen equivalent of 20/50). 10 of 16 patients (62.5%) had cataract in at least one eye. All patients had some degree of visual field constriction and moderately to severely reduced photopic and scotopic responses consistent with retinal degeneration. The degree of visual field constriction was dependent on age. Mutation detection rate and clinical findings in our patients are similar to a previously reported series. Long-term follow-up of USH2A patients are underway to better define the rate of retinitis pigmentosa progression in this group of patients.

671/W

Novel GJB2 mutations are associated with autosomal recessive non syndromic hearing loss. *E. Farrokhi[1], M. Hashemzadeh Chaleshtori[1], M. Shahrani[1], M. Dolati[2], L. Hoghoogi Rad[2], H. Pour-jafari[3], D. Farhud[4], A. Crosby[5], K. Ghatreh samani[1], M. Mansouri[1], D. Modaresinia[1], M. Jafari[1].* 1) Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran; 2) Department of Genetics,School of Medical Sciences,Qom University of Medical sciences,Qom,Iran; 3) Department of Genetics,School of Medicine, Hamadan University of Medicil Sciences, Hamadan, Iran; 4) Department of Genetics,School of Public Health,Tehran University of Medical Sciences, Tehran, Iran; 5) Medical Genetics,St Georges Hospital Medical School, University of London, London, United Kingdom.

Mutations of GJB2 gene encoding connexion 26 are the most common cause of hearing loss in many populatiions. We have provided evidence on the pathogenicity of our previously reported GJB2 allelic variants. We described the possibility of pathogenicity for the GJB2 allelic variants including 363delC, 327delGGinsA, H16R and G200R which have been co segregated with autosomal recessive and sporadic non syndromic hearing loss in different families and are not found in control subjects. We also found G130V and K102Q in heterozygous state in two deaf individuals. G130V results in an exchange a residue highly conserved among all the connexins but was found with a rate of 1% in control subjects and K102Q results in an exchange a residue not conserved among all the connexins and not identified in 100 control subjects. We conclude that, 363delC, 327delGGinsA, H16R and G200R may be pathogenic. However, the pathogenicity and inheritance of K102Q and G130V can not be assessed clearly and remains to be identified.

672/W

A series of 60 rhombencephalosynapsis cases and CGH array results lead to new phenotype and genotype considerations. *L. Pasquier[1,2], C. Bendavid[2], P. Loget[3], C. Dubourg[2], S. Jaillard[2,4], C. Henry[4], J. Lucas[4], J. Lespinasse[5], C. de la Rochebrochard[1], P. Marcorelles[6], F. Pelluard[7], D. Carles[7], M. Ferry[8], C. Fallet-Bianco[9], S. Odent[1], A. Laquerrière[10], V. David[2].* 1) Dept Clinical Genetics, Rennes Univ Hosp, Rennes, France; 2) Molecular genetics unit and UMR 6061 CNRS, IFR 140 GFAS, Rennes Univ Hosp, France; 3) Pathology laboratory, Le Mans Hosp, France; 4) Cytogenetics unit, Rennes Univ Hosp, France; 5) Cytogenetics unit, Chambéry, France; 6) Pathology laboratory, Brest Univ Hosp, France; 7) Pathology laboratory, Bordeaux Univ Hosp, France; 8) Radiology unit, Rennes Univ Hosp, France; 9) Pathology laboratory, Saint-Anne Hosp, Paris, France; 10) Pathology laboratory, Rouen Univ Hosp, France.

Rhombencephalosynapsis (RES) is a rare cerebellar malformation described by vermian agenesis, fusion of the hemispheres and dentate nuclei. RES is usually described as an isolated malformation which developmental and cognitive impairment are fuzzy. Except for 2 cases with chromosomal anomalies, genetic background is currently unknown. We initiated a database of RES cases throughout France to review the phenotype carefully including familial, clinical, radiological and pathological patterns. To date, 55 foetuses and 5 children were included and recurrences lead us to suggest phenotypical entities: 1- isolated, 2- syndromic with VACTER association, 3- syndromic with other cerebral malformations as Neural Tube Defect (NTD) or Holoprosencephaly (HPE), 4- others syndromic conditions (Gomez-Lopez-Hernandez syndrome was suspected once only). All children have motor delay and cognitive impairment, one with hypopituitarism and another with cataracts and polysyndactyly. Pathological review of cases confirmed rhombencephalosynapsis, isolated or associated with other cerebral lesions responsible for hydrocephalus. When DNA samples were available, CGH array technology was carried out leading to the discovery of 3 de novo microrearrangments. 2 cases of familial recurrences and 2 cases of consanguinity support the idea of a great genetic heterogeneity.

673/W

CLINICAL-GENETIC STUDY AND FOLLOW-UP OF INSTITUTIONALIZED PATIENTS FOR ESTABLISHING DIAGNOSIS AND RISK FACTORS FOR MENTAL RETARDATION. *L. Batista, L. Giuliani, J.M. Pina, G. Molfetta.* Dept Medical Genetics, Hosp das Clinicas - USP, Sao Paulo, Brazil.

In developed countries, mental retardation (MR) occurs in 2-3% of general population, this figure reaches up to 10% in developing countries. It is important to define the etiopathogenesis of this disorder in order to improve the treatment, to try to establish an accurate recurrence risk and to provide correct genetic counseling to families. Also, for inherited causes of MR, X-linked mental retardation is responsible for about 20-25% of cases in males, which the most common causes are FRAXA and FRAXE Syndromes. Screening for these syndromes is recommended for all children with learning disabilities or MR of unknown etiology associated to behavior disturbances or late language development. We have studied 430 institutionalized patients diagnosed as MR in a school for patients with learning disabilities. We have done an accurate birth and familial history as well as pedigree and physical examination; neuroimaging and cytogenetics exams are requested when necessary. Of the 430 patients, 70% were mild to moderate MR; 12% were severe and in the remaining 18% of patients we were not able to classify the MR severity because they had delayed neuropsychomotor development and we could not define yet if it would evolve into a MR. Etiopathogenic diagnosis was possible in 49% of the patients: 18.5% had genetic diseases divided in chromosomal disorders (15.4%) and monogenic disorders (20.2%); 3.2% had a single CNS anomaly; 16.2% showed environmental causes; 9.5% presented CNS multiple dysfunctions (MR with epilepsy or MR with brain palsy) and 0.9% had MR associated to psychosis. In order to screen for FRAXA and FRAXE syndromes, PCR technique were carried out in all male patients with unknown etiology. The laboratorial tests did not reveal positives cases for FRAXA or FRAXE syndromes. This work is important for defining the correct cause of the MR as well as defining better preventive approaching strategies for the families of the affect patient. It is also important to perform genetic counseling to every family and explain their accurate recurrence risks.

674/W

Array-based resequencing of *FMR1* in individuals presenting with a fragile X syndrome-like phenotype. *S.C. Collins, M.E. Zwick, S.T. Warren.* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome (FXS) is a common form of developmental delay resulting from the functional loss of the *FMR1* gene product, most often due to transcriptional silencing triggered by trinucleotide repeat expansion. However, there is one case in the literature of FXS caused by a missense mutation, wherein the well-characterized I304N mutation was found in a single patient with severe FXS. In comparison to this single clinically-relevant missense mutation in *FMR1*, there are 213 and 64 pathologic missense mutations respectively in *ABCD1* and *RPS6KA3*, two genes similar in size and genomic location to *FMR1*. Because there is no evidence suggesting that *FMR1* is less prone to conventional mutation than these similar genes, it is likely that a significant number of missense and nonsense mutations in *FMR1* have been missed, possibly due to the clinical laboratory standard of testing only for repeat expansion. To assess this hypothesis, we initiated resequencing of *FMR1* in males who present clinically with a FXS-like phenotype but test negative for CGG repeat expansion. All patients were diagnosed with mental retardation and demonstrated at least two additional features consistent with FXS, such as enlarged testes, FXS facies, autism-like behaviors, hyperactivity, and/or a similarly-affected male relative. Resequencing was performed with Affymetrix Resequencing Arrays (RAs), custom-designed to include all 17 exons of *FMR1* and substantial flanking sequence. RA data was analyzed with the automated statistical method ABACUS. Results from 31 patients and two controls indicate that an average of 95.9% of the nucleotides on each RA can be called with 99.9999% accuracy (i.e. quality score of 30). In this initial cohort, twelve novel variants were uncovered. While none of these variants are pathologic, they further define the normal sequence variation within the *FMR1* locus. Additionally, the detection of these variants provides evidence that the RA approach will successfully identify any conventional mutations in *FMR1* in this patient population. We are accepting samples into this ongoing NIH-sponsored study; more details can be found at www.fmr1resequencing.org.

675/W

Genotype, clinical presentation, neuropsychological profile, and brain pathology in 5 families with primary microcephaly due to ASPM/MCPH5 mutations. *A. Verloes[1,8], L. Titomanlio[2], F. Guimiot[3], A. Afenjar[4], L. Burglen[4], T. Billette deVillemeur[5], J-F. Gadisseux[1], S. Odent[6], A. Megarbane[7], B. Gerard[1].* 1) Genetics dept; 2) Child neurology dept; 3) Fetal pathology dept, Robert Debre Hospital, Paris, France; 4) Genetics dept,; 5) Child neurology dept, Trousseau Hospital, Paris, France; 6) Genetics dept, Rennes University, Rennes, France; 7) Genetics dept, Saint Joseph University, Beirut, Lebanon; 8) INSERM U676, Robert DEBRE hospital, Paris.

Human recessive primary microcephalies (MCPH) count at least six loci (MCPH1-6). MCPH5, caused by the ASPM gene(1q31) gene mutations is the most commonly involved. We report new mutations in 5 families with MCPH5. In family 1, the proband was born with an OFC of 32 cm. At age 4, OFC is -5SD and height is at -1SD. The boy shows mild MR. A further pregnancy was terminated. Neuropathology showed a small brain (biometry of 27-28 GW for 33 GW) with a simplified gyral pattern for age, decreased neuronal population and premature depletion of the germinal zone. In family 2, 2 brothers were affected with different severity. The eldest one, aged 25, had an OFC < -8SD. His IQ was 55, with homogeneous scores and preserved memory functions. The youngest, aged 10, has an OFC at -5SD. His IQ was 70 with normal memory functioning but weaknesses in executive functions. In family 3, 2 brothers aged 17 and 19 had microcephaly (-4 SD) with simplified gyral pattern, and mild to moderate MR. One of the sibs had seizures at age 14. In family 4, the affected girl was born with an OFC of 30cm. At age 12, OFC was at -7SD and associated with hypotelorism. Clinical and neuropsy details on the last family (with several affected patients) are currently gathered, and will be presented. Systematic sequencing of the whole coding sequence of ASPM demonstrated compound heterozygocyty or homozygocyty for non-sense mutations in the 5 families. Our patients illustrate inter- and intrafamilial variability of ASPM mutants, confirm surprisingly good preservation of cognition despite major reduction in brain size in some of them, and confirm the absence of specific histological anomalies of brain in ASPM-related MCPH.

676/W

The Xq28 inversion breakpoint interrupted a novel noncoding gene in a patient with Duchenne muscular dystrophy with severe mental retardation. *M. Yagi[1], H.T. Thi Tran[1], Z. Zhang[1], A. Nishiyama[1], Y. Oyazato[1], T. Okinaga[2], Y. Takeshima[1], M. Matsuo[1].* 1) Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; 2) Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan.

[Background] Duchenne Muscular Dystrophy (DMD) is the most common inherited muscle wasting disease and caused by mutation of the *dystrophin* gene. In one third of DMD patients, mental retardation (MR) is complicated. However exact mechanism that leads to MR is not yet known. We identified a pericentric inversion of the X chromosome in a DMD patient with severe MR and disruption of a novel noncoding gene cloned from the inversion breakpoint. **[Case]** The proband is a three-year-old Japanese boy. He was pointed out high CK nemia (25,510IU/l) at birth. At one year old muscle biopsy was performed to disclose no dystrophin staining and he was diagnosed as DMD. He complicated severe MR. **[Results and Discussion]** Neither Southern blot analysis nor sequencing of each 79 exons disclosed any responsible mutation in the *dystrophin* gene. A karyotyping disclosed 46,Y,inv(X)(p21.2q28). The breakpoint in Xp21.2 was supposed be within the *dystrophin* gene and dystrophin mRNA was found to be separated into two parts between exons 18 and 19. RACE assays was performed to clone the breakpoint in Xq28. 5'RACE analysis extending from exon 19 revealed an unknown fragment that was completely identical to the region of the Xq28. 3'RACE analysis extending from exon 18 revealed another unknown sequence that consisted of two separate parts derived from the two regions in Xq28. These three cloned sequences maintained characteristics of exons and the last one contained a polyadenylation signal, followed by poly A tail. Therefore, these three sequences represent a gene. However, this gene did not match any annotated gene but had no open reading frame, concluding a noncoding gene. As a conclusion the inversion breakpoints of inv(X)(p21.2q28) disrupted not only the *dystrophin* gene but also a novel noncoding gene located at Xq28. It was supposed that disruption of the novel gene located at Xq28 was responsible for the patient's severe MR.

677/W

Mannose binding lectin codon 54 polymorphism is associated with predisposition to Henoch-Schonlein purpura in childhood. *B. Durmaz[1], F. Ozkinay[1], M. Bak[2], A. Aykut[1], E. Serdaroglu[2], H. Onay[3], C. Ozkinay[3].* 1) Department of Pediatrics, Division of Genetics and Teratology, Ege University, Faculty of Medicine, Izmir, Turkey; 2) Department of Pediatrics, Behcet Uz Children's Hospital, Izmir, Turkey; 3) Department of Medical Genetics, Ege University, Faculty of Medicine, Izmir, Turkey.

Henoch-Schonlein purpura (HSP) is a systemic small vessel vasculitis characterized by purpura, arthritis, abdominal pain, and hematuria. It is acknowledged to be a type of hypersensitivity vasculitis with an abnormal inflammatory response within the blood vessel, however the etiology of HSP remains unknown. Mannose binding lectin (MBL) is a calcium dependent lectin that has an important role in innate immunity. The aim of this study is to determine the presence of any association between MBL gene variants and HSP in the child population. Codon 54 (allele B) polymorphism in the exon 1 of the MBL gene was investigated by PCR-RFLP method in 66 children diagnosed as HSP (mean age: 10.0±2.9) and 86 age matched healthy controls. The mutant B allele frequency was significantly higher in the patient group (17.4%) compared to the control group (8.1%); (p=0.014). AB genotype was found to be 28.8% and 14.0% in patient group and healthy control group respectively where the difference was statistically significant (p=0.024). AA genotype was found in 68.2% of the children with HSP and 84.9% of the healthy control group (p=0.014). These results suggest that codon 54 polymorphism in the MBL gene may play a role in susceptibility to HSP in children.

678/W

A neurological examination score for the assessment of spinocerebellar ataxia. *L. Jardim[1,3], C.R.M. Rieder[2], A. Chaves[1], C.R. Cecchin[1], T.L. Monte[2], R. Giugliani[1,4], C. Kieling[1].* 1) Medical Genetics Service, Hospital de Clinicas, Porto Alegre, RS, Brazil; 2) Neurology Service, Hospital de Clinicas, Porto Alegre, RS, Brazil; 3) Department of Internal Medicine, UFRGS, RS, Brazil; 4) Department of Genetics, UFRGS, RS, Brazil.

Objective: Spinocerebellar ataxias are a group of autosomal dominant disorders characterized by a highly heterogeneous set of genetic and clinical manifestations. The purpose of this work was to assess the neurological features of spinocerebellar ataxias, and to describe and test the feasibility, reliability and validity of a comprehensive neurological examination score (NESSCA). Methods: The NESSCA was administered to patients who were molecularly diagnosed with spinocerebellar ataxia type 3 (SCA3) at an outpatient neurogenetics clinic. The scale, based on the standardized neurological examination, consisted of 18 items that yielded a total score ranging from 0 to 40. The instrument's interrater reliability and internal consistency were investigated, and a principal components analysis and correlation with external measures were performed. Results: Ninety-nine individuals from 58 families were evaluated. Interrater reliability ranged from 0.8 to 1 across individual items ($p<0.001$); internal consistency, indicated by Cronbach's alpha, was 0.77. NESSCA scores were significantly correlated with objective measures of disease severity: disease stage ($rho=0.76$, $p<0.001$), duration ($rho=0.56$, $p<0.001$), and length of CAG tract ($rho=0.30$, $p<0.05$). Discussion: The NESSCA is a reliable instrument for the assessment of distinct neurological deficits in SCA3 patients. Global scores correlated with all external variables tested, showing the NESSCA to be a comprehensive measure of disease severity that is both clinically useful and scientifically valid. Further studies are required to evaluate its correlation with recently developed ataxia rating scales, as well as its suitability for rating other SCA.

679/W

Linkage of posterior amorphous corneal dystrophy to chromosomes 8q21.3-8q24.13 and 12q21.33-12q24.21 and exclusion of coding region mutations in *KERA*, *LUM*, *DCN* and *EPYC*. *A. Aldave[1], G. Rosenwasser[2], V. Yellore[1], J. Papp[3], E. Sobel[3], M. Chen[1], S. Rayner[1], J. Sassani[4].* 1) Cornea Service, Jules Stein Eye Inst/UCLA, Los Angeles, CA; 2) The Central Pennsylvania Eye Institute, Hershey, Pennsylvania; 3) Department of Human Genetics, David Geffen School of Medicine at UCLA; 4) Department of Ophthalmology, Milton S. Hershey Medical Center, Hershey, PA.

Purpose: To identify the genetic basis of posterior amorphous corneal dystrophy (PACD) through the performance of a genome-wide linkage analysis and to describe the clinical and histopathologic features of a large pedigree with PACD. **Methods**: Slit lamp examination of each study subject was performed to determine the affected status. Corneal pachymetry and topography were performed in affected individuals, and light and electron microscopic examination of corneal buttons excised at the time of penetrating keratoplasty were performed. DNA was obtained from affected and unaffected subjects, and a genome-wide linkage analysis was performed. The coding region of four positional and functional candidate genes, *KERA*, *LUM*, *DCN* and *EPYC*, were screened in affected and unaffected family members. **Results**: Slit lamp examination and DNA collection was performed for 53 individuals, 15 of whom were diagnosed as affected based on the presence of characteristic clinical features of PACD. Histopathologic examination of excised corneal specimens demonstrated disorganized stromal lamellae, and stromal staining with colloidal iron, but no staining with alcian blue or periodic acid-Schiff. A genome-wide linkage analysis demonstrated significant evidence of linkage with both single point and multipoint analyses to chromosomes 8 and 12, with the largest single point HLOD score of 3.05 obtained at marker D8S1784 and 2.92 obtained at marker D12S351. The largest multipoint HLOD scores obtained were 3.12 at D81784 and 3.44 at D12S78. The support intervals for PACD in the family we report are approximately 26 cM on chromosome 8, between the flanking markers D8S270 and D8S514, and approximately 30 cM on chromosome 12, between the flanking markers D12S351 and D12S79. No coding region mutations were identified in *KERA*, *LUM*, *DCN* or *EPYC*, which are located adjacent to D12S351. **Conclusions**: PACD is associated with characteristic corneal and extracorneal anterior segment abnormalities, indicating that PACD should be classified as an anterior segment dysgenesis, rather than a corneal dystrophy. Two genetic loci, a 26 cM interval on chromosome 8q and a 30 cM interval on chromosome 12q, have been identified, and coding region mutations have been excluded in several positional and functional candidate genes on chromosome 12q.

680/W

The importance of recurrent *CEP290* mutations for first-pass mutation screening in Leber Congenital Amaurosis. *F. Coppieters[1], T. de Ravel[2], I. Casteels[3], F. Meire[4], N. Van Regemorter[5], S. De Jaegere[1], A. De Paepe[1], P. Coucke[1], B.P. Leroy[1,6], E. De Baere[1].* 1) Ctr for Medical Genetics, Ghent Univ Hosp, Ghent, Belgium; 2) Ctr for Human Genetics, Catholic Leuven University, Belgium; 3) Dept of Ophthalmol, Catholic Leuven University, Belgium; 4) HUDERF, Brussels, Belgium; 5) Centre de Génétique, Free University of Brussels, Belgium; 6) Dept of Ophthalmol, Ghent Univ Hosp, Belgium.

Recently, the *CEP290* (*NPHP6*) gene was identified as a novel gene for Leber Congenital Amaurosis (LCA), representing one of the most frequent causes of LCA. The major objective of this study was to determine the proportion of *CEP290* mutations in a cohort of 62 LCA patients, mainly of Belgian origin, in whom a first-pass mutation screening was negative for mutations in known LCA genes.

At first, we screened for the recurrent *CEP290* mutation c.2991+1655A>G. This mutation was identified in 17/62 patients (27%), of which 2 were homozygous and 15 heterozygous. Sequencing of the total coding region revealed a second mutation in 14/15 patients. Secondly, the 45 LCA patients who did not carry c.2991+1655A>G, were screened for 4 other recurrent *CEP290* mutations (c.[3310-1G>A;3310C>A], c.5587-1G>C, p.Lys1575X and p.Thr1722GlnfsX2). We found 3 distinct heterozygous mutations in 7 individuals, showing that c.2991+1655A>G is not present in all *CEP290*-related LCA cases. A second mutation was identified in 2 cases. In one patient no second mutation was found. Screening of the remaining 4 is ongoing. Overall, 10 novel *CEP290* mutations were identified. Individuals in whom no second *CEP290* mutation could be identified by screening of the coding region at the genomic level, are being analysed by cDNA screening.

These findings confirm the importance of *CEP290* in LCA, as its mutations were identified in 24/112 (21%) patients from our entire LCA patient population. In addition, we have shown that a significant fraction of mutations (24/62; 39%) can be found through screening of only 4 recurrent mutations in our pre-screened mutation-negative LCA cohort.

681/W

Association between the p53 codon 72 polymorphism and primary open angle glaucoma in the Japanese population. *F. Mabuchi[1], K. Kashiwagi[1], Z. Yamagata[2], H. Iijima[1], S. Tsukahara[1].* 1) Dept Ophthalmology, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Dept Health Sciences, Univ Yamanashi, Chuo, Yamanashi, Japan.

Purpose Previous studies looking at the association of the p53 gene polymorphism and primary open angle glaucoma (POAG) revealed conflicting results. Additionally, there have been no studies in the Japanese population. We thus assessed whether genetic polymorphism of p53 was associated with POAG in the Japanese population.

Methods Genomic DNA was examined in a cohort of 426 Japanese patients with POAG, including 213 patients with normal tension glaucoma (NTG) and 213 patients with high tension glaucoma (HTG), and 188 control subjects. The average age was 63.7 ± 13.6 years (mean ± SD) for the NTG patients, 62.9 ± 14.8 years for the HTG patients, and 65.9 ± 11.2 years for the control subjects. The p53 genotype (a G to C substitution at codon 72 which changes an arginine to a proline residue) was determined using allele specific primer PCR analysis, and compared between POAG patients and control subjects.

Results No significant difference (NTG vs. control, P = 0.98, and HTG vs. control, P = 0.69, Chi-square test) was observed regarding the frequencies of the p53 genotype between the NTG (GG: 43.2%, GC: 44.6%, CC: 12.2%) or HTG (GG: 40.4%, GC: 47.9%, CC: 11.7%) patients and the control subjects (GG: 44.1%, GC: 43.6%, CC: 12.3%). Additionally, there was no significant difference (NTG vs. control, P = 0.94; and HTG vs. control, P = 0.66, Fisher's exact test) in the frequencies of the p53 alleles between the NTG (G allele: 65.5%, C allele: 34.5%) or HTG (G allele: 64.3%, C allele: 35.7%) patients and the control subjects (G allele: 66.0%, C allele: 34.0%).

Conclusion The p53 codon 72 polymorphism was not found to be associated with POAG in the Japanese population. Further studies in the other ethnic populations should be performed to elucidate the relationship between the p53 gene and POAG.

682/W

Persistent Hyperplastic Primary Vitreous and Tuberous Sclerosis. *J.G. Pappas[1], K. Daley[1], M.A. Steele[2].* 1) Pediatrics, Human Genetics, NYU, School of Medicine, New York, NY; 2) Ophthalmology, NYU, School of Medicine, New York, NY.

We describe a 5 month old boy that presented at birth with left leukocoria and left microphthalmia. Ophthalmology evaluations including slit lamp, ultrasound and visual evoked response. The right eye was normal for age. The examination of the left eye revealed persistent fetal vasculature from the optic disc to the posterior lens capsule, severe cornea opacification with reduced corneal diameter and disorganized anterior chamber and the diagnosis of persistent hyperplastic primary vitreous (PHPV) was made. The patient was referred to us because of seizures at age 3 months and Woods lamp examination of the skin reveled hypopigmented spots. The brain MRI revealed scattered patchy areas of abnormal signal within both cerebral hemispheres, predominantly involving the gray and subcortical white matter consistent with tubers as well as nodular contour of the bodies of the lateral ventricles consistent with subependymal nodules. It also reported left sided phthisis bulbi. The findings were typical of tuberous sclerosis (TS). DNA sequencing of the TSC1 and TSC2 genes revealed no mutations in TSC1 and a deletion of T at position 3218 of the exon 10 of the TSC2 gene. Renal sonogram revealed multiple renal cysts and angiomyolipomata in both kidneys consistent with TSC2 associated TS. One case of PHPV in a child with retinal tumor and TS has been reported in the medical literature (Milot J et al 1999). The etiology of unilateral PHPV is unknown and it is not hereditary. PHPV usually occurs together with other ocular abnormalities and it has been described in the autosomal recessive oculopalatocerebral syndrome. Our case is the second reported case of PHPV in tuberous sclerosis. PHPV is readily recognizable in the newborn and we suggest examination of newborns with PHPV for ocular, cutaneous and other signs of TS.

683/W

Opitz-Kaveggia (FG) syndrome revisited: The clinical phenotype in 10 affected males with MED12 mutation R961W. *R.D. Clark[1], J.M. Graham[2], R.E. Stevenson[3], R.C. Rogers[3], K.L. Jones[4], J.B. Moeschler[5], M.J. Friez[3], C.E. Schwartz[3].* 1) Division of Genetics, Department of Pediatrics, Loma Linda University Children's Hospital, Loma Linda, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Greenwood Genetic Center, Greenwood, SC; 4) Department of Pediatrics, Division of Dysmorphology, UCSD School of Medicine, San Diego CA; 5) Clinical Genetics, Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Given the recent report by Risheg et al. that a recurrent R961W mutation in MED12 causes Opitz-Kaveggia (FG) syndrome, we examined 10 affected males in 7 families to delineate the phenotype of this X-linked mental retardation syndrome. Eight of the 10 cases were previously reported including one 40-year old man from the original FG family and 2 brothers published by Keller et al. (1976). All mothers were heterozygotes for the R961W mutation. Heterozygote females had normal intelligence. The cases show a striking resemblance most evident in childhood. Typical features are small, simple, prominent, cupped or low set ears, narrow external auditory canals, frontal upsweep of hair, dolicocephaly, tall forehead, flat broad thumbs, distally adherent nails and hypotonia. Short stature and relative macrocephaly are common but normal height and HC are also seen. Several cases had anal anomalies, cardiac lesions, typically ASD or VSD, and hypo/aplasia of the corpus callosum. Minor anomalies include inguinal hernias, cryptorchidism, ptosis and single palmar crease. Feeding problems, tracheomalacia and constipation improved with time. Most multiplex families had miscarriages or death of affected males. Mental retardation is moderate to severe but one child has an IQ of 84. An affable personality is characteristic. We will present the evolution of the phenotype over time.

The Opitz-Kaveggia (FG) syndrome has a recognizable phenotype with characteristic facies and a social-oriented affect. Although the diagnosis can be made clinically, the extent of the phenotype is still emerging and molecular testing is recommended on suspect cases.

684/T

Single cell microRNA and mRNA profiling reveals global gene expression changes during mouse ES differentiation. *R. Tan[1], L. Bahreinifar[1], D. Ridzon[1], K. Guegler[1], W. Strauss[2], C. Chen[1].* 1) Applied Biosystems, Foster City, CA; 2) Department of Molecular, Cellular, & Developmental Biology, University of Colorado, Boulder, CO 80309.

We describe a new method for simultaneously quantifying mouse microRNA (miRNA) and target messenger RNA (mRNA) genes from each of 70 single cells. The method is based on multiplex RT, multiplex preamplification, and singleplex real-time TaqMan® PCR assays. Single cell expression signature could classify individual ES, embryoid body (EB), and somatic cells. Significant inter-cell variations of both miRNA and mRNA expression were observed within or between ES cell lines, indicating the heterogeneity of ES cells. Highest variability was observed among EB cells, demonstrating that EB cells undergo differentiation at different stages. Interestingly, expression of ES marker gene OCT4 and signaling gene Tdgf1 was absent in 3T3 and splenocyte cells, highly expressed in ES cells, and significantly reduced in EB cells. Furthermore, there is no correlation in expression levels between miRNAs and their predicted target mRNAs, supporting translational repression model. Our results gain new insight of both miRNA and mRNA expression patterns at a single cell level.

685/T

Mapping the Location of Acetylated H3 Histones as a Method to Identify Transcriptional Regulatory Elements in Risk Genes: Application to Reading Disabilities. *C. Barr[1,2,3], I. Livne-Bar[1], Z. Xu[1], T. Cate-Carter[2], R. Tannock[2], E. Kerr[2], M. Lovett[2], R. Bremner[1], J. Couto[1,2,3].* 1) Genetics and Development, Toronto Western Hosp, UHN, Toronto, ON, Canada; 2) Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, Canada; 3) Institute of Medical Sciences, University of Toronto, Toronto, Canada.

Introduction: Specific histone modifications (e.g. H3 and H4 acetylation, methylation) mark transcription regulatory elements (e.g. promoters, enhancers) and this property of histones has recently been used as a means for identifying regulatory regions across large genomic regions. Evidence for linkage/association to reading disabilities (RD) on chromosome 6p22 has been supported by multiple studies with recent studies pointing to DCDC2 and KIAA0319 as the most likely candidate. Further, a correlation with reduced gene expression and a KIAA0319 risk haplotype has been reported, indicating a change in gene expression as contributing to risk. Methods: To identify gene regulatory elements for these genes, we used chromatin immunoprecipitation to acetylated histone 3 (H3ac) coupled with genomic tiling arrays (ChIP-chip) to identify regions marked by acetylated histones across a 500 kb genomic region in mouse, syntenic to the region on 6p22. Results: We identified a region marked by H3ac spanning the first untranslated exon of KIAA0319. Five markers previously associated with RD in independent studies were located within this acetylated region. An additional 4 polymorphisms associated to RD, including the most significant marker in our families, were located within a 22 kb haplotype block that encompassed the acetylated region. Conclusions: These results support this putative regulatory region as the likely site of genetic variation contributing to RD on 6p and narrowing the region necessary to screen for the susceptibility alleles. This study is the first to use the position of modified histones as a method for identifying gene regulatory regions as the site of DNA variation contributing to a genetic trait.

686/T

***Mecp2* deficiency leads to altered *Htr2c* pre-mRNA editing and HTR2C isoform distribution in mouse hippocampus and cerebellum.** *M. Landers[1], Z. Yu[1], I. Van den Veyver[1,2].* 1) Obstetrics and Gynecology; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Rett Syndrome (RTT) is a neurodevelopmental disorder caused by mutations in *MECP2*, a methyl-CpG binding protein and transcriptional repressor. CpG methylation plays an important role in genomic imprinting since imprinted genes are regulated by regions of differentially methylated CpGs (or ICs). A well studied imprinted region is the one on chr 15q11-q13, involved in Prader-Willi (PWS) and Angelman (AS) syndromes, disorders characterized by several degreees of mental and motor retardation. Many AS cases are caused by deletions or mutations of the maternal copy of *UBE3A*. *UBE3A* regulation has been linked to a brain-specific paternally expressed antisense transcript (*UBE3AAts*) in human and mouse. *Ube3aATS* in mouse appears to be controlled by the PWS-IC and exons (*U*) upstream of *Snrpn.* By a complex splicing pattern, *Ube3aATS* also serves as host for several types of paternally expressed snoRNAs: *MBII13,MBII52* and *MBII85. MBII52* has been shown to affect pre-mRNA editing of the serotonin receptor 2C (*Htr2c*). Combinations of *Htr2c* editing (sites A,B,E,C,D) result in the expression of up to 24 HTR2C isoforms with different G protein-coupling functions. Since RTT and AS share autism-spectrum disorder features we decided to assess *MBII52* expression in a mouse model of RTT. qRT-PCR assays showed no significant differences in *MBII52* levels in hippocampus (Hp) and cerebellum (Cb) from P53-P64 *Mecp2*$^{-/y}$ and *Mecp2*$^{+/y}$ mice. We identified, however, higher edited *Htr2c* mRNA levels over sites A,B and C in *Mecp2*$^{-/y}$ Hp and lower levels over sites A,B,E and D in *Mecp2*$^{-/y}$ Cb. Further analysis revealed that hyperediting of *Htr2c* mRNA in *Mecp2*$^{-/y}$ Hp leads to a 20% increase in the levels of HTR2C INV,VNI,VNV and VSV isoforms and a 58% decrease of the unedited INI isoform. Also, altered editing levels in *Mecp2*$^{-/y}$ Cb lead to a slight decrease of the INV,VNI,VNV and VSV isoforms and a 2.5-fold increase of the INI isoform. Since HTR2C edited isoforms display a reduced ability to activate the phospholipase C signalling cascade, our results support a role for the serotonergic pathway in the pathology of RTT.

687/T

Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range regulation of active genes. *S. Peddada[1], D.H. Yasui[1], M.C. Bieda[2], R.O. Vallero[1], A. Hogart[1], R.P. Nagarajan[1], K.N. Thatcher[1], P.J. Farnham[2], J.M. LaSalle[1].* 1) Medical Microbiology and Immunology and Rowe Program in Human Genetics, School of Medicine, University of California, Davis, CA; 2) Department of Pharmacology and Genome Center, School of Medicine, University of California, Davis, CA.

Mutations in *MECP2* cause the autism-spectrum disorder Rett syndrome. MeCP2 is predicted to bind methylated promoters and silence transcription. Contrary to this model, the first large-scale mapping of neuronal MeCP2 binding sites on 26.3 Mb of target imprinted and non-imprinted loci revealed that 59% of MeCP2 binding sites are outside of genes and only 5.9% are in CpG islands. Furthermore, integrated genome-wide promoter analysis of MeCP2 binding, CpG methylation, and gene expression revealed that 66% of MeCP2-bound promoters are actively expressed and only 6% are highly methylated. *JUNB*, an immediate early gene relevant to the pathogenesis of Rett syndrome, is one example of a highly active gene whose expression is modulated by distal and proximal binding to a partially methylated promoter. Therefore, these results support a predominant role for MeCP2 as a long-range modulator rather than a proximal silencer of gene expression.

688/T

New MeCP2-target neuronal genes potentially associated with Rett syndrome. *C. Yang[1], M. Soutome[1], K. Endoh[1], S. Yokoi[2], I. Imoto[2], T. Taira[3], J. Inazawa[2], T. Kubota[1].* 1) Epigenetic Medicine, Univ Yamanashi, Yamanashi, Japan; 2) Mol Cytonetics, MRI, Tokyo Medical Dental Univ, Tokyo, Japan; 3) Mol Cell Biol, Univ Yamanashi, Yamanashi, Japan.

Rett syndrome (RTT) is an X-linked dominant disease caused by *MEPC2* mutations. MeCP2 protein is exclusively expressed in neurons in the brain and bound to the methylated promoters of genes to regulate their expression, indicating that pathogenesis of RTT is deregulation of the target genes in neurons. Although several targets have been reported, identification of MeCP2 targets remains important issue for better understanding of RTT. To identify new targets, we first searched “triple-positive” BAC clones which contain MeCP2 binding, DNA methylated and histone H3K9 dimethylated sites using ChIP-on-chip technology. Within the regions of the 22 BAC identified, we found five neuronal genes. Of these, we found that four genes had the MeCP2-binding sequence motif (CpG-A/T runs) in their upstream regions. Out of these four genes examined, we confirmed MeCP2-binding and promoter methylation in three genes (which encode two neural cell-cell interaction molecules and one transport associate molecule in neuronal dendrites) in SH-SY5Y neuronal cultured cells by ChIP analyses and bisulfite-sequencing, respectively. These preliminary data suggest that the three neuronal genes may be new MeCP2 targets and potentially contribute to symptoms in RTT, such as autism and epilepsy.

689/T

Hypomethylation of H19 differentially methylated region in a patient with a 46,XX,t(8;11)(q24.1;p15.4)pat karyotype and a Silver-Russell syndrome phenotype. *K. Izumi[1,2], Y. Morinishi[3], M. Hattori[1], K. Kosaki[1].* 1) Dept Pediatr, Keio Univ, Tokyo, Japan; 2) Dept Genetics, Case Western Reserve Univ., Cleveland, OH; 3) Dept of Pediatr, National Defence Med Coll,Tokorozawa, Japan.

Silver-Russell syndrome (SRS) is characterized by severe intrauterine growth retardation and relative macrocephaly with triangular facies and other minor malformations. Recently, a subgroup of SRS patients has been found to exhibit aberrant imprinting at the H19 differentially methylated region (DMR) on chromosome 11p15. The underlying mechanism leading to the aberrant methylation has not yet been delineated. Here we report an aberrant hypomethylation of H19 DMR in a 2-year-old girl with SRS features and a de novo balanced reciprocal translocation of which breakpoint was near the H19 locus. The patient was born at 35 weeks of gestation and had a birth weight of 1060 g. She fulfilled the Preece diagnostic criteria of SRS. G-banding analysis revealed a 46,XX,t(8;11)(q24.1;p15.4) karyotype. Reiterative FISH experiments and genome walking revealed that the breakpoint on chromosome 11 resided in an intron 1 of the MRPL23 gene, while the breakpoint on chromosome 8 resided in the midst of a large gene desert. Analsys of SNPs near the breakpoint revealed that the translocation occurred during the spermatogenesis. Methylation analysis of the H19 DMR using bisulfite sequencing revealed that only 3 (13%) of 23 randomly chosen clones were methylated across the 18 CpG sites. The observation that the presently reported proband with an SRS phenotype and a balanced translocation, of which the breakpoint was only 25K bases from the H19, had an H19 DMR that was mostly unmethylated strongly suggests that the translocation event is causally related with the aberrant matylation pattern. The balanced translocation might have separated H19 from a putative cis-acting regulatory element that is responsible for the methylation of the paternally derived H19 DMR allele. The successful precise localization of the translocation breakpoint in the present case has yielded a promising clue to the identification of such a regulatory element whose existence on the telomeric side to of H19 has never been previously.

690/T

Disruption Of Epigenetic Regulatory Elements And Chromosomal Alterations In Patients With Beckwith-Wiedemann Syndrome. *A. Smith[1,2], M. Suzuki[3], R. Thompson[3], C. Shuman[4], J. Greally[3], J. Squire[5], R. Weksberg[1,2,4].* 1) Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Inst. of Med. Science, University of Toronto, Canada; 3) Albert Einstein College of Med., Bronx, NY, USA; 4) Div of Clinical and Metabolic Genetics, Hosp for Sick Chidlren; 5) Dept of Lab Medicine and Pathobiolgy, University of Toronto, Canada.

Beckwith-Wiedemann syndrome (BWS) is characterized by somatic overgrowth, macroglossia, omphalocele, and a thousand-fold increased risk for embryonal tumors. It is associated with dysregulation of gene expression of an imprinted gene cluster on chromosome band 11p15. This dysregulation occurs by several mechanisms including changes in DNA methylation, uniparental disomy, microdeletion, duplications and translocations or inversions. The 11p15 region is divided into two domains each controlled by an imprinting centre for each domain. Imprinting centres can be differentially methylated and associated with regulatory non-coding RNA transcripts that can regulate the expression of neighboring genes in cis over large distances up to one megabase. The KCNQ1 differentially methylated region (DMR2) is found within intron 10 of KCNQ1 in Domain 2. DMR2 also contains the promoter for KCNQ1OT1, a paternally expressed, untranslated anti-sense transcript, which is believed to suppress the expression of nearby genes on the paternal chromosome. We have 9 translocation or inversion patients that have breakpoints within 500kb of DMR2. We expected that translocations and inversions associated with BWS would disrupt the imprinting centre in Domain 2; however, we found that BWS patients with translocations have normal DMR2 methylation and expression of KCNQ1OT1. Our data suggest that there are as yet unidentified mechanisms that can cause BWS and its associated tumors. Thus, we hypothesize that physical disruption of the region and regulatory signals other than DMR2 methylation and KCNQ1OT1 transcription in Domain 2 can alter imprinted gene expression in cis. Complete genomic hybridization by array using a Nimblegen custom oligonucleotide array representing 33 megabases of the p-terminal of chromosome 11 was performed on the translocations.

691/T

The relationship between cleft lip and palate and methylation of an IAP transposon insertion at Wnt9b in the A/WySn mouse model. *D.M. Juriloff[1], M.J. Harris[1], L. Gagnier[2], D.L. Mager[1,2].* 1) Dept Medical Genetics, U British Columbia, Vancouver, BC, Canada; 2) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada.

The A/WySn mouse strain has a high frequency of cleft lip and palate (CLP) of complex genetic etiology. It is a very good model for human CLP, a common birth defect with complex etiology that is poorly understood. Previously, the CLP of A/WySn was shown to be caused by the joint effect of two unlinked recessive loci, clf1 and clf2, and a very strong maternal effect of the A/WySn strain; clf1 is a malfunction of the Wnt9b gene, caused by the insertion of an IAP transposon 6.6 kb 3' of the gene (hereafter, Wnt9b-IAP). Complex inheritance patterns for other mutations due to IAP insertions are due to parental effects on methylation of their IAP. Therefore, we have examined the relationship between phenotype and methylation of this individual inserted IAP in this model. Methylation of the 5'LTR of the IAP was assessed by COBRA assay and validated by bisulfite sequencing in some cases; faces of E12 embryos, or heads of E11.5 embryos were used. A/WySn embryos with normal faces showed consistent methylation of 50% (n=10), whereas CLP littermates were consistently lower, at 0-5% (n=12). In order to detect parent-of-origin effects on methylation of the IAP, various crosses were used to produce the compound mutant, Wnt9b-IAP/Wnt9b-null, which has a high risk of CLP and has received the Wnt9b-IAP mutation from one known parent. Consistently across several experiments, phenotypically normal compound-mutant embryos had IAP methylation levels of 40-60% (n=29), whereas their CLP littermates had 0-10% (n=7). In normal embryos, the IAP from A/WySn mothers tended to be less methylated (40-55%) than the IAP from A/WySn sires (50-60%). However, nearly all CLP (with near 0% methylation) was associated with maternally-derived IAPs, and this indicates that for this IAP the population of maternal gametes as a group must be less methylated than their paternal counterpart. In summary, the complexity of heredity of CLP in the A/WySn mouse model is due to epigenetics and points to similar mechanisms in human.

692/T

Paternally Transmitted Haplotypes of the Imprinted Insulin Gene are Associated with Size for Gestational Age and Umbilical Cord IGF-II Levels. *R.M. Adkins[1], J. Krushkal[2], C. Klauser[3], E.F. Magann[4], J.C. Morrison[3], J. Fain[5], G. Somes[2].* 1) Pediatrics, University of Tennessee Health Science Center, Mephis, TN; 2) Preventive Medicine, UTHSC; 3) Obstetrics & Gynecology, Univ. MS Medical Center; 4) Naval Medical Center Portsmouth, VA; 5) Molecular Sciences, UTHSC.

Objective: To test the association between haplotypes in the insulin-IGF2 locus and both risk of small for gestational age birth and umbilical cord IGF-II levels, as well as the effect of the parental origin of haplotypes. **Subjects:** 207 pairs of healthy African-American full-term newborns and mothers were recruited from Memphis TN and Jackson MS with birth weights ranging from 2210g to 4735g. **Methods:** Six single nucleotide polymorphisms (SNPs) located in the insulin (INS) and insulin-like growth factor 2 (IGF2) genes were genotyped in all mothers and newborns. Associations of individual SNPs and inferred haplotypes in the newborns and mothers with risk of small for gestational age (SGA) birth were tested using logistic regression, and mean umbilical cord IGF-II levels were compared by ANOVA. The risk of SGA and differences in cord IGF-II were also compared according to the parental origin of haplotypes. **Results:** In newborns three INS SNPs exhibited significant ($p<0.01$) association with reduced SGA risk. Two of these SNPs also were significantly associated with umbilical cord IGF-II levels. The alternate alleles at these SNPs were associated with reduced risk of SGA when present in the mother. No maternal SNPs associated with cord IGF-II levels. When analyzed according to parental origin of haplotypes, paternally-transmitted haplotypes significantly associated with risk of SGA and cord IGF-II levels, but maternally-transmitted haplotypes were not significantly associated. **Conclusion:** Newborn genotypes for polymorphisms near the 5' end of the insulin gene are significantly associated with size for gestational age and cord IGF-II levels, with a major effect due to the paternally inherited allele, which is preferentially expressed due to imprinting. There is some evidence that complementary haplotypes confer reduced risk of SGA in mothers and newborns.

693/T

QUANTIFICATION OF microRNA DURING CENTRAL NERVOUS SYSTEM DEVELOPMENT. *D.B. Dogini, P.A.O Ribeiro, T.C. Pereira, C.S. Rocha, I. Lopes-Cendes.* Medical Genetics, FCM - UNICAMP, Campinas, Sao Paulo, Brazil.

MicroRNAs (miRNAs) are a recently discovered class of non-coding RNA molecules of 21-24nt that regulate the expression of target genes in a post-transcriptional manner. This regulation is likely to be mediated by translational repression or target mRNA degradation. miRNAs are though to be involved in several important biological processes, including cell differentiation and embryonic development. In order to better investigate the role of miRNAs in central nervous system (CNS) development, we quantified 104 different miRNAs in mouse brain during development. We obtained RNA from mouse in four stages of development (E15, E17, P1 and P7) and used it in real-time PCR reactions with a stem-loop RT based TaqMan MicroRNA Assay. Bioinformatics analysis identified four clusters (C1, C2, C3 and C4) of miRNAs expression. In addition, we found a significant decrease in expression of 12 miRNAs (Cluster C1; $p<0,05$) in latter stages of development. Our results suggest the presence of a specific expression pattern in cluster C1, indicating that these miRNAs are involved in regulation of genes related to neurogenesis. Supported by CAPES and FAPESP.

694/T

MicroRNA expression profiling in human embryonic stem cells using universal bead arrays. *J. Fan[1], J. Chen[1], J. Loring[2], L. Laurent[2].* 1) Dept Genetic Analysis, Illumina, Inc, San Diego, CA; 2) Burnham Institute for Medical Research, La Jolla, CA.

We have developed a very sensitive and reproducible method for microRNA expression profiling. The method is a modification of the high throughput gene expression profiling assay, the DASL® Assay that we developed previously (Fan et al., Genome Research 14:878-885. 2004). It applies a solid-phase primer extension (after target hybridization) to enhance the discrimination among homologous miRNA sequences. In addition, universal PCR is used to amplify all targets prior to array hybridization. Currently, assays are designed to simultaneously analyze 470 well-annotated human miRNAs (miRBase: http://microrna.sanger.ac.uk/), and additional 273 human miRNAs compiled from the literature. Highly reproducible miRNA expression profiles (R^2 >0.98) were generated with as little as 200 ng total RNA input. Furthermore, very similar expression profiles were obtained between total RNA and enriched small RNA species (R^2 = 0.96). High concordance (R^2 = 0.8) was obtained between the array results and quantitative RT-PCR results, when "fold-difference" was compared.

We have used this method for global miRNA profiling of human embryonic stem cells (hESC), neural stem cells (NSC), and differentiated cells to probe the differences in miRNA usage leading to self-renewal and pluripotence. Unsupervised clustering analysis separated all samples into four distinct groups: hESCs, fetal NSCs, adult NSCs and differentiated cells. Interestingly, miRNAs differentially regulated in hESCs are distributed in large genomic clusters. Moreover, previously described "oncogenic" miRNAs are over-expressed in hESCs, while "tumor suppressor" miRNAs are depleted in hESCs compared to the differentiated cell types. Let-7, a miRNA shown to be necessary for cellular differentiation in *c. elegans*, is strongly down-regulated in hESCs. This suggests that miRNAs play important regulatory role in maintaining the stem cell state, and in directing stem cell differentiation.

695/T

Methylation pattern of several tumor suppressor genes in various cell lines. *Y. Lee, K. Uhm, E. Lee, S. Park, H. Kim.* anatomy, human genetics, col. of medicine, korea univ., Seoul, Korea.

Gene expression is mostly controlled at the level of the transcription initiation. It is well known that thousands of genes are deregulated in cancer cells. During malignant transformation, the malignant cell accumulates epigenetic abnormalities that do not alter the DNA sequence but modify genes expression. It is established that epigenetic alterations and the resulting inactivation of tumor suppressor genes often contribute to the development of various cancers. Until now, however, there is lack of information about methylation profiles differences between normal and tumors. In order to gain insights into the function of DNA methylation, here, we investigated the methylation profile of several tumor suppressor genes in 8 normal and 8 cancer cell lines. Several tumor suppressor genes among tested, such as ATM, DLC-1, SFRP-1 were hypermethylated in breast tumor MCF7 cells. However, these were not methylated in Cos7, ST3L1, NIH3T3, C2C12 normal cells. Unexpectedly, p16INK4a, well known tumor suppressor genes, was hypermethylated in 7 normal cell lines. In summary, this genome-wide epigenetic approach to the methylation patterning of tumor suppressor genes will accelerate understanding of causation and will impact on clinical assessment in the areas of both prevention and treatment. Our findings also emphasize the usefulness of DNA methylation as a marker for differential environment for normal and tumor, and as a tool for evaluation of tumor progression.

696/T

Aberrant DNA hypermethylation of Integrin a4 in cholangiocarcinoma. *E. Lee, K. Uhm, Y. Lee, H. Kim, S. Park.* anatomy, college of medicine, korea univ., seoul, Korea.

Aberrant DNA methylation of 5'-CpG islands located at gene promoter region has been identified as a mechanism for transcriptional inactivation of genes. To ascertain the DNA hypermethylation in cholangiocarcinoma (CC), we investigated promoter methylation status of Integrin a4 in 19 CCs, 19 adjacent non-tumor tissues, and 7 normal liver tissues using methylation-specific PCR (MSP). The frequencies of DNA methylation of Integrin a4 were: 57.9% (11 of 19) in CCs, 5.3% (1 of 19) in adjacent non-tumor tissues, and 0% (0 of 7) in normal liver tissues respectively. There was a statistically significant difference between CCs and adjacent non-tumor tissues ($p<0.0001$). In additionally, restoration of Integrin a4 expression in CC cell lines was achieved by treatment with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine. These results suggest that the transcriptional inactivation by aberrant DNA methylation of Integrin a4 may contribute to the tumorigenesis of CC.

697/T

Aberrant DNA methylation of multiple genes in myeloid leukemia. *K. Uhm, Y. Lee, E. Lee, H. Kim, S. Park.* Dept Anatomy, Col Medicine, Korea Univ, Seoul, Korea.

DNA methylation in the promoter region of a gene plays an important role in gene silencing. To examine whether promoter methylation is involved in the tumorigenesis of hematologic malignancies, I investigated promoter methylation status of the 6 multiple genes in 20 acute myeloid leukemias (AML) and 20 chronic myeloid leukemias (CML) by methylation-specific PCR (MSP) method. Fifty-five of normal peripheral blood samples were included as controls. The frequencies of DNA hypermethylation in AML were: 43% for SFRP1, 85% for SHP1, 86% for Integrin a4, 93% for RUNX3, 29% for H-cadherin, and 0% for DAB2IP, respectively. The DNA methylation frequencies in CML were; 6% for SFRP1, 25% for SHP1, 13% for Integrin a4, 31% for RUNX3, 35% for H-cadherin, 0% for DAB2IP, respectively. In contrast, DNA hypermethylation of genes, excepted H-cadherin, was not detected in 55 normal peripheral blood samples. The DNA methylation frequencies of SFRP1, SHP1, Integrin a4, and RUNX3 genes were higher in AML than in CML, significantly ($p<0.05$). These results suggest that the transcriptional inactivation by aberrant methylation of SFRP1, SHP1, Integrin a4, and RUNX3 genes may contribute to the tumorgigenesis of AML and CML. Also, the aberrant DNA methylations of these 4 genes are more frequent event in AML than CML.

698/T

The interaction between a functional variant in *F5* gene and maternal smoking during pregnancy on preterm delivery. *Y.X. Yu[1], H.J. Tsai[1], S.C. Zhang[1], X. Liu[1], C. Pearson[2], K. Ortiz[2], X.B. Wang[1].* 1) Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Northwestern University Feinberg School of MedicineCenter, Chicago, IL; 2) Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA.

Introduction: We previously reported that factor 5 (*F5*) genetic polymorphisms were associated preterm delivery (PTD, gestational week <37). *F5* gene plays a critical role in the regulation of blood coagulation. Women smoking during pregnancy had higher levels of blood coagulation than those who did not. We hypothesize that *F5* gene and maternal smoking during pregnancy may synergistically increase the risk of PTD. **Methods:** A functional SNP (rs6019) in *F5* gene was genotyped in 548 PTD mothers and 1,770 mothers who delivered full-term recruited from a case-control study at Boston Medical Center. The individual effects of SNP rs6019 and maternal smoking, and their interactive effect on PTD and gestational age were examined using logistic regression and multiple linear regression models after adjusting potential confounders. **Results:** Maternal smoking was associated with PTD and gestational age. Additionally, SNP rs6019 was associated with PTD (OR [95% CI]: 1.3 [1.1-1.6]) and gestational age (β[SE]: -0.5 [0.1]; $p < 10^{-4}$), respectively. More importantly, we found significant interactive effects of SNP rs6019 and maternal smoking on PTD. Comparing with non-smoking mothers with the CC genotype, those with the CG or GG genotype was associated with increased risk of PTD (OR [95%CI]: 1.5 [0.8-2.9] for CG; 2.5[0.8-7.9]) for GG) when smoking intermittently, and even higher risk when smoking continuously (OR [95%CI]: 2.6 [1.7-4.2] for CG; 4.1 [1.9-8.8] for GG). Similarly, significant interactive effects were observed for gestational weeks (β[SE]: -1.8 [0.4]; $p < 10^{-4}$ for CG; -2.4 [0.6]; $p = 10^{-4}$ for GG). **Conclusions:** We confirmed that maternal smoking and *F5* genetic variant each was associated with increased risk of PTD. Moreover, we found significant *F5* gene-maternal smoking interaction on the risk of PTD.

699/T

Location analysis of E2F4 binding sites by high-density oligonucleotide human promoter arrays. *S. Song[1], C. Brueck[2].* 1) Agilent Technologies, Santa Clara, CA; 2) Sigma-Aldrich, St. Louis, MO.

Regulatory proteins bind to genomic DNA to control chromosome replication and gene activity, thereby functioning as switches in the regulatory circuitry of cells. This network of circuits is uncharted in many instances and its understanding will aid researchers in identifying new target genes and therapeutics capable of modulating these pathways. ChIP-on-chip (chromatin immunoprecipitation-on-chip) also known as Location Analysis (LA), is the powerful technology to analyze how regulatory proteins interact with the genome of living cells driving the next generation microarray platform (Boyer, L.A. et al., Cell, v122, p947-956). This advanced technology provides insight into key mechanisms of methylation, histone modification, as well as DNA replication, modification, and repair. The E2F4 family is a ubiquitous family of transcription factors involved in regulating basic cellular processes. Here we take an unbiased, sensitive, and comprehensive approach towards identifying E2F4 target genes by examining localization of E2F4 binding sites using high-density oligonucleotide human promoter arrays and integrated ChIP analytics software.

700/T

Heritability of metabolic syndrome and its phenotypic components in Chinese female twins aged 20 to 60 years. *S.C. Zhang, X. Liu, Y.X. Yu, H.J. Tsai, X.B. Wang.* Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL.

Introduction: Metabolic syndrome (MS) is defined as a cluster of metabolic abnormalities including central obesity, dyslipidemia, hypertension and hyperglycemia. The prevalence of MS has increased rapidly worldwide in the past decades. However, the role of genetic and environmental influence on MS remains unclear. This study investigates the heritability of MS and its five phenotypic components using a twin design. **Methods:** A total of 1,606 female twin pairs, 1,108 monozygotic (MZ) and 498 dizygotic (DZ) twin pairs, aged 20 to 60 years, were recruited from a rural area in China. Zygosity was determined using 10 polymorphic microsatellite markers, with an accuracy exceeding 99.5%. MS is defined as follow: central obesity (waist circumference ≥80 cm), plus any two of the following factors: triglyceride (TG)≥1.7m mMol, high density lipid (HDL) < 1.1m mMol, systolic blood pressure (SBP) ≥ 130 or diastolic blood pressure (DBP)≥ 85 mmHg, fasting plasma glucose (FPG) ≥ 5.6m mMol (ref). Heritability was estimated using structure equation modeling (Mx software). We fitted an ACE model that estimates additive genetic (a^2), common (c^2) and specific (e^2) environmental components of MS and its five components. **Results:** The prevalence of MS in this study population is 3.2%. The correlation coefficients of MS and its five components among MZ (ranging from 0.54 to 0.79) are higher than those among DZ (ranging from 0.19 to 0.58). Consistently, heritability (95%CI) estimates of MS, central obesity, high TG, low HDL, high BP and high FPG are 0.79 (0.17-0.88), 0.52 (0.19-0.79), 0.53 (0.04-0.65), 0.66 (0.32-0.76), 0.46 (0.10-0.84) and 0.13 (0.00-0.52), respectively. **Conclusions:** This large twin study demonstrated strong genetic influence on MS and its components, except for high FPG. It underscores that further investigation of MS should consider both genetic predisposition, environmental factors, and GxE interactions. Ref: Bayoumi A et al.Obesity, 2007;15(3):551-556.

701/T

A rapid flow cytometry test based on histone H2AX phosphorylation for the sensitive and specific diagnosis of Ataxia Telangiectasia. *C. Giachino[1], V. Turinetto[1], A. Brusco[2], S. Cavalieri[2], E. Lantelme[1], L. Orlando[1], U. Ricardi[3], M. De Marchi[1], A. Amoroso[2], D. Gregori[4], P. Porcedda[1].* 1) Department of Clinical and Biological Sciences, University of Turin, Italy; 2) Department of Genetics Biology and Biochemistry, University of Turin, Italy; 3) Department of Medical and Surgical Disciplines, University of Turin, Italy; 4) Department of Public Health and Microbiology, University of Turin, Italy.

Background: Ataxia Telangiectasia (A-T) is a progressive neurodegenerative disease with onset in early childhood, caused by mutations in the ATM (ataxia-telangiectasia mutated) gene. Clinical diagnosis relies on laboratory tests showing high levels of serum alphafetoprotein (AFP), cell sensitivity to ionizing radiation (IR) and absence or reduced levels of ATM protein. Many tests, however, are not sufficiently sensitive and/or specific for A-T, have long tournaround times or require large blood samples. We have therefore developed a new flow cytometry method for the diagnosis of A-T through the measurement of histone H2AX phosphorylation. Methods: Forty-six healthy donors, 20 genetically proven A-T patients, 19 with suspected A-T and 1 with Friedreich Ataxia were recruited. Histone H2AX phosphorylation in T-cell lines, lymphoblastoid cell lines (LCLs) and peripheral blood mononuclear cells (PBMCs) was evaluated by flow cytometry after 2 Gy IR. Results: Phosphorylated histone H2AX mean fluorescence intensity of irradiated A-T cells was significantly lower than that of healthy donors. The intra-staining, intra-assay and inter-assay imprecisions were ≤15.5%. Sensitivity and specificity were virtually 100% when the test was performed on PBMCs. Screening of the 19 patients with suspected A-T classified fifteen patients as non-A-T. The four classified as A-T were subsequently confirmed by ATM mutation analysis. The Friedreich Ataxia patient was classified as non-A-T. Conclusions: This flow cytometry test is very sensitive, specific and rapid (two days), and requires only 1-2 ml of blood. It may thus be proposed for the early differential diagnosis of A-T.

702/T

A High Resolution Oligonucleotide CpG Island Microarray for Relative DNA Methylation Measurement. *D. Roberts[1], C. Foo[2], S. Giles[1], E.L LeProust[1], S. Milligan[1], C. Hopkins[1], R.M. Saxena[1], D. Roberts[1].* 1) Dept Research & Development, Agilent Technologies, Santa Clara, CA; 2) University of California, San Francisco, CA.

CpG islands are stretches of high GC content DNA containing multiple CpG dinucleotides. When CpG dinucleotides within these islands are methylated, especially in promoter regions, expression of the corresponding downstream genes is often repressed. Aberrant CpG island methylation is implicated in cancer. We have developed an oligonucleotide microarray that specifically represents the CpG islands in the human genome. This microarray contains ~230,000 oligo probes tiling the 21 megabases of 27,800 CpG islands, with an average spacing between probes of 95 base pairs. The microarray is designed to be compatible with several published methods for the genome-wide detection of methylated CpG islands. To demonstrate the ability of this microarray to accurately detect methylated DNA, we performed analysis of human genomic DNA samples after methylated DNA immunoprecipitation (mDIP). Additionally, we developed and tested "spike-in" control DNA that was in vitro methylated to varying degrees. The mDIP method combined with CpG island microarray analysis accurately differentiated between partially and fully methylated spike-in DNAs. We then applied the whole-genome assay to the prostate cancer cell line PC3, where we detected methylated CpG islands upstream of cancer related genes including CDKN2A/p16. We extended the study to other cancer cell lines and determined relative methylation at multiple cancer related genes. Finally, using male and female embryonic lung fibroblast cell lines, we demonstrate that many CpG islands on the female X chromosome are more methylated than the corresponding islands on the male X chromosome. In comparison, methylation of CpG islands on the autosomes is essentially the same for both the male and female samples. This supports a role for CpG methylation in silencing the inactive X chromosome in females.

703/T

Hypomethylation of the *H19* imprinting control region in Silver-Russell syndrome. *S. Bruce[1,2], K. Hannula-Jouppi[2], C.M. Lindgren[3,4], M. Lipsanen-Nyman[5], J. Kere[1,2].* 1) Karolinska Institutet, Department of Biosciences and Nutrition, Huddinge, Sweden; 2) University of Helsinki, Department of Medical Genetics, Helsinki, Finland; 3) University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford, UK; 4) University of Oxford, Oxford Centre for Diabetes, Endocrinology and Medicine, Oxford, UK; 5) University of Helsinki, Hospital for Children and Adolescents, Helsinki, Finland.

Silver-Russell syndrome (SRS) is a congenital growth retardation syndrome that has recently been found associated to hypomethylation of an imprinting control region (ICR) on chromosome 11p15.5 (*H19* ICR). In this study we investigated the methylation status of the *H19* and *KCNQ1OT1* ICRs in 39 SRS patients and the *H19* ICR in 84 children born small for gestational age (SGA). The methylation status was investigated using methylation-sensitive restriction enzyme digestion of genomic DNA from whole blood, followed by quantitative real-time PCR. We further genotyped 4 microsatellites in the SRS patients and their parents to search for maternal duplications of the 11p15 region. The normal range of methylation was 46% (standard deviation (SD) ±6%) at both the *H19* and *KCNQ1OT1* ICR as established by screening 40 normal length individuals. This corresponds well to the expected 50% methylation at an imprinted locus. Twenty-five of the SRS patients (65%) were found to have methylation percentages below 35% (-2SD) at the *H19* ICR, while only one of the SGA children (1%) had an abnormal methylation profile. This patient had many dysmorphic features compatible with SRS. Methylation at the *KCNQ1OT1* ICR was within the normal range for all investigated patients. Among the hypomethylated SRS patients, a strong negative correlation between the degree of hypomethylation and minus SD in length at birth and at 2 years was found (P= 0.001, P=0.002, respectively). This suggests that among the hypomethylated SRS patients, the hypomethylation seem to explain a large part of the variation in length up till the age of 2 years. No maternal duplications for the region were found, and therefore the mechanism of hypomethylation remains unexplained.

704/T

Are H19 mutations involved in Silver-Russell syndrome? *N. Schoenherr[1], G. Binder[2], E. Korsch[3], H.A. Wollmann[2], T. Eggermann[1].* 1) Institute of Human Genetics, Unversity Hospital RWTH Aachen, Aachen, Germany; 2) Children's Hospital, University of Tübingen, Germany; 3) Children's Hospital, University of Köln, Germany.

(Epi)mutations of 11p15 are associated with the overgrowth disorder Beckwith-Wiedemann syndrome (BWS) and with the primordial growth retardation disease Silver-Russell syndrome (SRS). In 11p15 two imprinting control regions (ICR1 and ICR2) regulate the expression of 14 imprinted genes. While (epi)mutations in the ICR2 are responsible for ~50% of BWS cases, in SRS only one case with a maternal duplication restricted to ICR2 was published. More than 35% of SRS patients show a ICR1 hypomethylation. The ICR1 is paternally methylated and regulates the expression of the oppositely imprinted genes IGF2 and H19. The function of the untranslated RNA H19 is still unknown but the finding that H19 is relative highly conserved among mammals indicates a profound functional relevance. Due to the supposed function of the H19 sequence in the regulation of the imprinted region 11p15 we searched for mutations in this gene in 44 SRS patients. We detected three SRS patients with variants in the transcribed region of H19. In two cases (SR17; SR81) different 3 bp deletions in exon 1 could be identified (g.8616_8618delGGG; g.8818_8820delAGG (AF087017)). Patient SR93 carried a 39 bp duplication affecting exon 2 and intron 2 (g.9867_9906dup39). All three variants were not detected in controls and are localised in evolutionary conserved regions. SR93 additionally showed a ICR1 hypomethylation. We performed splicing as well as expression analyses to figure out the functional consequences of these mutations. Splicing studies revealed a deviation from the normal H19 splicing behaviour in SR81 and SR93. Expression analysis on blood lymphocytes carried out in SR93 did not verify an altered expression pattern of H19. Nevertheless, our results indicate a relevant role of H19 mutations in the aetiology of SRS: functional effects of these variants, e.g. chromatin restructuring of the ICR1 or an altered function of the antisense RNA, are well conceivable and make further investigations of the biological role of H19 necessary.

705/T

Sequence-based bioinformatic prediction and QUASEP identify genomic imprinting of the KCNK9 potassium channel gene in mouse and human. *U. Zechner[1], S. Bähring[2], D. Galetzka[1], G. Plyushch[1], F.C. Luft[2], P. Nürnberg[3], T. Haaf[1], G. Kelsey[4], N. Ruf[5].* 1) Institute of Human Genetics, Johannes Gutenberg University Mainz, Mainz, Rheinland-Pfalz, Germany; 2) Franz Volhard Clinic, Charité, University Medical School, Berlin, Germany; 3) Cologne Center for Genomics and Institute for Genetics, University of Cologne, Germany; 4) Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge CB22 3AT, United Kingdom; 5) Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany.

Genomic imprinting is the epigenetic marking of gene subsets resulting in monoallelic or predominant expression of one of the two parental alleles according to their parental origin. We describe the systematic experimental verification of a prioritized 16 candidate imprinted gene set predicted by sequence-based bioinformatic analyses. We used Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) and discovered maternal-specific imprinted expression of the Kcnk9 gene as well as strain-dependent preferential expression of the Rarres1 gene in E11.5 (C57BL/6 × Cast/Ei)F1 and informative (C57BL/6 × Cast/Ei) × C57BL/6 backcross mouse embryos. For the remaining 14 candidate imprinted genes, we observed non-imprinted biallelic expression. In adult mouse tissues, we found that Kcnk9 expression was restricted to the brain and also was maternal-specific. QUASEP analysis of informative human fetal brain samples further demonstrated maternal-specific imprinted expression of the human KCNK9 orthologue. The CpG islands associated with the mouse and human Kcnk9/KCNK9 genes were not differentially methylated but strongly hypomethylated. Thus, we speculate that mouse Kcnk9 imprinting may be regulated by the maternal germline differentially methylated region (DMR) in Peg13, an imprinted non-coding RNA gene in close proximity to Kcnk9 on distal mouse chromosome 15. Our data have major implications for the proposed role of Kcnk9 in neurodevelopment, apoptosis, and tumorigenesis, as well as for the efficiency of sequence-based bioinformatic predictions of novel imprinted genes.

706/T

The ATRX chromatin remodeling protein regulates the expression of specific imprinted genes in the murine brain. *D.C. Tremblay[1], M.A. Levy[1], N.G. Bérubé[1,2,3,4].* 1) Biochemistry, University of Western Ontario, London, Ontario, Canada; 2) Paediatrics, University of Western Ontario, London, Ontario, Canada; 3) Children's Health Research Institute, London, Ontario, Canada; 4) Lawson Health Research Institute, London, Ontario, Canada.

X-linked α-thalassaemia mental retardation (ATR-X) syndrome is characterized by severe cognitive delay, a broad range of developmental abnormalities, and α-thalassaemia. Disease-causing mutations in the *ATRX* gene give rise to a malfunctioning protein and result in aberrant DNA methylation at specific repeat sequences. ATRX is an ATP-dependant chromatin remodeling protein that targets α-globin gene expression, leading to α-thalassaemia in affected patients. To identify additional genes that are regulated by Atrx, we compared the transcriptional profile of Atrx-null and control mouse forebrain samples. We utilized a mouse model system in which Atrx is conditionally deleted in the developing cortex and hippocampus starting at embryonic day 8.5. This analysis revealed that specific imprinted genes are upregulated in the Atrx-null newborn forebrain. Using quantitative RT-PCR, we established that alterations in imprinted gene expression occurred in a progressive manner, suggesting that Atrx might contribute to the maintenance of imprinting marks, such as DNA methylation. We therefore performed bisulfite mutagenesis to identify potential DNA methylation changes at differentially methylated regions (DMRs). Subtle alterations in methylation patterns at imprinting control regions were identified, and additional studies will be required to clarify whether these methylation changes are related to aberrant gene expression. Taken together, our findings establish for the first time a link between Atrx and the control of imprinted gene expression in the murine brain, providing further evidence that imprinting mechanisms are an important facet of brain development and cognitive functions.

707/T

Genome-wide profiling of epigenetic modifications in postmortem brain using microarray based methods: Use of the PWS/AS domain as a proof of feasibility. *R. Person, X. Zhang, Y-H. Jiang, A.L. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Epigenetics is the study of stable and potentially heritable changes in gene expression that do not entail changes in DNA sequence. The majority of epigenetic studies focus on analysis of DNA methylation and of chromatin modifications using chromatin immunoprecipitation (ChIP). We wish to test the hypothesis that epigenetic abnormalities in brain may cause disorders such as autism and schizophrenia. As a proof of feasibility, we have analyzed DNA methylation using three methods and shown that the DNA abnormalities in Prader Willi syndrome (PWS) and Angelman syndrome (AS) brain can be detected robustly using genome-wide Agilent CpG island arrays. We have found that methods to detect methylated DNA distinguish AS but not PWS well from control brain and that methods to detect unmethylated DNA distinguish PWS but not AS well from controls; this means that searches for DNA methylation differences should utilize at least two methods, one to detect methylated DNA and another to detect unmethylated DNA. Similarly, using ChIP on microarrays (ChIP-chip) with multiple antibodies to histone modifications, we have characterized the PWS/AS domain in depth using a dense array focused on this region, and also shown that the abnormalities in PWS and AS can be detected using genome-wide Agilent promoter arrays. Findings of note are the occurrence of sharp peaks of H3K4 methylation at all active promoters in the PWS/AS domain and the presence of a broad peak of H3K9 trimethylation over the paternal but not the maternal copy of the HBII-85 snoRNA cluster. A modest amount of data comparing autism and control brain is available, and more extensive studies are ongoing, but no definitive abnormalities have been detected in autism brain to date. These results demonstrate the feasibility of detecting epigenetic defects causing autism or schizophrenia using genome-wide methods and postmortem brain, if such abnormalities do occur.

708/T

DNA methylation profiling in Hodgkin lymphomas using BeadArray technology. *M. Bibikova[1], J.I. Martin-Subero[2], E. Wickham-Garcia[1], J. Richter[2], J.-B. Fan[1], D. Barker[1], R. Siebert[2].* 1) Illumina, Inc., San Diego, California 92121, USA; 2) Institute of Human Genetics, Christian-Albrechts University, Kiel, Germany.

DNA methylation is an epigenetic modification which does not affect the genetic code, but affects gene transcriptional regulation and can be heritable. Epigenetic profiles have been used as markers for disease identification or progression. Tumor suppressor gene inactivation by DNA hypermethylation is a well known phenomenon in most solid and hematological malignancies. In the case of Hodgkin lymphoma (HL), however, the DNA methylation changes have not been well studied. We have performed DNA methylation profiling of HL cell lines using the GoldenGate® Assay for methylation (Illumina, Inc.), which allowed the measurement at 1505 individual CpG sites from regulatory regions of 807 genes involved in cell cycle control, differentiation, apoptosis, DNA repair and imprinting. Four classical HLs (cHL) and one nodular lymphocyte predominant HL (NLPHL) as well as eight DNA samples from normal hematological tissues were studied in duplicate. A methylation level ranging from 0 (unmethylated) to 1 (methylated) was calculated, and differences between entities above 0.5 were considered differentially methylated. In comparison with the controls, cHL showed 241 hypermethylated and 40 hypomethylated gene promoters whereas 154 and 80 promoters were hyper- and hypomethylated in NLPHL, respectively. CHL displayed a high number (n=87) of hypermethylated promoters in comparison to NLPHL. Hypermethylated gene promoters in HL included known tumor suppressor genes (e.g. p16, p73, DAPK). Interestingly, CpG-islands from genes involved in B-cell specific pathways (e.g. BCAM, BLK, MME and SYK) were exclusively hypermethylated in cHL. Such epigenetic silencing of B-cell specific genes may be the cause of loss of the B-cell identity characteristic for cHL, and thus, might play a key role in its pathogenesis. The methylation data generated by the array were validated by methylation specific PCRs and bisulfite sequencing. In conclusion, genome-wide methylation profiling revealed a global alteration of the methylome in cHL, being mostly characterized by promoter hypermethylation of a large number of genes.

709/T

Whole genome methylation profiling of aortic smooth muscle cells as a model for the development of atherosclerosis. *D. Biscocho[1], J.J. Connell[1], E.R. Hauser[1], W.E. Kraus[2], S.G. Gregory[1].* 1) Department of Medicine and Center for Human Genetics; 2) Department of Medicine and Division of Cardiology.

DNA methylation is a mechanism used by the cell to control transcription through modification of chromatin structure in specific regions of the genome. This type of transcriptional control allows for heritable epigenetic inactivation of a gene, as well as temporal control of gene activation. DNA methylation changes have been shown to play a role in the proliferation of smooth muscle cells (SMCs) during the initiation of coronary artery disease. However, these changes have only been characterized in a limited number of genetic loci. We have carried out genome-wide profiling of DNA methylation in proliferating aortic SMCs to identify novel markers for use in diagnosis of severity of disease and to identify atherosclerosis-susceptibility and atherosclerosis-protective genes. DNA isolated from aortic SMCs from passages (p) 5, 6, 7 and 8 was digested with methylation sensitive enzymes and linker mediated PCR was carried out to discriminate between differentially methylated loci. A self-self experiment from p5 provided the baseline for clone variation of a genomic tiling path array. PCR products of DNA from p6, 7, and 8 were co-hybridized with DNA from p5 to the genomic array. Differentially methylated regions were correlated with annotated genes, predicted regulatory regions and CpG islands. Analysis of genome-wide methylation patterns showed an expected general trend towards hypomethylation with progressive passaging. We have begun to investigate two loci, one hypomethylated and one hypermethylated, that are adjacent to the transcription factor Fli-1 and the ITGB1 gene, respectively. Both genes represent candidates involved in the etiology of SMC proliferation. Over expression of Fli-1 has been shown to lead to erythroblast survival and proliferation, while ITGB1 belongs to a family of proteins that have major roles in biological processes including cell migration, tissue organization, growth and differentiation. Here we describe the detailed analysis of our genome-wide methylation profiling of aortic SMCs and their implication in the development of atherosclerosis.

710/T

Hypomethylation at the *H19/IGF2* ICR1 in the human placenta is associated with fetal intrauterine growth restriction. *D.K. Bourque[1], L. Avila[1], M.S. Peñaherrera[1], P. von Dadelszen[2], W.P. Robinson[1].* 1) Medical Genetics; 2) Obstetrics and Gynaecology, Univ of British Columbia, Canada.

Placental insufficiency can lead to pre-eclampsia (PET) and/or intrauterine growth restriction (IUGR), each affecting 5% of pregnancies. Methylation at the *H19/IGF2* imprinting control region 1 (ICR1) is thought to play an important role in fetal and placental development. As methylation at this site is inversely correlated with expression of the important growth promoting gene *IGF2*, abnormalities may affect fetal and placental growth. To evaluate the hypothesis that abnormal levels of methylation at the *H19/IGF2* ICR1 significantly contribute to pre-eclampsia and IUGR, we collected two chorionic villous samples each from control (N= 20), PET (N=11), IUGR (N=8), and combined PET+IUGR placentas (N=13). We assessed methylation status at two CpG sites (C10 and C12) within the ICR using a SNuPE assay. There was low correlation in level of methylation between samples from the same placenta (R^2=0.34, p<0.001), but high correlation between C10 and C12 CpG sites from one sample (R^2=0.81, p<0.001). There was a reduced mean percent methylation in the IUGR group (29.7%) as compared to the control (36.5%, p<0.01), PET (37.1%, p<0.01) and PET+IUGR (37.1%, p<0.01) groups. These results suggest that IUGR with and without PET may be different in etiology and that decreased expression of *IGF2* in the placenta may be involved in isolated IUGR. As a marker of fetal methylation status, we assessed methylation in amnion samples (N=5 from each group). Mean methylation was 35.8% for control placentas, 36.5% for IUGR, 34.5% for PET and 37.2% for IUGR/PET. Methylation level in amnion was not correlated with clinical group or with corresponding villi samples. This indicates that changes in methylation may be confined to the placenta. We also assessed *H19/IGF2* ICR1 methylation in whole villi from 16 placentas with confined trisomy 16, a situation often associated with fetal IUGR. The mean methylation in these placentas was 30.8% (p=0.009, vs. controls, one-way ANOVA). Decreased methylation at the *H19/IGF2* ICR1 may occur as a response to poor placental implantation rather than being a spontaneous error.

711/T

Optimized methylated DNA analysis of formalin fixed paraffin embedded tissue samples by bisulfite sequencing. *V. Boyd, M. Barker.* Applied Biosystems, Foster City, CA.

The tissue preservation process compromises genomic DNA from formalin fixed paraffin embedded (FFPE) samples. However, FFPE samples remain the most abundant tissue available to researchers and are extremely valuable due to the associated clinical records. A workflow enabling researchers to reliably extract the gDNA, perform bisulfite conversion and obtain sequence information that provides clues to the methylation status of a FFPE sample would be a unique contribution to the field of epigenetics. There are multiple steps that have been optimized and consolidated to achieve this goal. Genomic DNA extracted using a commercially available kit that provides DNA suitable for the bisulfite conversion by thoroughly removing protein associated with the DNA. Residual protein associated with the DNA reportedly impairs bisulfite conversion. A mild bisulfite conversion with a commercially available bisulfite conversion kit limits fragmentation of the already fragmented gDNA from FFPE samples by avoiding temperatures in excess of 50 degrees. Our recent results include a time-course study showing efficient bisulfite conversion occurs within three hours. The bisulfite converted gDNA, present as fragments due both to FFPE preservation and bisulfite treatment, is readily purified without bias or sample loss using a spin centrifugation device. All fragments and methylation states are recovered using this unique purification protocol based on size-cutoff and provides accurate representation of the methylation states present. A “percentage of functionality” test can be performed on the extracted DNA prior to bisulfite conversion which provides an indication of DNA quality, and correlates well with the expected success of analyzing the same sample after bisulfite conversion. PCR conditions have been optimized prior to bisulfite sequencing using tailed primers and a two-tiered thermal cycling program. A new commercially available sequencing clean-up matrix provides both ease of use and sensitivity.

712/T

Genome-Wide Analysis of Alterations in Histone Methylation and Gene Expression in Hutchinson-Gilford Progeria Syndrome. *K. Cao, D. Faddah, M.R. Erdos, B.C. Capell, F.S. Collins.* National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder with widespread phenotypic features of premature aging. Classic HGPS is caused by a de novo point mutation in exon 11 of the LMNA gene, activating a cryptic splice donor and resulting in a mutant lamin A protein termed “progerin” that lacks 50 amino acids near the carboxyl terminus. During interphase, progerin anchors to the nuclear membrane, disrupting the nuclear scaffold and causing nuclear blebbing that has been referred to as the cellular hallmark of HGPS. Given the known interactions between the nuclear lamina and transcription factors, as well as the evidence that changes in modified histones predate the blebbed nuclear morphology in HGPS, we hypothesized that progerin causes cell damage not only by its structural effects, but in the way it alters chromatin structure and transcriptional regulation. To test our hypothesis, we have implemented a combined approach using expression array analysis and ChIP-chip (chromatin immunoprecipitation coupled with DNA microarray technology). We studied fibroblasts from normal and HGPS individuals, and generated tet-inducible cultured cells expressing progerin to assess the early events following progerin expression. Expression microarray analysis defined a set of 235 genes that show at least a two-fold, statistically significant change in HGPS. Parallel ChIP-chip analysis using ENCODE and human promoter arrays generated high-resolution maps for the distribution of H3K4, H3K27, and H3K36 trimethylation. Combining these data sets led to the identification of an initial list of differentially active and suppressed genes that may explain some of the cellular phenotypic features of HGPS. Furthermore, we have recently employed the Illumina/Solexa sequencing technology to map histone methylation patterns across the entire genome in HGPS. This study provides novel insights into the complex relationship between transcriptional regulation and chromatin organization in both HGPS and normal aging.

713/T

Rapid prenatal confirmation of ultrasound-impressed Beckwith-Wiedemann syndrome caused by hypomethylation of LIT1 by quantitive endonuclease-polymerase chain reaction. *S.P. Chang[1], G.C. Ma[1], C.W. Yang[1,2], D.J. Lee[1], M. Chen[1,3,4].* 1) Center for Medical Genetics, and Department of Medical Research, Changhua Christian Hospital, Changhua, Taiwan; 2) Graduate Institute of Molecular Medicine, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; 3) Department of Obstetrics and Gynecology, and Department of Medical Genetics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; 4) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua 500, Taiwan.

Beckwith-Wiedemann syndrome (BWS) is a rare congenital overgrowth disorder associated with abnormalities of imprinted gene expression at chromosomal region 11p15. We propose a rapid molecular test for evaluating the statuses of DNA methylation at 11p15 by using methylation-sensitive endonuclease-coupled quantitative polymerase chain reaction (E-Q-PCR) in fetuses affected with BWS. E-Q-PCR involved two steps: (1) methylation-sensitive endonuclease NotI treatment, and (2) quantitative real-time PCR performance. PCR was achieved by use of two pairs of primers that specified amplifications of 2 distinct regions (with and without NotI cutting sites, respectively) surrounding an imprinting center LIT1/KCNQ1OT1 of 11p15. PCR-amplification ratio of the NotI-cut region to the NotI-uncut region was calculated as a methylation index in LIT1. We tested this novel strategy (E-Q-PCR) in 2 fetuses clinically diagnosed with BWS and 9 unaffected fetuses from cultured amniocytes.The E-Q-PCR analysis can assess DNA methylation changes in LIT1 between unaffected individuals and BWS fetuses. The methylation indices detected in both BWS fetuses (9.0% and 9.1%)were apparently lower than that of unaffected fetuses (56.9%~67.8%). E-Q-PCR is a novel method for quantitative analysis of methylation status of LIT1, which accounts for 60% detectable molecular pathology of BWS cases. This methodology is easily performed and suitable for rapid confirmation of BWS fetuses impressed by obstetric ultrasound.

714/T

A Genome-wide methylation study in Epstein-Barr Virus Oncoprotein Latent Membrane Protein 1 Transfected Lymphoma Cells. *Y.F. Chen[1], W.C. Hsiao[2], C.L. Tung[2], I.J. Su[3], H.S. Sun[1, 2].* 1) Institute of Basic Medical Sciences, National Cheng Kung University Medical College, Tainan, Taiwan; 2) Institute of Molecular Medicine, College of Medicine, National Chung Kung University, Tainan, Taiwan; 3) Division of Clinical Research, National Health Research Institutes, Tainan, Taiwan.

Epigenetic mechanisms, which involve DNA and histone modifications, result in the heritable silencing or activation of genes without a change in their DNA sequences. Recent studies have revealed the changes in DNA methylation that include influence genome integrity, hypermethylations of tumor suppressor genes and/or hypomethylation of oncogenes may lead to tumor development. Epstein-Barr virus (EBV) is a γ herpesvirus that infects more than 90% of the human population and mainly through the infections of B-lymphocytes and epithelial cells. Although EBV was associated with many human malignancies including nasopharyngeal carcinoma (NPC), Burkitt's lymphoma and T cell lymphoma, the underlying mechanism of EBV-associated tumorigenesis remains unclear. It was reported that EBV-encoded latent membrane protein (LMP1) can affect DNA methyltransferases 1 expression and alter mRNA level of few specific genes in LMP1-overexpressed NPC cells. These results may partially explain the EBV effect in tumor development. To examine whether similar effect exists in LMP1-overexpressed lymphoma cells, this study investigates the whole genome methylation profile in LMP1-transfected BAJB and H9 cells. The global DNA methylation degree, methylcytosine level and expression of different DNA methyltransferases were determined in LMP1-expressing BAJB and H9 cells. Furthermore, we have established a database of genome-wide NotI-tagging sites to document the whole genome methylation profile based on differentially methylated CpG island sequences. Our current data showed the global methylation patterns, unlike the epithelial cell model, is not significantly changed in LMP1-overexpressed lymphoma cells.

715/T

DNA methylation alterations in males with Klinefelter syndrome. *B. Coffee, I. Albizua, S. Warren.* Dept Human Genetics, Emory University, Atlanta, GA.

Klinefelter syndrome (47,XXY) is the most common chromosome abnormality in humans with an incidence of 1 in 600 males. The phenotype in Klinefelter syndrome is relatively mild with males presenting with hypogonadism, learning difficulties, gynecomastia after puberty and infertility in adulthood. Because of the mild phenotype, approximately 75% of males with Klinefelter syndrome go undiagnosed. Males with the Klinefelter variants 48,XXXY, 48,XXYY, and 49,XXXXY have a similar, but a more severe, phenotype than 47,XXY males. The mechanism of how additional sex chromosomes leads to the phenotypic features exhibited by Klinefelter males in not known. We present here evidence that the presence of supernumerary sex chromosomes result in the alterations of DNA methylation at various loci in the genomes of Klinefelter patients. Aberrant changes in DNA methylation can result in alterations in chromatin structure leading to changes in gene expression. Aberrant DNA methylation changes are associated with many human diseases, such as imprinting disorders (Prader-Willi, Angelman, Beckwith-Wiedemann and Russell-Silver syndromes), fragile X syndrome, and many types of cancers. The alteration of DNA methylation in Klinefelter patients suggest that additional sex chromosomes may act as a methylation sink, interfering with the establishment and maintenance of DNA methylation patterns in the human genome altering gene expression leading to the phenotypic features seen in Klinefelter patients.

716/T

A flexible, high-density array platform for genome-wide characterization of epigenetic mechanisms. *L. Dannenberg, H. Liu, H. Holster, J. Kitzman, L. Iniguez, R. Green, X. Zhang.* NimbleGen Systems Inc., Madison, WI.

Epigenetic mechanisms, such as DNA methylation and histone modification, play important roles in the control of eukaryotic cellular functions. High-density DNA microarrays, derived from a digital design process, have allowed researchers to examine epigenetic events at an unprecedented scale and resolution. Several methods for measuring the status of genome-wide cytosine methylation have been developed on this platform, and side-to-side comparison demonstrated how these methods can complement each other. Besides DNA methylation, the state of histones provides key information regarding chromatin structure. Multiple array based technologies are available to study different aspects of chromatin structure, including histone modification, histone replacement, and nucleosome positioning. New development on the platform, specifically the 2.16 million feature long-oligo arrays, has expanded the horizon of studies on chromatin structure. The latest progress on epigenetic applications of this unique array platform will be discussed.

717/T

Novel approaches to whole genome methylation profiling applied to epigenetic alterations in breast cancer. *J. Edwards[1], A.H. O'Donnell[2], C. Lee[4], H. Peckham[4], F. Haghighi[3], T.H. Bestor[2].* 1) Columbia Genome Center; 2) Genetics and Development; 3) Psychiatry, Columbia University, New York, NY; 4) Applied Biosystems, Foster City, CA.

The nature of the methylation abnormalities undergone by cancer genomes is of great importance to cancer research and treatment, but one major limitation has been the lack of a method for analyzing the methylation patterns of the entire genome simultaneously in a rapid, cost-effective, unbiased manner. Other methods, such as array-based techniques, by their very nature, cannot be used to look at the methylation status of repetitive elements, which are known to undergo methylation changes in cancer. The novel method presented here combines fractionation of DNA according to methylation status and ultra-high throughput DNA sequencing using ABI's SOLiD platform to allow efficient whole-genome methylation profiling even when the amount of DNA available is limited. A new pipeline that can organize the flood of sequence data generated during this study has been implemented. These tools have allowed us to examine for the first time the complete methylation profile of a panel of breast cancer samples including the MCF7 cell line, primary tumors and matched controls. Data pertaining to differential methylation in mammary carcinoma has been compiled and annotated, and zoomable chromosome ideograms have been created that allow immediate comparison of methylation patterns from two or more sources. Data is also presented as tracks on the UCSC genome browser that show the relationship of methylation profiles to a large number of annotated features. New software has been developed to map sequence tags and for higher-level analyses, such as the search for genomic signatures that may regulate methylation patterns and the identification of key pathways which undergo methylation changes that may play a role in breast cancer. This project, via an unbiased, whole-genome methylation profiling method, capable of investigating methylation status of all sequences, has provided a first look into the complete methylation landscape of the human genome and provides new insights into epigenetic abnormalities in cancer.

718/T

Epigenomic profiling of major depression. *F. Haghighi[1], J.R. Edwards[3], A.H. O'Donnell[2], A.J. Dwork[4], J.J. Mann[1], T.H. Bestor[2].* 1) Psychiatry; 2) Genetics and Development; 3) Columbia Genome Center; 4) Pathology, Columbia University, New York, NY.

The etiology of psychiatric disorders such as Major Depression has genetic, environmental, and epigenetic components. The epigenetic component has received very little attention but is likely to involve pathological abnormalities in genomic methylation patterns that regulate genes involved in the development or physiology of the brain. We have developed new methods for the characterization of genomic methylation patterns and for the purification of sequences that are differentially methylated between control and depressed brains. Application of these methods will improve our understanding of the causes of a major and too often fatal psychiatric disorder. As the aim of this study, we explore the epigenetic profile of major depression; postmortem brain specimens were ascertained with comprehensive clinical and toxicological profiles. We used brain tissues from the ventral prefrontal cortex that is thought to be involved in depression based on neuroanatomical studies. Using experimentally and computationally validated methods, genomic DNA from brain tissue of normal and depressed subjects were digested using methylation sensitive and dependent enzymes, thus fractionating the genome into methylated and unmethylated compartments. The sequence fragments corresponding to these compartments were used for paired-end library construction and subsequent sequencing via the high-throughput 454 sequencing platform. The paired-end sequences were then mapped to the human genome to detect potential disease specific DNA methylation profiles. We have developed statistical and computational tools to analyze such data and reveal novel genomic patterns concerning the form and function of DNA methylation in the human brain. This provides the first systematic and unbiased investigation into the potential epigenetic basis of major depression, a disease of critical public health importance.

719/T

Analysis of methylation status in the promoter of interferon regulatory factor-2 gene in patients with late-onset psoriasis. *N. Hosomi[1,2], K. Fukai[1], N. Oiso[3], Y. Kira[4], T. Ohshimo[1], A. Umekoji[1], M. Ishii[1].* 1) Dermatology, Osaka City University, Osaka, Japan; 2) Dermatology, Kashiwara Municipal Hospital, Kashiwara, Japan; 3) Dermatology, Kinki University, Osakasayama, Osaka, Japan; 4) Central Laboratory, Osaka City University, Osaka, Japan.

Interferon regulatory factor 2 (IRF2) is a transcriptional regulatory protein which represses the expression of interferon-alpha/beta genes. In mice lacking IRF2, the inflammatory skin disease very similar to human psoriasis develops spontaneously. In addition, IRF2 gene is located at human chromosome 4q35, where a familial psoriasis susceptibility locus has been mapped. Thus, the IRF2 gene is a strong candidate gene for psoriasis. We hypothesized that the methylation of the promoter of the gene is associated with type 2 psoriasis in Japan. To analyze IRF2 promoter methylation status, we investigated genomic DNA in peripheral leukocytes of patients with late-onset psoriasis and healthy controls using bisulfite-sequencing method. We enrolled ten late-onset psoriasis patients (mean age; 72.4 years, mean affected age; 49.8years) and five healthy controls (mean age; 63 years) in this study. The genomic DNAs were treated by sodium bisulfite, and the region from -508 to -22 of the IRF2 promoter was amplified by PCR in two fragments. They were cloned into TA-cloning vector, and fifteen clones for each fragment were sequenced. We identified 16 methylated CpG sites (-78, -122, -126, -153, -157, -232, -240, -261, -290, -306, -364, -369, -377, -407, -430 and -456; numbered from the transcriptional initiation site) in the IRF2 promoter of late-onset psoriasis. We also identified 7 methylated CpG sites in the promoter of healthy controls (-126, -167, -232, -235, -253, -306 and -379). The frequencies of the methylated CpGs were approximately 5% both for the psoriasis patients and the normal controls.

720/T

Detection sensitivity of DNA methylation status using high resolution melting on the LightScanner. *C. Hough, J. McKinney, L. Cutler, D. Teng.* Idaho Technology, Inc. 390 Wakara Way, Salt Lake City, Utah 84108.

DNA methylation is a frequent epigenetic modification of CpG dinucleotides. Aberrant DNA methylation patterns within CpG islands of many gene promoters have been associated with increased cancer risk. CpG island hypermethylation results in stable gene silencing as noted in cancer and aging as well as in normal development, imprinting, and X-chromosome inactivation. In contrast, global genomic CpG hypomethylation has been demonstrated in aging and early neoplasia. Traditional methods for determining methylation status include bisulfite conversion of all unmethylated cytosines to uracil followed by PCR amplification and direct sequencing. Methylated cytosines remain unconverted and can be easily identified by sequencing. We sought to systematically evaluate whether the methylation status of DNA could be reliably detected by high resolution melting using the LightScanner instrument. Such pre-sequencing detection could allow for a decreased sequencing effort if methylation detection using Hi-Res melting is robust and sensitive. To create methylation status controls, untreated and SssI-treated pBluescript II plasmid were bisufite converted using the Qiagen EpiTect kit. Sequencing of a representative region confirmed complete methylation and bisulfite conversion of the samples. Primers were designed to amplify 7 different fragments with a size range of 79-216 bp and 1-8 CpG sites interspersed throughout. The 100 percent methylated and unmethylated samples were run straight and mixed at the following percentage of methylated sample: 50, 40, 30, 20, 15, 10, 5, and 2 percents. The 100 percent methylated and unmethylated samples were easily distinguishable. When the two samples were mixed at the above ratios, the melting profiles showed a reproducible dose-response type relationship, with the 50 percent mix having the greatest deviation from the unmethylated standard. Hi-Res melting was sensitive enough to detect methylation status down to 2 percent in certain fragments. Hi-Res melting appears to be a sensitive method for pre-sequencing detection of methylation status.

721/T

Identification of genes silenced by methylation on head and neck tumor cell lineages. *C. Kaneto[1], G. Molfetta[1], M. Calmon[3], R. Moura[2], J. Kaiano[2], R. Rodrigues[4], C. Zanelli[5], H. Brentani[6], A. Camargo[2], E. Tajara[4], D. Carraro[2], P. Rahal[3], S. Valentini[5], W. Silva-Jr.[1].* 1) Depto Genética, Faculdade de Medicina de Ribeirão Preto/USP. CTC/CEPID/FAPESP; 2) Instituto Ludwig de Pesquisas sobre o Câncer-SP; 3) Depto Biologia, Universidade Estadual Paulista-IBILCE/UNESP; 4) Faculdade de Medicina de São José do Rio Preto-SP; 5) Depto Ciências Biológicas. Faculdade de Ciências Farmacêuticas-UNESP; 6) Hospital do Câncer AC Camargo-SP.

Abnormalities in the normal pattern of DNA methylation have been characterized as an important mechanism on carcinogenesis. It is called epigenetic modification as it does not change DNA sequence and can be defined as a heritable change in gene expression. Epigenetic alterations observed in cancer include hypermethylation of selected CpG island gene promoters and simultaneous global hypomethylation. The aim of this project was to identify, by Rapid Subtraction hybridization (RaSh) method, putative genes silenced by methylation in four head and neck cancer lineages. These lineages were also treated with demethylating agent in order to evaluate changes in gene expression after treatment. A total of 480 genes were analysed, 186 genes had enhanced expression after treatment with demethylating agent and, of these, 169 present CpG island in their promoter region. RT-PCR assay was chosen to validate differential expression of genes selected by RaSH. The genes that showed differential expression were: PLAU, CD82, RBBP4, AOF2, TMSB10, HSPA5, LAMC2 and POU2F3. For all the selected genes an enhanced expression was observed after treatment with demethylating agent on, at least, one of the analysed lineages. POU2F3 showed enhanced expression after treatment in FaDu and UM-SCC-38A lineages. In UM-SCC-14, the genes CD82, RBBP4, AOF2, HSPA5 and LAMC2 showed the same effect, but HSPA5 and LAMC2 had their expression enhanced in UM-SCC-17 too. In UM-SCC-38, all genes showed enhanced expression after treatment with demethylating agent. Our work is another evidence that these genes may be regulated by methylation and silenced by epigenetic changes in head and neck cancer. Financial Support: CNPq, FAPESP.

722/T

Chromosome-Wide Methylation Analysis Gives New Insights on the Effectiveness of DNA Promoter Methylation in Transcriptional Repression in Melanoma Cells Compared to Normal Melanocytes. *Y. Koga[1], M. Pelizzola[2], A. Molinaro[2], M. Krauthammer[3], S. Ariyan[4], D. Narayan[4], R. Halaban[5], S.M. Weissman[1].* 1) Departments of Genetics; 2) Epidemiology; 3) Pathology; 4) Surgery; 5) Dermatology, Yale University School of Medicine, New Haven, CT.

Altered gene expression due to aberrant modifications of chromatin is involved in tumorigenesis and maintenance of the malignant phenotype. One such process involves methylation/demethylation of cytosine at cytosine-guanine (CpG) pair rich islands in promoter regions of genes. We, therefore, examined genome-wide DNA promoter methylation coupled with gene expression analysis to determine melanoma specific epigenetic profiles and identify epigenomic markers in melanoma development. The methylcytosine immunoprecipitation method known as MeDIP (Weber et al., 2005) was adapted to enrich for methylated DNA. MeDIP coupled with DNA hybridization to microarrays was employed to generate methylation profiles in normal melanocytes compared to melanoma cells. The DNA methylation profiles were compared to global gene expression studies in these cells. A preliminary analysis of the entire X-chromosome demonstrated complexity of DNA methylation patterns across the different coding and non-coding genomic regions. DNA from melanoma cells was hypermethylated in the upstream, 5'UTR and coding exon regions compared to normal melanocytes. Bioinformatic analysis showed that hypermethylation of high CpG content (CpGr>0.4), in upstream and 5'UTR regions appeared to be highly indicative of transcriptional repression. DNA methylation profiles combined with gene expression analysis highlighted several interesting regions of specific hyper/de-methylation in melanoma cells. Bisulfite sequencing of specific regions confirmed the MeDIP results and showed strong correlation between DNA methylation and gene expression. Our studies demonstrate that novel insights can be derived from global DNA methylation and gene expression analyses that can be the basis for predicting epigenomic markers in melanoma.

723/T

Methylation Analysis of the Fmr-1 Promoter Region in Fragile X Patients. *B. López, E. Velasco, M.J. Alonso, M. Durán, J. Tellería, I. Fernández.* Human Genetics Lab, IBGM, Valladolid, Spain.

Fragile X syndrome (FRAXA) is the most common cause known of inherited mental retardation. It is origined by an expansion of an unstable CGG-repeat tract in the 5'-untranslated region of the Fmr-1 gene on the X chromosome. According to repeat size, four allele categories have been established: normal (<45 CGG), grey zone (45-54 CGG), premutated (55-200 CGG), and full mutated (>200 CGG). The massively expanded CGG repeat leads to promoter hypermethylation and transcriptional inhibition. We have studied forty male patients classified according to repeat size: 10 X-Fragile full mutated (FM), 10 premutated (PM), 10 grey zone (G) and 10 normal by methylation-specific PCR (MSP) and bisulphite sequencing in order to study Fmr-1 promoter methylation status and specific methylation at CpG sites. Bisulphite sequencing was performed by sodium bisulphite treatment with CpGenome DNA Modification Kit (Chemicon International) followed by PCR reaction using primers similar to that of Weinhäusel et al. described for antisense strand. Purified PCR products were then used for the sequencing reaction and sequenced with an ABI 3100 machine. After bisulphite treatment, all cytosines must be converted to uracil except those that are mehylated (5' methylcytosine).We have observed that C-T conversion performance of non methylated cytosines was total in controls and XF excluding three specific cytosines at 13518, 13551, 13561 positions (GenBank accession number L29074 antisense strand) that remain still partialy not converted for FM patients. Comparative study between N, G and PM alleles and FM alleles shows that this phenomenon is exclusive for methylated promoter, and suggests the possibility that Fmr-1 methylated promoter could have targets for methyl specific transcription factors at this sites. This fact would explain the bisulphite treatment protection of these three cytosines surrounding CpG sites. The finding that all studied FM patients have the same not converted cytosine pattern at three cytosines next to methylated CpG sites opens the possibility of a coordinated transcription inhibition between methylation and methyl-binding protein repressors.

724/T

The Methylation Status of Transcribed Alu Repeats in Neuroblastoma Cell Lines. *L. Manzella, J. Bischof, M.F. Bonaldo, M.B. Soares.* Cancer Biology and Epigenomics, Children's Memorial Research Center, Chicago, IL.

DNA methylation is tightly associated with gene expression. Alterations in DNA methylation are one of several mechanisms involved in cancer development and progression. One epigenetic alteration typically associated with cancer is genome-wide hypomethylation which can de-repress the transcription of oncogenes and retrotransposable elements. It is known that retrotransposition and transcription of retroelements are highly suppressed in somatic and mature germ cells by hypermethylation of those elements, avoiding de novo retrotransposition. Our hypothesis is that the hypomethylation and subsequent transcription of Alu repeats in cancer cells could result in retrotransposition events and thus interfere with gene expression. In order to have a better understanding of the correlation between Alu expression and methylation in somatic cancer cells, we selected transcribed Alu repeats (tAlu) in an aggressive neuroblastoma cell line (LA1-55n). This selection allowed us to identify 136 clones that met strict criteria and gave us a high level of confidence to assign the tAlu's localization in the genome. The tAlus identified were mapped to ninety-nine genes, twenty-one in intergenic regions and sixteen in ESTs, including clusters in all categories. The localization in genes included: thirty-four within 3' UTRs, fifty-nine in intronic regions, five in an intron /3'UTR, and one in an intron/exon region. We are currently in the process to validate the Pol III transcription by specific RT-PCR. The methylation patterns of fourty-three tAlus have been analyzed using by bisulfite conversion methodology. We intend to concentrate our analysis at first, onto the Alu promoters regions (A and B boxes) of the verified ones. Our further analyses will include compare the methylation patterns of each CG position among the tAlus to identify whether some specific positions play a role in expression.

725/T

DNA demethylation in breast cancer. *A.H. O'Donnell[1, 2], R.A. Rollins[3], T.H. Bestor[2].* 1) MD/PhD Program; 2) Genetics and Development, Columbia University, New York, NY; 3) Current address: Wyeth Research, Pearl River, NY.

DNA hypomethylation was first identified in primary tumors by Feinberg and Vogelstein in 1983. Demethylation is especially prominent at retrotransposons, pericentric satellite sequences, and cancer-testis genes. No progress in elucidating the mechanism of demethylation in tumors has been made to date, although mutations or dysregulation of the DNA methyltransferases have been ruled out. It is not known whether cancer-specific demethylation is the result of gain-of-function or loss-of-function pathways. In order to address this issue, cell hybrids were generated by fusion of a demethylated and a normally methylated breast cancer cell line. While maintaining relatively stable DNA content over 160 generations, the hybrid cells show persistence of parental methylation patterns and a slow trend towards demethylation of pericentric satellite DNA. Dysfunction of the DNMTs is not responsible for the demethylation phenotype as the DNA methylation machinery of the normally methylated cell line is unable to methylate the demethylated DNA. While DNA demethylation has been shown to increase genomic instability, it may also have an anti-cancer role. Demethylation may activate a demethylation checkpoint mediated by local inflammatory response via the TLR9 innate immunity pathway and through the expression of neoantigens that are attacked by components of the adaptive immune system. In addition, low methylation levels have been shown to induce apoptosis. Identification of the mechanism and genes involved in DNA demethylation will facilitate the development of novel therapeutics in the treatment of breast cancer.

726/T

Hypomethylation of the *H19/IGF2* ICR1 in Russell-Silver Syndrome. *M.S Penaherrera[1], S. Weindler[2], M.I. Van Allen[1], S. Langlois[1], W.P. Robinson[1].* 1) Dept. Medical Genetics, University of British Columbia, Canada; 2) Fac. of Medicine, University of Leipzig, Germany.

Russell-Silver Syndrome (RSS) is characterized by pre- and post-natal growth deficiency, dysmorphic facial features, relative macrocephaly and body asymmetry. Around 10% of RSS cases are associated with maternal uniparental disomy for chromosome 7 (UPD7). Methylation sensitive Southern Blot analysis of the telomeric imprinting center region (ICR1) of *H19/IGF2* has previously shown that 20-55% of RSS cases (n=89) are associated with epimutations at 11p15. To further evaluate this we assessed the methylation status of two CpGs within ICR1 in peripheral blood of 22 RSS patients and 22 unaffected, age-matched controls using Single-Nucleotide Primer Extension (SNuPE). The methylation status at both sites was highly correlated (r^2=0.95; p<0.0001). The mean percent methylation at ICR1 in the patients (29.9%, SD +/-11.21) was significantly lower than that of the controls (37.8%, SD+/-5.69) (p= 0.005; Student's t-test). If hypo- and hypermethylation are defined as a value of more than 2 standard deviations (SD) below and above the mean of the controls, then 8 of 22 (36%) patient samples but no control samples in our series showed evidence of hypomethylation. The distribution of the patient methylation values is consistent with at least two distinct etiologies of RSS, in which approximately 1/3 of cases are associated with ICR1 hypomethylation. In this subgroup, the mean methylation value was 15.8% (SD+/-4.93). UPD11p15 was excluded in all cases. Two cases with UPD7 had normal methylation values. Pairwise comparisons between the presence or absence of a series of clinical features between the hypomethylated and normally methylated group showed no statistically significant differences. Methylation analysis is a useful tool to confirm the clinical diagnosis of RSS; however, it is not clear if such errors arise due to some other underlying factor. We are currently extending the methylation analysis of this region to several other CpGs located within the ICR1 using pyrosequencing.

727/T

GAA repeat expansion-associated epigenetic changes in Friedreich ataxia. *M.A. Pook, O. Ismail, D. Varshney, S. Lymperi, R. Mouro Pinto, S. Al-Mahdawi.* CCCB/BICGP, Division of Biosciences, School of Health Sciences & Social Care, Brunel University, Uxbridge, UK.

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder that is primarily caused by a GAA repeat expansion mutation within intron 1 of the FXN gene, leading to a decreased level of frataxin protein expression. The mechanism by which this mutation acts is currently unknown, but two models have been put forward. Firstly, it has been suggested that the GAA repeat expansion may adopt an abnormal triplex structure that interferes with FXN gene transcription. Secondly, there is evidence that the GAA repeat expansion is associated with epigenetic changes, such as DNA methylation and modification of histones, producing a heterochromatin-mediated gene silencing effect. In support of this second hypothesis, we have recently obtained data that shows increased DNA methylation of specific CpG sites immediately upstream of the expanded GAA repeat sequence in FRDA patient autopsied brain tissue, compared with non-GAA repeat expansion containing brain tissue. In contrast, no such changes were identified in the FXN promoter region. We have also identified similar DNA methylation increases in brain and heart tissues from our recently established GAA repeat expansion-containing FXN YAC transgenic mouse model, compared with similar non-GAA repeat expansion FXN YAC transgenic mice. These studies will be detailed, together with our more recent investigations to identify potential GAA repeat expansion-associated changes in methylation and acetylation of histones at the FXN locus. Such epigenetic studies to identify the potential GAA repeat expansion mechanism of action will provide valuable information for novel FRDA therapies.

728/T

METHYLATION ANALYSIS OF CANDIDATE GENES IN AUTISM. *M. Shinawi[1], R. Zascavage[1], P. Fang[1], A. Porter[2], D. Treadwell-Deering[2], A.L. Beaudet[1].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dep Psychiatry & Behav Science, Baylor Col Medicine, Houston, TX.

The genetic predisposition to autism is thought to be substantial with an estimated heritability of more than 90%. However, genome-wide linkage studies have not shown strong evidence for major autism-related loci. While mutations in few genes have been found in a small number of families with autism, studies of larger series of patients indicate these are very rare causes of autism. The high male-to-female sex ratio in autism has been replicated and confirmed in several epidemiologic studies. We are testing the hypothesis that de novo or inherited epimutations of sex chromosome-linked genes are responsible for the disease in a subset of autistic individuals and contribute to male susceptibility to autism. Our focus is on sex chromosome-linked candidate genes that are: 1) expressed mainly in the brain or involved in neuronal function; 2) not subject to X-inactivation with or without a homologue on the Y chromosome; and/or 3) subject to sexual dimorphism. The methylation status is being analyzed by using gel-based radioactive bisulfite sequencing or Southern blot analysis in the CpG islands of the following genes: *NLGN4X*, *NLGN4Y*, *PCDH11X*, *PCDH11Y*, *MAOA*, and *MAOB*. Blood samples from 15 affected females, 30 affected males and 30 controls and brain samples from 9 autistic individuals and 5 controls are being examined and compared. The preliminary data on all brain samples and on blood samples from 15 affected females and 12 affected males did not show significant differences between patients and controls. In all samples from males, the DNA was completely unmethylated. The data for the pairs *NLGN4X*/*NLGN4Y* and *PCDH11X*/*PCDH11Y* in females were consistent with the interpretation that the inactive and active X chromosomes are unmethylated. For the *MAOA* and *MAOB* the data were consistent with the interpretation that the inactive X chromosome was fully or partially methylated and the active X unmethylated. Very high desity custom Agilent arrays of the X and Y chromosome are now being used to analyze copy number, DNA methylation and chromatin modification.

729/T

DNA methylation profiles in diffuse large B-cell lymphoma and their relationship to gene expression status. *X. Wang[1], T.C. Greiner[2], B.L. Pike[1], D.D. Weisenburger[2], Y. Hsu[1], G. Renaud[3], T.G. Wolfsberg[3], M. Kim[1], D.J. Weisenberger[1], K.D. Siegmund[1], W. Ye[1], S. Groshen[1], R. Mehrian-Shai[1], W.C. Chan[2], P.W. Laird[1], J.G. Hacia[1].* 1) Biochemistry & Molecular Biology, University of Southern California, los angeles, CA; 2) University of Nebraska Medical Center, Omaha, NE; 3) National Institutes of Health, Bethesda, Maryland.

While gene expression, genomic copy number, and mutational analyses have provided key insights into the genetic basis for the extensive pathologic and biologic heterogeneity in diffuse large B-cell lymphoma (DLBCL), considerably less is known about its epigenetic underpinnings. Here, we evaluated the DNA methylation levels of over 500 unique gene-associated CpG islands in fourteen DLBCL tumors using McrBC-based CpG island microarray, MethyLight, and bisulfite sequencing analyses. Although we observed variation in DNA methylation across all DLBCL, we identified twelve CpG islands (*AR*, *CDKN1C*, *DLC1*, *DRD2*, *GATA4*, *GDNF*, *GRIN2B*, *MTHFR*, *MYOD1*, *NEUROD1*, *ONECUT2*, and *TFAP2A*) showing significant methylation in greater than 85% of the tumors surveyed. Interestingly, we found that the methylation levels of CpG islands proximal to *FLJ21062* and *ONECUT2* differed between activated B-cell-like (ABC-DLBCL) and germinal center B-cell-like (GCB-DLBCL) subtypes, which have distinct clinical outcomes. In addition, we compared the methylation and expression status of sixty-seven genes located within 500-bp of our methylation assays. Our observations are more consistent with the potential involvement of DNA methylation in the maintenance relative to the initiation of gene silencing. Nevertheless, the proportional reductions in *BNIP3*, *MGMT*, *RBP1*, *GATA4*, *IGSF4*, *CRABP1* and *FLJ21062* expression with increasing methylation suggests that epigenetic processes could be causally involved in the initial stages of gene silencing. Overall, the genes highlighted in our analyses warrant further investigation into their roles in the development and progression of DLBCL and potential as clinical biomarkers.

730/T

A New Method for Detecting CpG Methylation Status Using High-Resolution Melting. *L. Zhang, S. Dandekar, J.C. Papp.* Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA.

Cytosine methylation of CpG dinucleotides in CpG islands is an important mechanism of gene regulation in vertebrates. CpG methylation plays a role in both developmental and cancer biology. Abnormal methylation of CpG sites results in developmental abnormalities in humans. Abnormal methylation of CpG islands in tumor-suppressor genes promotes the development and growth of cancerous cells. Current methods available for detecting CpG methylation patterns require extensive manual processing or the use of expensive dual-labeled probes. We describe a new high-throughput, quantitative, low-cost technique which utilizes recent developments in high-resolution melting (HRM) technology to ascertain methylation status with no further manual manipulation after the PCR step. Unmethylated cytosine in the DNA sample is deaminated to form uracil by treatment with bisulfite. In subsequent PCR amplification the uracil is replicated as thymine. Methylated cytosine in the DNA sample is protected from reacting with the bisulfite, and the cytosine remains after PCR amplification. A pure sample of PCR product from bisulfite treated unmethylated DNA, with its cytosine converted to thymine, has a markedly different melting profile from the methylated DNA PCR product with its unconverted cytosines. In addition, the heterogeneous mixture of unmethylated and methylated DNA has a different melting profile from either of the pure samples. Using HRM analysis software, the degree of methylation of an unknown sample can be quantified by comparing the melting profile of the unknown sample to standards with known percent methylation. Using this technique on two genes which are commonly hypermethylated in a wide variety of cancers, hMLH1 and $P16^{INK4}\alpha$, we are able to detect as little as 1% of the methylated allele.

731/T

Position-dependent cancer hyper- and hypomethylation in a DNA repeat array linked to FSH dystrophy. *M. Ehrlich[1], L. Qi[1], K. Jackson[1], C. Shao[1], K. Tsumagari[1], M. Lacey[2].* 1) Hayward Genetics Prog, SL31, Tulane Medical Sch, New Orleans, LA; 2) Dept. of Mathematics, Tulane University, New Orleans, LA.

Short subtelomeric arrays of tandem 3.3-kb units, called D4Z4, are linked to the enigmatic facioscapulohumeral muscular dystrophy (FSHD). D4Z4 arrays with 1 to 100 units are on 4q35 and 10q26 but only a 4q35 array of 1-10 units is pathogenic. D4Z4 is normally dependent on DNMT3B for most of its methylation. By blot-hybridization analysis with various CpG methylation-sensitive restriction endonucleases, we found hypomethylation of D4Z4 in some ovarian carcinomas and Wilms tumors and hypermethylation in others, as previously seen for NBL2, another tandem repeat that is normally methylated mostly by DNMT3B. Surprisingly, in cancers with D4Z4 hypermethylation, there seems to be a barrier to spreading of methylation to the beginning of the repeat array despite the very high sequence conservation throughout the array. This suggests differences in chromatin structure affecting methylation at the interface of the (G+C)-rich D4Z4 array and the proximal sequence. In addition, several unusual CpG methylation patterns correlated with atypical local sequences. A tenaciously methylated CpG site proximal to the array is surrounded by repeated T-containing oligonucleotide motifs. Conversely, resistance to cancer-associated hypermethylation was seen at D4Z4 CpG sites near runs of G that can form G-quadruplexes, a non-B DNA structure. G-quadruplexes can regulate transcription and interactions between DNA duplexes in vivo. Therefore, G-quadruplexes in D4Z4 may not only decrease the probability of cancer-linked DNA hypermethylation, but more importantly, also play a central role in the topological constraints that confer pathogenicity on short 4q D4Z4 arrays and make long ones phenotypically neutral. (Supported in part by NIH Grant NS048859 and an FSH Society Grant.).

732/T

Integrative Whole Genome Genetic and Epigenetic Analysis of Lung Tumor Genomes. *E. Vucic, W.W. Lockwood, I.M. Wilson, R. Chari, B.P. Coe, C. MacAulay, S. Lam, W.L. Lam.* British Columbia Cancer Research Centre, Vancouver, BC, Canada.

Background: Lung cancer (LC) is the leading cause of cancer mortality worldwide. Understanding molecular mechanisms driving LC development and progression will lead to rational development of diagnostics and intervention based on a fuller understanding of disease biology. Although previous studies have yielded loci specific surveys of genetic and epigenetic changes, no study to date has simultaneously analyzed genetic and epigenetic alterations at the DNA level on a whole genome scale. Objective: To comprehensively characterize the underlying molecular alterations driving LC development using an integrative genomic and epigenomic analysis. Methods: A whole genome tiling path comparative genomic hybridization (CGH) array was used to generate high resolution copy number (CN) profiles of 161 lung tumors and 20 carcinoma in situ (CIS) lesions. Whole genome methylation profiles were determined by Methylation Dependent Immunoprecipitation (MeDIP) array CGH. Array data was visualized using SeeGH software and subjected to a segmentation algorithm to computationally determine regions of gain and loss and areas of differential methylation. Results: Complementary genomic and epigenomic profiles highlighted numerous CN and DNA methylation changes. In addition to novel regions of recurrent gain and loss, complex rearrangements with multiple segmental alterations present on the same chromosome arm highlight the instability of the tumors. Focal high level amplifications were characteristic of advanced tumors whereas whole arm changes were more common in the CIS lesions. Interestingly, LC subtypes were defined by unique patterns of CN and methylation changes, indicating their differential development. Lastly, concerted regions of DNA hypermethylation and segmental loss, as well as hypomethylation and gain signified novel two hit mechanisms for both gene silencing and activation respectively. Conclusions: Discovery of these novel features may shed light on disease mechanisms and identify new molecular targets for therapy and early diagnosis. Work supported by Genome Canada and CIHR.

733/T

Elevated mutation rate in late-replicating regions of the human genome. *J. Stamatoyannopoulos[1], I. Adzhubey[2], S. Sunyaev[2].* 1) Dept. of Genome Sciences, Univ Washington, Seattle, WA; 2) Div. of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Most human mutations arise as replication errors which escape DNA repair. The rate at which mutations arise in different genomic regions is known to be heterogenous in primate and rodent genomes, although the explanation for this is unknown. Regional mutational variation cannot be explained by male bias or by nucleotide contexts. Evidence from yeast suggests that DNA repair may become defective in late S-phase, with correspondingly higher mutation rate. We therefore hypothesized that a similar mechanism may underlie human mutation rate variation. To test this, we analyzed human nucleotide diversity and human-chimpanzee divergence across 44 diverse genomic regions (500kb-1.8Mb in size, collectively 1% of the genome) in which replication timing was measured at high resolution by the ENCODE Consortium. We find markedly elevated levels of human-chimpanzee divergence and human nucleotide diversity in late replicating regions compared to early replicating regions (21% increase in substitution rate and 47% increase in SNP density). Both substitution rate and SNP density closely parallel replication timing in a step-wise gradient from early to late S-phase. We demonstrate that this relationship cannot be explained by either G+C content or recombination rate, nor by the effect of hypermutable CpG di-nucleotides. The data suggest the existence of corresponding gradient in the effectiveness of DNA repair throughout S-phase in human cells. Significantly, the results indicate that the interplay between mutation and selection may vary markedly and predictably between different human gene loci, particularly those located within late replicating regions. Among the latter are numerous genes involved in early development and primitive cellular differentiation, mutations of which are disproportionately implicated in the genesis of human malignancies.

734/T

Changes in histone modifications reactivate the expression of epigenetically silenced tumor suppressor genes in cancer cells. *S. Fukushige, E. Kondo, A. Horii.* Department of Molecular Pathology, Tohoku University School of Medicine, Sendai, Miyagi, Japan.

Epigenetic modifications such as DNA methylation and histone modification play crucial roles in the pathogeneses of cancer by transcriptional silencing of tumor suppressor genes. Although the use of DNA hypomethylating agent such as 5-azacytidine effectively reactivates the expression of methylated tumor suppressor genes, the inhibitors of histone deacetylase alone normally do not have much effect on it. Here we report that the combination of demethylation at histone H3 lysine 9 and histone acetylation, which changes major histone modifications of most epigenetically silenced genes in cancer cells, reactivates the expression of densely methylated genes. To accomplish these changes in histone modifications, we used the JMJD2D which is a histone demethylase specific for dimethylated and trimethylated histone H3 lysine 9 and the NFκB transcriptional activation domain which recruits p300 histone acetyltransferase. These two components were linked to the methyl-CpG binding domain (MBD) to be recruited at the methylated promoter. As the *MLH1* gene is epigenetically silenced in HEK293T and AN3CA cells, we performed transfection experiments of this DNA construct and found the reactivation of the *MLH1* gene. Because MBD is used to recruit this construct at the *MLH1* methylated promoter, it is thought that this reactivation occurred without DNA demethylation of promoter region. Furthermore, the *CDKN2A* gene in DLD1 cell and the *GSTP1* gene in LNCaP cell were also reactivated by the use of above mentioned construct. These results suggest that changes in histone modifications are sufficient for reactivation of these methylated tumor suppressor genes without the use of DNA hypomethylating agent such as 5-azacytidine.

735/T

A *cis*-regulatory map of the human embryonic stem cell genome. *R.D. Hawkins[1, 2], G. Hon[1, 2], J.E. Antosiewicz[3], J.A. Thomson[3], B. Ren[1, 2].* 1) Laboratory of Gene Regulation, Ludwig Institute for Cancer Research, La Jolla, CA; 2) Department of Cellular and Molecular Medicine, UCSD School of Medicine, La Jolla, CA; 3) The Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI.

The self-renewal capacity and pluripotency of embryonic stem cells (ESC) depend on the complex interactions between transcription factors and genomic regulatory sequences in these cells. Our ability to exploit the human embryonic stem cells for therapeutic purposes is currently limited by an incomplete understanding of the *cis*-regulatory elements in the human genome. As a first step towards elucidating the mechanisms of self-renewal and cell fate commitment by human ESC, we have systematically identified and characterized the promoters, enhancers and insulator elements in the human genome in undifferentiated human ESC and in differentiated ESC after BMP4 treatment. We accomplish this by locating the genomic binding sites of specific histone modifications and the insulator binding protein CTCF in these cells using ChIP-chip analysis and genome tiling microarrays. We find that the patterns of insulator protein binding to DNA and chromatin modifications at promoters are nearly invariant before and after differentiation. By contrast, the chromatin modifications at enhancers undergo significant changes during this process. We identified specific enhancers that correspond to genes involved in self-renewal as well as differentiation and development. We find that a single gene is often driven by multiple enhancers, and that the effect of multiple enhancers is generally additive. This genome-wide map of *cis*-regulatory elements will provide insights on the regulatory mechanism for stem cell maintenance and differentiation.

736/T

Multi-faceted gene silencing mechanism of MeCP2. *H. Soejima[1], S. Yakabe[1,2], H. Yatsuki[1], K. Joh[1], K. Miyazaki[2], T. Mukai[3].* 1) Division of Molecular Biology and Genetics, Department of Biomolecular Sciences, Faculty of Medicine, Saga University, Saga, Japan; 2) Division of General Surgery, Department of Surgery, Faculty of Medicine, Saga University, Saga, Japan; 3) Saga University, Saga, Japan.

For epigenetic gene silencing, cooperation of methyl-CpG binding proteins (MBDs) and chromatin modification factors, which are recruited to methylated DNA, is required. We have screened genes, which are suppressed by MeCP2, one of the MBDs, by MeCP2 knockdown (KD) experiments combined with microarray gene expression analyses. We found that expression of 46 genes elevated more than three times in common with two independent KD experiments with different siRNA sets. Among the 46 genes, 24 had CpG islands (CGIs) within their putative promoter regions. We examined MeCP2 binding and DNA methylation at promoter CGIs of 10 genes. Three showed MeCP2 binding before KD and release from promoter CGI after KD, whereas others did not show MeCP2 binding even before KD. Among the three genes, two showed promoter DNA methylation but one did not. Furthermore, DNA methylation of the two genes was not changed after KD. These results suggested that a majority of genes are indirectly, rather than directly, suppressed by MeCP2, that DNA methylation itself is insufficient for the silencing when both DNA methylation and MeCP2 are involved, and that MeCP2 can bind to unmethylated CGI, leading to the silencing.

737/T

Identification of microRNAs involved in hematopoietic stem cell differentiation. *G.A. Molfetta[1,3], D.L. Zanette[2,3], D.G. Pinheiro[1,3], M.A. Zago[2,3], W.A. Silva-Jr[1,3].* 1) Dept of Genetics, School of Medicine from Ribeirao Preto-USP, Brazil; 2) Dept of Clinical Medicine, School of Medicine from Ribeirao Preto-USP, Brazil; 3) Center for Cell-Based-Therapy/CEPID/FAPESP, Brazil.

miRNAs are a class of small endogenous non-coding RNAs that recognize target sequences of imperfect complementarity in cognate mRNAs and either destabilize them or inhibit translation. Emerging evidences show that miRNAs play important role in stem cell self-renewal and differentiation. We sought to identify miRNAs that regulate the early stage of hematopoietic stem cell differentiation. CD34+ cells were purified from bone marrow and differentiated into myeloid and erythroid lineages; we have extracted RNA after 12h and 40h of culture. Analysis of gene expression was carried out by SAGE using I-SAGE Kit and to access a miRNA profile we used TaqMan® MicroRNA Assays Human Panel. Fold-change was calculated using $2^{-\Delta\Delta Ct}$ method where undifferentiated CD34+ cells were used as calibrator. A higher set of expressed miRNAs was found in myeloid 40h and in erythroid 12h. The two most highly expressed miRNAs in both myeloid and erythroid samples were miR-124a and miR-15b. In myeloid sample both miRNAs were expressed only at 40h while erythroid sample showed higher expression of miR-15b at 12h and miR-124a at 40h. We also looked for predicted miRNA target genes in our SAGE libraries and correlated miRNA expression with the expression of its target genes. For myeloid sample, the majority of miRNA target genes are transcription factors. We selected TFDP2 as a target for myeloid lineage; DP2 function as binding partner for E2F transcription factor regulating its activity. E2F inhibits the NFkB survival signal suggesting NFkB role in myeloid differentiation. We selected EVI1 as a target for erythroid lineage. EVI1 has important role in leukemogenesis and megakaryocytic differentiation. This gene is downregulated by its miRNA in erythroid sample raising the hypothesis of miRNAs blocking myeloid commitment and activating erythroid commitment. We describe that the same miRNA is required for modulation of different target genes depending on the differentiation pathway taken by the cell. Financial Support: FAPESP.

738/T

Sequence analysis of small non-coding RNAs present in preimplantation mouse embryos. *Y. Ohnishi[1,2,4], A. Toyoda[3], Y. Sakaki[3], K. Tokunaga[1], H. Hohjoh[2].* 1) Department of Human Genetics, Graduate School of Medicine, Univ. Tokyo, Tokyo, Japan; 2) National Institute of Neuroscience, NCNP, Tokyo, Japan; 3) RIKEN Yokohama Institute, Yokohama, Japan; 4) JSPS Research Fellow.

Small non-coding RNAs (18-30 nucleotides in length) including small interfering RNAs (siRNAs) and microRNAs (miRNAs) are thought to play an essential role in biological functions related to development, differentiation and proliferation. Recent studies suggested that such small-sized RNAs most likely contributed to gametogenesis and embyogenesis in vertebrates. In this study, we focused on the early development of mouse embryos and investigated small-sized RNAs present in the course of the development of the mouse preimplantation embryos. We cloned and sequenced small-sized RNAs isolated from unfertilized mouse eggs, morula-stage embryos (2.5 d.p.c.) and blastocysts (3.5 d.p.c.). A total of 2880 clones derived from the mouse eggs and embryos were isolated and sequenced. After annotation of the clones, we found that 531, 342 and 490 clones isolated from unfertilized mouse eggs, morula-stage embryos, and blastocysts, respectively, were mapped to the mouse genome. In addition, 20 clones (4%) derived from 19 miRNA genes, 19 clones (6%) from 9 miRNA genes, and 239 clones (49%) from 54 miRNA genes were detected in unfertilized mouse eggs, morula-stage embryos, and blastocysts, respectively. Interestingly, we noticed that there was a difference in size population of the cloned sequences among the three stages. The small-sized RNA clones in unfertilized eggs and blastocysts appeared to have a peak around 21-24 nucleotides in length, which were similar to those of repeat-associated siRNAs (rasiRNAs) and microRNAs. In contrast, the small-sized RNA clones in morula-stage embryos displayed a bimodal distribution peakede around 20-24 nucleotides and 30 nucleotides in length. Altogether, the data present here suggest that a dramatical alteration of small-sized RNA molecules most probably occurs in the course of the mouse preimplantation embryos.

739/T

The mammalian genome expresses an abundance of small RNAs. *R.J. Taft[1], L.J. Croft[1], M. Askarian-Amiri[1], C. Simons[1], J.M.G. Szubert[2], X. Zhou[3], J.S. Mattick[1].* 1) ARC Special Research Center for Functional and Applied Genomics, Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia; 2) Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom; 3) LC Sciences, Houston, TX, USA.

We have previously shown that the proportion of non-protein-coding DNA consistently scales with increasing biological complexity. We hypothesize that these vast sections of the mammalian genome encode many vital genetic elements, including small regulatory non-coding RNAs. Using criteria based on known microRNAs, we identified approximately 1.7 million sequences encoding putative stem-loop structures in the mouse genome, which may act as small RNA precursors. Thousands of randomly chosen predictions were tested using custom microarrays containing modified oligonucleotides, and were interrogated with small RNAs less than 300 nt. Controls were included for known small RNAs, particularly miRNAs, and for ubiquitous and highly expressed mRNA contamination. We found that 15% of mouse predictions were detected in whole 10-12 day embryo, and a total of 19% were detected in five separately polled tissues. Many of the predictions were differentially detected between tissues. There was also no difference in the validation rates between sequences exhibiting conservation and those that did not, suggesting that many may be clade-specific. Northern blot analysis of a random selection of 50 of the array-positive mouse predictions confirmed that 75% were detectable as small RNAs whose sizes ranged from ~20-110 nt, and include a potential new class of small RNAs at ~100 nt. Tissue panel expression analysis by Northern indicates that a least a subset of these small RNAs are highly expressed across a range of tissues. We have also performed experiments interrogating a prediction set of ~1.6 million putative stem-loop precursors in human, and found that ~35% returned positive results. We predict that, at a minimum, the mammalian genome expresses hundreds of thousands of small RNAs.

740/T

Identification of the genes modulating the activity of RNAi pathway using whole-genome shRNA library. *C. Pak, P. Jin.* Department of Human Genetics and Graduate Program in Genetics & Molecular Biology, Emory University School of Medicine, Atlanta, GA 30322.

RNA interference (RNAi) is a well-conserved mechanism that uses small noncoding RNAs to silence gene expression post-transcriptionally. Gene regulation by RNA interference (RNAi) has been recognized as one of the major regulatory pathways in eukaryotic cells. The endogenous small RNAs can shape diverse cellular pathways, including chromosome architecture, development, growth control, apoptosis and stem cell maintenance. RNAi operates through two post-transcriptional mechanisms: targeted mRNA degradation (siRNA) and suppression of translation (miRNA). The RNAi mechanism has been co-opted by researchers and has achieved broad utility in gene-function analysis, drug-target discovery and validation, and therapeutic development. Although several major components of the endogenous RNAi machinery, including Dicer, Argonaute proteins and TRBP, have been identified, little is known about the regulation of the RNAi pathway itself. In this study, using a cell-based RNAi reporter system to monitor modulation of RNAi activity, we are conducting a genome-wide RNAi screen to identify the genes that could modulate the activity of the RNAi pathway. In this system, an HEK 293-derived stable cell line expressing a GFP reporter gene (293-EGFP) was infected with a lentivirus expressing a short-hairpin RNA (shRNA) that resembles endogenous miRNA precursors and are processed by the endogenous miRNA machinery into siRNAs that specifically target to GFP. Upon transduction of GeneNet™ human 50K siRNA library (200,000 siRNA complexity targeting 47,000 transcripts), target cell populations with selected GFP fluorescence intensity have been recovered by flow cytometry. The corresponding target genes are being identified by sequencing and expression arrays. Subsequently, candidate genes will be further validated using independent siRNA duplexes. This study will assist in further advancement in understanding the importance of small regulatory RNAs in biology and human diseases.

741/T

Cis-elements, DNA replication and repeat instability at the human myotonic dystrophy type 1 locus. *J.D. Cleary[1,2], L. Foiry[3], G. Gourdon[3], C.E. Pearson[1,2].* 1) Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada; 2) Department of Molecular & Medical Genetics, University of Toronto; 3) Inserm U781, Hôpital Necker-Enfants Malades, Université Paris V, Paris, France.

Trinucleotide repeat instability is the cause of a growing number of human disorders including myotonic dystrophy type 1 (DM1). Depending upon the disorder, repeat instability can be observed in somatic and germline tissues for both non-proliferative and proliferative cells suggesting that a variety of DNA metabolic processes contribute to the mutational process. The distinct pattern of instability between tissues suggest a role for epigenetics. The pronounced instability that occurs within proliferative tissues and during periods of rapid proliferation supports a role for DNA replication, either independently or in conjunction with DNA repair, in instability. Proliferation was shown to be required for spontaneous repeat instability in cultured human DM1 patient-derived fibroblasts; this instability was enhanced by agents known to alter DNA synthesis and replication fork progression. We have determined the replication profile at the human DM1 chromosomal locus by quantitative competitive PCR analysis of nascent DNA derived from our patient-derived fibroblasts. In parallel, a similar analysis was done in transgenic mice with 45 kb of human DM1 locus with a tract of 300 CTG repeats. In humans, this analysis suggests that the DM1 locus is located within a region of abundant replication activity, with replication origins located proximal to the repeat. Interestingly, reduced replication activity is associated with the expanded DM1 repeat tract and is demarcated by CTCF binding site flanking the repeat tract. Further analysis of transgenic mice indicates differences in the replication profile between tissues. Taken together these data support a proximal 3' origin of replication at the expanded unstable DM1 repeat tract such that the CAG repeat is the lagging strand template. Changes in the replication origin location, utilization or changes in the progress of the replication fork through the repeat tract may be a significant factor in repeat instability.

742/F

RAPP-HODGKIN SYNDROME.CASE REPORT. *G. Garcia-Sanchez[1], C.F. Martínez-Cruz[2,3], M.C. Mata-Rivera[4], L. Hernandez-Gomez[4].* 1) Servicio de Genética.Direccion de Investigacion.Instituto Nacional de Rehabilitación, México, D.F; 2) Servicio de Comunicacion Humana.Depto de Seguimiento Pediatrico.Instituto Nacional de Perinatologia. México D.F; 3) Servicio de Pediatría Instituto Mexicano del Seguro Social.HGZ 53. México, D.F. email. guillegs@yahoo.com.mx; 4) Servicio de Audiologia.Instituto Nacional de Rehabilitación, México, D.F.

Rapp-Hodgkin syndrome (RHS), was first described over 30 years ago in an affected mother, son, and daughter with a combination of anhidrotic ectodermal dysplasia, cleft lip, and cleft palate (Rapp and Hodgkin, 1968). The clinical syndrome is comprised of a characteristic facies (narrow nose and small mouth), wiry, slow-growing, and uncombable hair, sparse eyelashes and eyebrows, obstructed lacrimal puncta/epiphora, bilateral stenosis of external auditory canals, microsomia, hypodontia, cone-shaped incisors, enamel hypoplasia, dystrophic nails, and cleft lip/cleft palate. Approximately 45 cases of this developmental disorder, usually with autosomal-dominant inheritance, have been reported. Several ectodermal dysplasia syndromes, including Rapp-Hodgkin, syndromes, are known to result from mutations in the p63 gene. A 7-year-old Mexican boy was seen in The Department of Genetics. Instituto Nacional de Rehabilitación. The proposito was the first child of nonconsanguineous Mexican parents. The pregnancy was uncomplicated with no known exposure to teratogens. The child was born at term with a birth weight of 3,600 g. His clinical findings included characteristic facies, sparse hair, eyebrows and eyelashes. Obstructed right lacrimal puncta and epiphora. Repaired right cleft lip and cleft palate, multiple caries, unerupted upper central incisors. The skin was dry. Hands with some hyperpigmented areas. Diffuse dermatitis of the scalp and face was present. All fingernails and toenails were dystrophic. He had hearing loss. Bilateral ear discharge at 3 and 6 years old. No other family member is affected. The clinical presentation of ectodermal dysplasia with cleft palate was consistent with Rapp-Hodgkin syndrome, which is one of several allelic diseases associated with mutations in the TP63 gene. sweating.

743/F

Geleophysic Dysplasia: clinical, radiological and ultrastructural studies. *C.A. Bacino[1], N. Brunetti-Pierri[1], J. Hicks[2], L. Potocki[1], J.G. Leroy[1], B. Lee[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pathology, Baylor College of Medicine, Houston, TX.

Geleophysic dysplasia (GD; MIM 231050) is an autosomal recessive disorder characterized by short-limbed dwarfism, brachydactyly and a "happy-looking" facial appearance. Based on the detection of lysosome-like inclusions in different tissues, the underlying cause of GD is considered to be a lysosomal storage defect. We report the clinical and radiological features of four GD cases confirmed by the presence of skin fibroblast inclusions on electron microscopy (EM) analysis. Two of the four cases are siblings and exhibited significant variability especially with regards to the cardiac involvement. Short stature and brachydactyly were present in all four patients, while laryngeal stenosis was present in two patients and Perthes disease in one patient. Taken together, these findings show a significant interfamilial and intrafamilial clinical variability consistent with a broad disease spectrum. No gene is currently known for this disorder and the diagnosis of GD is often difficult and mostly based on clinical and radiological findings. We provide evidence that EM of cultured skin fibroblasts is useful and reliable as an adjuvant tool for the diagnosis of GD. In an attempt to identify the defective pathway and ultimately the gene responsible for GD, using a proteomic approach, we have evaluated the differential expression protein profiles of GD fibroblasts and controls. This analysis allowed us to identify up to 24 proteins that are differentially expressed between GD patients and controls. Mass spectrometer analysis is currently in progress to determine the identity of the affected proteins.

744/F

X-Linked Mental Retardation Snyder-Robinson Type, second report in a Mexican family. *L.E. Becerra[1,3], G. Castañeda-Cisneros[2,3], J.E. García-Ortíz[1,3], J. Sánchez-Corona[2,3].* 1) Division de Genética, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) División de Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, México; 3) Doctorado en Genética Humana, CUCS-UdeG, Guadalajara, Jalisco, México.

Snyder and Robinson syndrome (OMIM 309583) is defined as a X-linked mental retardation syndrome with characteristic habitus (marfanoid), diminished muscle bulk, osteoporosis, facial asymmetry, a prominent lower lip, nasal voice, narrow or cleft palate, and long, thin fingers and toes; in some an unsteady gait, nonspecific movement disorder, and seizures. By linkage analysis was identified the related gene on Xp21.3-p22.12(spermine synthase gene). Here we described a Mexican family with Snyder-Robinson syndrome (SRS) with two individuals aged 28 and 21 year-old, respectively, born from healthy parents (at present mother 47 and father 60 year-old, respectively). They showed psychomotor development delay, thin habitus, facial asymmetry with prominent lower lip (more evident in younger brother), thoracic kyphoscoliosis, thin finger and toes, osteoporosis, and fractures (the older was more affected). In both: normal karyotype, negative molecular X-fragile test, lower bone density (-3.22 SD), and lower platelet count. X-ray studies shows: normal cranial computed axial tomography; thickened calvarium; lower bone density, long and thin tubular bones with thin cortex, the older had thoracic kyphoscoliosis, and asymmetric bent femur. The differential diagnosis included: Lujan-Fryns syndrome (OMIM 309520) tshares thin habitus, mental retardation, speech defects, X-linked recessive inheritance, but macrocephaly, long and triangular face, delayed skeletal maturation, hypoplasia of corpus callosum and heart anomalies are not recorded in SRS; Fragoso syndrome (OMIM 248770) shares thin habitus, lower bone density, scoliosis, hypotonia and mental retardation but facial asymmetry, prominent lower lip, and fractures are not referred, and its inheritance is recessive; Martin-Bell syndrome (OMIM 309550) sharing mental retardation, X-linked inheritance, but not macrocephaly, heart anomalies, and the molecular studies were negative.

745/F

Osteoporosis: a new feature of Bardet Biedl syndrome. *E. Heon[1, 4], W. Cole[2, 4], A. Daneman[3], J. Bin[4], G. Billingsley[4], E. Sochett[5].* 1) Ophthalmology and Vision Scien, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Orthopedic Surgery, Surgery, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Diagnostic Imaging, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Genetics and Genomics Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 5) Endocrinology, Pediatrics, The Hospital for Sick Children, Toronto, Ontario, Canada.

Bardet-Biedl syndrome (BBS: OMIM 209900) is a genetically heterogeneous disorder characterized by the primary features of progressive retinal dystrophy, obesity, polydactyly, renal malformations, cognitive impairment and hypogenitalism. 12 BBS genes have been identified to date. Identification of 2 BBS patients with severe, early-onset osteoporosis led us to explore the incidence of this finding in a cohort of Canadian patients affected with BBS. Forty patients affected with Bardet-Biedl syndrome were studied including 16 males, 24 females. Investigations included: thoracic and lumbar spine X rays, bone mineral density assessment, documentation of the following parameters: BMI, height, genotype, serum creatinine, PO4, ALP, PTH, Calcium, Vit D and electrolytes. Data was interpretable in 37 cases. Only one child (8 yrs) had normal x-rays. The others showed a variable combination of findings including: Osteoporosis, degenerative changes and vertebral deformity. Osteoporosis was observed in all cases (age range 3.4-40 yrs), degenerative changes were seen in 14/37 patients while vertebral deformities such as kypho scoliosis was seen in 23/37 patients. History of fracture was documented in 4 cases (age range 9-15 yrs) while a history of bone pain was present in 15 cases. There was no significant correlation with the genotype, gender, weight, height, or biochemical anomalies. However, the incidence and severity of the changes observed correlated with age. In summary, impairment of the function of BBS proteins appears to predispose patients to develop osteoporosis and to interfere with bone health. Documentation of this clinical feature in BBS will be important to optimize patient management and improve our understanding of the molecular pathways involved.

746/F

Cleidocranial dysplasia: the use of a specific protocol to detect atypical cases and new findings in eight Brazilian cases. *P.J.G. Pereira, L.A.N. Oliveira, D.R. Bertola, R.S. Honjo, C.A. Kim, L.M.J. Albano.* Genetics Unit, Instituto da Criança, Sao Paulo, SP, Brazil.

Cleidocranial dysplasia (CCD) constitutes a generalized autosomal dominant skeletal dysplasia with variable expression, affecting membranous bone ossification. It is characterized by short stature, patent fontanels, tooth anomalies, hypoplastic clavicles and other skeletal changes. We applied a specific radiological protocol in eight cases with clinical diagnosis of CCD from 4 families to amplify the CCD phenotypic spectrum and also to detect atypical cases. Six out of eight patients were females, and two were males. All cases were familial but one (7/8 - 87.5%). Main clinical findings were: short stature (3/7 - 43%); typical face (6/8 - 75%); midfacial hypoplasia (6/8 - 75%): delayed closure of the fontanels (5/7 - 71.5%); patent fontanels (2/7 - 28.5%); late teeth eruption (7/8 - 87.5%); delayed eruption of permanent teeth (7/7 - 100%); approximation of the shoulders (6/8 - 75%); genu valgus (6/8 - 75%); flatfeet (5/8 - 71.5%). Main X-ray findings were: hypoplasia of the clavicles (6/8 - 75%); aplasia of clavicles (1/8 - 12.5%); small scapulae (5/7 - 71.5%); hypoplasia of the iliac wings (4/7 - 57%); wide pubic symphysis (6/7 - 86%); delay of the pubic bone ossification (6/7 - 86%); broad femoral head and short femoral neck (6/7 - 86%); coxa valga (4/8 - 50%); coxa vara (2/8 - 25%); underpneumatization of the sinuses air cells (4/7 - 57%); lack of ossification of the calvaria (4/8 - 50%). No atypical CCD case was found. However, one case presented a duplication of the calcaneus, which is an unusual finding. We consider that integrated radiological and genetic evaluations are important to a better delineation of this skeletal dysplasia. Thus, atypical cases and new findings would be easily and promptly detected.

747/F

Job Syndrome masquerading as Non-Accidental Injury. *W. Reardon[1], F. Stewart[2].* 1) Dept Clin Gen, Ctr Medical Gen, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) Dept Medical genetics, City Hospital, Belfast BT9 7AB.

Syndrome diagnosis, particularly in the absence of objective laboratory analysis, is challenging, requiring specialist insights and experience. Job syndrome is a rare primary immunodeficiency disorder, classically described in association with recurrent staphylococcal skin abscesses, eczema and a predisposition to mucocutaneous candidiasis. IgE levels are massively elevated. Awareness of the syndrome is low among paediatricians, even among geneticists, many of the cases diagnosed having had several years of symptoms prior to recognition. Spontaneous bone fractures are a recognised aspect of the syndrome, even in the absence of an associated demonstrable osteoporosis. This predisposition to fractures was pivotal to our recent recognition of a case of Job syndrome at an advanced stage in child protection proceedings, when a permanent care order was being sought in respect of an 18 month old girl with unexplained fractures. Radiological, child protection and orthopaedic experts all agreed that the findings were consistent woth non-accidental injury. Several aspects of the history, including eczema, has been assumed to be co-incidental. The confirmation of IgE levels in excess of 100 fold the normal age range strongly supported the diagnosis of Job syndrome and led to the withdrawl of the case against the parents.

748/F

Phenotypic variability in the CDAGS syndrome: Report of an additional family. *R.L. Sparkes, A.M. Innes, D.R. McLeod.* Department of Medical Genetics, Alberta Children's Hospital, Calgary, AB, Canada.

A distinct, autosomal recessive genetic syndrome was recently characterized and genetically mapped to 22q12-q13[1]. The name "CDAGS" was suggested for its commonly associated features: **c**raniosynostosis and **c**lavicular hypoplasia; **d**elayed fontanel closure, cranial **d**efects and **d**eafness; **a**nal anomalies; **g**enitourinary malformations; and **s**kin eruption. Seven patients from four families with diverse ethnic backgrounds were described. The most consistent clinical features included coronal synostosis, wide open fontanels with parietal foramina, sparse brows and lashes, imperforate anus and hyperkeratotic skin eruptions.

We report the eighth and ninth individuals with the CDAGS phenotype: a brother and sister born to consanguineous Pakistani parents. Our patients lack coronal synostosis, genitourinary malformations and anal anomalies. Features shared with the published cases include sagittal and lambdoidal craniosynostosis, cranial defects, facial dysmorphism, dental anomalies, deafness, developmental delay, hypoplastic clavicles and skin eruptions, the latter being mild. Unreported features seen in one or both of our siblings include bilateral paresis of the ocular depressor muscles, Chiari I malformation, occult spinal dysraphism and chronic pancreatitis.

Although the genetic defect in CDAGS is not known, because of overlapping features with other genetically characterized conditions including cleidocranial dysplasia (*RUNX2* gene), it was suggested that the molecular pathogenesis involves disruption of multiple signaling pathways important for osteoblast differentiation, chondrocyte maturation and craniofacial morphogenesis, thereby explaining the paradoxical occurrence of both delayed ossification and accelerated suture closure. Identification of the gene for CDAGS and phenotypic characterization of additional families will facilitate further understanding of these developmental mechanisms in this interesting and variable genetic condition.

[1]Mendoza-Londono et al. *Am J Hum Genet* 77:161-168,2005.

749/F

Moebius syndrome: report of a new case. *G.Juarez Garcia[1], L.Hdez Gomez[2], D.Gomez Torres[3], F. castillo Lorca[4].* 1) Dept Genetics Neuropsicologia, Inst Nacional de Rehabilitacion, Mexico, D.F; 2) Dept Genetics, Audiologia, Inst Nacional de Rehabilitacion, Mexico, D.F; 3) Dept Genetics, Investigación, Inst Nacional de Rehabilitacion, Mexico, D.F; 4) Servicio de Audiologia de Gaes. México.

Moebius syndrome is an extremely rare disorder characterized by a lifetime facial paralysis, involving sixth and seventh cranial nerves with malformations of orofacial structures and the limbs. A number of mechanisms have been proposed to explain the pathogenesis, including prenatal ischemia. In some patients, the dysgenesis is genetically determined and can be isolated or form part of a more extensive polymalformation syndrome (mutations of organizing or regulatory genes). In most patients with brainstem dysgenesis, however, the disorder is caused by prenatal destructive or disruptive lesions of vascular origin. We present a new case: female mexican child 4-year 7 moths -old is the first child of young non consanguineous parentes, obteind pretermino. She presented paralysis facial left. That presents inconvenience of language, characterized by being found to level at level of sentences, well directed and structured with articulatory distortions. EEG normal. ABR responses in 30dB heard right, and 40dB heard left. transient evoked otoacoustic emissions 95% in both hearings. Normal intellectual capacity.

750/F

Mosaic neurofibromatosis type 1 (NF1) in Finland. *J. Ruohonen[1], S. Peltonen[2], J. Peltonen[3], M. Poyhonen[1,4].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Department of Dermatology, University of Turku, Turku, Finland; 3) Institute of Biomedicine, Department of Anatomy, University of Turku, Turku, Finland; 4) Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland.

Background Neurofibromatoses are hereditary diseases that belong to the group of phakomatoses. Neurofibromatosis type 1 (NF1) has distinctive cutaneus features and NF type 2 has mainly intracranial lesions. In the segmental or mosaic type (formerly known as NF5) the disease features of NF1 are distributed regionally on the body. It is caused by a gonosomal mosaicism of the NF1-gene. There is no previous data on mosaic NF1 in Finland. **Methods** Patients' disease features were collected from hospital files. A large literature search was made and the cases were compared with the Finnish patients. We used several different criteria found in the literature to classify the patients. The objective was to study the incidence of mosaic NF1 in Finland and the disease features in Finnish patients. **Results** Classification of patients unequivocally according to existing criteria for mosaic NF1 proved difficult. The incidence of mosaic NF1 in Finland (0,0005%) was lower than that found in other countries (0,0014%-0,002%), but in areas where the material was the most comprehensive (Turku and Oulu university hospital districts) the incidences (0,0013% and 0,0011% respectively) were very similar to the other studies. The most common disease feature was the neurofibroma, which was found in 81% of the Finnish patients and in 76% of the cases in the literature. The frequence of café-au-lait-spots was respectively 39% and 35% and that of freckles 26% and 38%. Lisch nodules, mainly unilateral, presented in 15% of the Finnish patients and in 8% of the literature cases. **Conclusions** There is a need for more unified criteria for classifying mosaic NF1. The incidence and disease features of mosaic NF1 in Finland are similar to those found in other countries and in the literature. This material can base further molecular genetic studies on mosaic neurofibromatosis type 1.

751/F

Postural Orthostatic Tachycardia is an age dependent manifestation of Ehlers-Danlos Syndromes. *C. Slemenda[1], B.F. Griswold[2], L. Sloper[2], C.A. Francomano[3], N.B. McDonnell[2].* 1) LI, NIA/NIH, Baltimore, MD; 2) LCI, NIA/NIH, Baltimore, MD; 3) GBMC, Baltimore, MD.

Postural Orthostatic Tachycardia (POTS), defined as a heart rate increase greater than thirty beats per minute from supine to standing, has been reported to be associated with joint hypermobility. We studied the prevalence of POTS among 61 consecutive patients with hypermobile and classical forms of Ehlers Danlos syndrome seen at the National Institutes of Health. Supine, sitting, and standing heart rate measurements were obtained for each subject with five minutes of rest between each position. Thirty eight percent (23/61) of the subjects met criteria for POTS. The condition was significantly more common ($p<0.001$) in patients under the age of 25, with 72% of such patients being affected, as compared to 13.9% of persons over the age of 25. The presence of POTS was associated with a reduction in quality of life, including inability to maintain gainful employment or attend school. The etiology and natural history of POTS in this cohort is not well understood and merits further investigation.

752/F

Duplication 22q11.2: Clinically Heterogeneous New Syndrome or Genetic Polymorphism? *J.A. Bernstein[1], F.S. Alkuraya[2], L. Armstrong[3], K.C. Chen[1], C. Clericuzio[4], J.M. Graham[5], J. Stoler[2], H.M. Saal[6], C.A. Stevens[7], A.M. Cherry[1], H.E. Hoyme[1].* 1) Stanford School of Medicine, Stanford, CA; 2) Children's Hospital Boston, Boston, MA; 3) B.C. Children's Hosptial, Vancouver, B.C. Canada; 4) University of New Mexico, Albuquerque, NM; 5) Cedars-Sinai Medical Center, Los Angeles, CA; 6) Cincinnati Children's Hospital, Cincinnati, OH; 7) T.C. Thompson Children's Hospital, Chattanooga, TN.

Duplication of 22q11.2 has been described as the mechanism underlying a recently recognized condition featuring a range of physical and developmental abnormalities. We are aware of 50 reported cases. Duplication of 22q11.2 is expected to occur at equal frequency as its deletion, however, the duplication syndrome has been diagnosed much less frequently. This apparent underdetection may result from the clinical variability of the syndrome. In case reports common features include dysmorphic facies, cleft palate, congenital heart disease, poor postnatal growth, seizures and cognitive impairment. However, significant inter- and intrafamilial variation has been described.

In an effort to refine our understanding of duplication 22q11.2, we present twelve new cases from eight families. Our cases demonstrate many recognized characteristics of the syndrome. However, they also include manifestations not previously described: cyclic vomiting, hemihyperplasia, congenital hypothyroidism and radial aplasia. Notably, three of our cases are parents of affected children who carry duplication 22q11.2 without significant clinical sequelae.

Our observations expand the range of anomalies associated with duplication 22q11.2. The finding of unaffected parents carrying the duplication suggests complicated inheritance for this syndrome. Alternatively, duplication 22q11.2 may represent a polymorphism that has incidentally been detected in patients with unrelated conditions. We expect duplication 22q11.2 will be identified increasingly with greater clinical awareness and expanded use of array-CGH. Further study of families with this duplication will be needed to distinguish a new syndrome from a possible polymorphism.

753/F

Revisiting genetic influences on neural tube defects: extended evaluation of a large dataset with evidence supporting maternal effects. *K.L. Deak[1], T.M. George[2], D.S. Enterline[1], G. Worley[1], D.G. Siegel[1], J.R. Gilbert[1], M.C. Speer[1], the NTD Collaborative Group.* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Children's Hospital, Austin, TX.

Neural tube defects (NTDs) are the second most common birth defect with an incidence of about 1/1000. We analyzed the family structure, genetic inheritance patterns, and evidence for maternal effects in our collection of 1066 NTD families (1467 affected patients). Of these families, 307 are multiplex and have two or more individuals with a neural tube defect. The majority of the patients had myelomeningocele or spina bifida (66.9%) and the next largest group had cranial defects (17.7%). The overall male to female ratio for all affected individuals was 0.82 and when the sex ratios are analyzed according to the NTD level, those individuals with an upper NTD had a greater female excess, with a M:F sex ratio of 0.66. Of the twins in these families, two of the five sets of monozygotic twins and 27% of the like sex twins are concordant for the disease, while only three of 35 (8.57%) dizygotic twin pairs are concordant. Notably, none of the unlike sex twins were concordant for disease and all affected twin pairs were of the same sex and had the same NTD type. We estimated a 6.3% recurrence risk to siblings (CI 0.04 - 0.08), consistent with other reports. In families with two or more related affected individuals, the sex ratios of affected and normal gene transmitting or connecting family members show a higher proportion of female transmitters ($P = 0.0002$), suggesting that affected individuals are more likely to inherit NTD-associated genes from their mother. There were also more than twice the number of affected relatives on the maternal side of the family ($P = 0.006$). We did not observe a difference in the average sibship size of maternal and paternal relatives, however there were significantly more miscarriages, infant deaths, and stillborn pregnancy outcomes in the mothers of aunts and uncles ($P < 0.0001$) and of first cousins ($P = 0.04$) of the probands. Together, these suggest a role for a maternal or imprinting effect in the etiology of NTDs.

754/F

Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF) Associated with Congenital Anomalies. *E. Font-Montgomery[1], H. Edwards[1], D. Adams[1], P. Held[1], P. Choyke[2], L. Guay-Woodford[3], T. Heller[4], P. Mohan[5], K. Daryanani[6], W. Gahl[1,7], M. Gunay-Aygun[1,7].* 1) MGB, NHGRI/NIH, Bethesda, MD; 2) NCI, NIH; 3) University of Alabama, Birmingham AL; 4) NIDDK, NIH; 5) CNMC, Washington, DC; 6) NIH CC; 7) Intramural Office of Rare Diseases, NIH.

ARPKD/CHF, the most common childhood ciliopathy, is characterized by dilated renal collecting ducts resulting in renal insufficiency and ductal plate malformation of the biliary system resulting in CHF. It is caused by mutations in PKHD1, which encodes fibrocystin, a protein located on the primary cilia-basal body/centriole. Other ciliopathies, commonly associated with overlapping features, include Joubert Syndrome (JS) and related cerebello-oculo-renal syndromes (CORS), Bardet-Biedl (BBS), Meckel-Gruber (MGS) and Oral-Facial-Digital-1 (OFD1) syndromes and potentially other, yet-to-be-discovered disorders. Although many ciliopathy genes have been identified, for most of these disorders the processes of gene identification and phenotype delineation remain incomplete. The current consensus clinical diagnostic criteria for ARPKD/CHF require characteristic kidney and liver involvement, family history consistent with autosomal recessive inheritance, and absence of congenital anomalies. In the ongoing NIH natural history study on ARPKD/CHF and other ciliopathies (www.clinicaltrials.gov, trial NCT00068224), we have evaluated 88 patients, 72 of whom were referred with a diagnosis of ARPKD/CHF. NIH evaluations including high resolution ultrasound, MRI and sequencing of the PKHD1 gene confirmed ARPKD/CHF in 59 of the 72 patients. Here we present 5 of the 72 patients who had congenital abnormalities in addition to the typical kidney and liver disease of ARPKD/CHF. These include a patient with tetralogy of Fallot and another with unilateral cleft lip/palate, both of whom have two pathogenic mutations in PKHD1. PKHD1 sequencing was negative in the other 3 patients, one of whom had craniofacial dysmorphism associated with enlarged basilar cisterns. We continue enrolling patients to better delineate the phenotypic spectrum and improve diagnostic accuracy of these disorders.

755/F

Macrocephaly in autism is not a homogeneous marker phenotype. *M.M. Keegan, T.N. Takahashi, J.H. Miles.* Thompson Center for Autism, University of Missouri Hospital, Columbia, MO.

Autism spectrum disorders (ASD) are a broad category of neurodevelopmental disorders which can originate from a variety of genetic and environmental causes. To delineate this heterogeneity we have looked for biologically based phenotypes occurring in a significant proportion of individuals with ASD. One informative phenotype is macrocephaly defined as head circumference (HC) ≥ 97% which occurs in 25-35% of individuals with autism and in 37-47% of parents of ASD children (Miles et al. 2002). Longitudinal data, however, are conflicting; some studies report normal or low head size at birth with accelerated head growth in the first few months or between 2 and 3 years, hypothesizing that sudden increase in growth velocity is the correct autism associated phenotype. We examined longitudinal HC curves of 55 children (49 males, 6 females) with classical autistic disorder (AD) with an essential phenotype, who attend a large research based autism center, had birth HC and at least 3 more measurements. Our results indicate that the majority of AD children (62%) have normal HC at birth (Z= -1.0 to 0.5) and continue to have head growth consistently within the normal range with no period of excessive growth. The remaining 38% have normal HC at birth (Z= -1.3 to 1.4) but become macrocephalic. This group is also heterogeneous with 31% having surpassed 97% by age three, 31% in mid-childhood (ages 3-7), and 37.5% after age 8 years. Parents of both groups were considerably more apt to be macrocephalic than expected (60% fathers, 12% mothers in macrocephalic group, 54% fathers, 10% mothers in normocephalic group). These results indicate that macrocephaly is clearly an ASD risk factor, but is a heterogeneous phenotype which undoubtedly has a variety of developmental origins. Previous conflicting reports can usually be attributed to known pitfalls in the study of pediatric head growth including unreliability of birth HC due to molding during delivery, lack of consistent measurement techniques, the dearth of longitudinal studies in typically developing populations and the lingering misperception that ASDs are one homogeneous disorder where averaging measurements is valid.

756/F

Preferential petterns of association of cleft lip with or without cleft palate with others major congenital malformations. *J.J. Morales[1], L. Luna[1], A.R. Villa[2], O.M. Mutchinick[1].* 1) Depto of Genetics, INCMNSZ; 2) Clinical Epidemiology, INCMNSZ, Mexico City.

Approximately 25% of newborns with CL(P) are multiple malformed (MM). The aims of the present study is to determine preferential patterns of association (PPA) of CL(P) with other MCM and to propose a method for the identification of such patters in any type of MM individuals. Data was obtained from the database of the Mexican program of Registry and Epidemiologic Surveillance of Congenital Malformations (RYVEMCE), a case-control hospital-based study. We analyzed 154 cases with CL(P) selected from a total of 588 non-syndromic MM newborns. We observe a total of 1640 MCM corresponding for 31 differences diagnosis. To identify possible preferential associations of CL(P) with other MCM we decide to include only those cases observed at least twice. The analysis was based on the estimation of the respective observed to expected (O/E ratio) method. The expected value was the result of the product of the frequencies observed for CL(P) and each of the MCM included. For this pairwise approach, were obtained the prevalence of CL(P) and each one of the MCM using as denominator the total number of MCM (1640). An O/E value higher than 1and statistically significant according to Poisson test (P<0.05) was considered a preferential association. To avoid spurious associations due to the clustering among all defects we use multiple regression logistic (MLR) analysis. Those cases that showed to be preferentially associated were included in a subsequent analysis to further delineate specific patterns creating a correlation matrix . Our result show that CL(P) was significantly associated with 8 different MCM: anencephaly (AN) (15), encephalocele (EC) (7), hydrocephaly (HC) (), microphthalmia (MP) (), severe nose anomalies (SNA) (), microtia (M) (), polydactyly (P) (), and hypospadias (HP) () (P<0.05 - P<0.001). Among those 3 were specific dyads CL(P)+ AN, CL(P) + P and CL(P) + HP, 2 were triads CL(P)+ M + MP and CL(P) + EC + MP and CL(P) + HC + SNA + MP. This is the first report using the methodology here in described that importantly avoids many spurious associations that are not real PPA.

757/F

Linear Scleroderma "en coup de sabre" (LScs). Report of 2 cases and a literature review. *E.J. Ramirez-Lizardo[1,2,3], S.E. Totsuka-Sutto[1,3], M.C. Islas-Carbajal[1], T.A. Garcia-Cobian[1], E.G. Cardona-Muñoz[1].* 1) Unidad de Investigación Cardiovascular. Centro Universitario de Ciencias de la Salud. Universidad de Guadalajara; 2) Departamento de Genética Instituto Jalisciense de Cirugía Reconstructiva. Secretaria de Salud Jalisco; 3) Instituto de Genética "Dr. Enrique Corona" CUCS. Universidad de Guadalajara. Guadalajara Jalisco México. elizardo@cucs.udg.mx.

Scleroderma is a rare disease of unknown etiology in which increased collagen deposition occurs and results in dermal thickening. Involvement may be diffuse (systemic sclerosis) or localized to skin (localized scleroderma). Linear scleroderma represents a unique form of localized scleroderma that primarily affects the pediatric population, with 67% of patients diagnosed before 18 years of age. When linear scleroderma occurs on the head, it is referred to as linear scleroderma "en coup de sabre", given the resemblance of the skin lesion to the stroke of a sabre. LScs is a descriptive term denoting linear scleroderma of the frontoparietal or frontal area of the face and scalp. In most cases with LScs, disorders such as seizures, uveitis, dental anormalities, cerebral abnormalities and ocular muscle dysfunction are associated We describe 2 pediatric patients with linear scleroderma "en coup de sabre". Our patients presented a typical skin lesion on the frontoparietal area. One patient presented furthermore skin discoloration and depression on her chin. Both family noted echymotic color change and depression becoming progressively more prominent affecting theirs forehead, nasal and chin areas. The present cases show 2 families everyone with one proposita with LScs support on the clinical date of skin discoloration and depression on the typical area for the LScs. Both are sporadic case. The differential diagnosis included the Parry-Romberg syndrome. The clinical presentation and etiology hypotheses are reviewed.

758/F

CHARGE Syndrome Masquerading as the 22q11.2 Deletion Due to Significant Immunodeficiency. *K.E. Sullivan[1], D.M. McDonald-McGinn[1], S. Bale[2], E.H. Zackai[1].* 1) Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) GeneDx, Gaithersburg, MD.

CHARGE syndrome occurs in approximately 1:9000 births and is associated with a CHD7 mutation in 60% of cases. CHD7 is a chromatin remodeling protein expressed at low levels in many tissues including the thymus. There are few data describing the immune system in patients with CHARGE although the second most common cause of death is infectious. Individual case reports with CHARGE-like features and profound immune deficiency have sometimes been referred to as DiGeorge syndrome. Of note, the expression of CHD7 in pharyngeal mesenchyme parallels that of TBX1, the major gene contributing to the phenotype of DiGeorge syndrome/the 22q11.2 deletion. With this in mind, we carefully defined the immune deficiency in four patients who presented in infancy with phenotypic features of CHARGE as well as significant infection leading to a concern for a 22q11.2 deletion. All patients had normal 22q11.2 deletion studies by FISH but were in fact found to have CHD7 mutations. Three of four patients had total T cell counts of less than 20% of normal. In spite of dramatically depressed T cell counts, two patients had relatively normal T cell function as measured by proliferative responses to T cell mitogens. Two patients also had markedly impaired antibody production presumably due to decreased T cell production. In an effort to determine how frequently T cell decrements are found in patients with CHARGE, we subsequently measured absolute lymphocyte counts (comprised largely of T cells) in eight other patients with a CDH7 mutation. Of these, three had persistently low absolute lymphocyte counts which are strongly suggestive of T cell compromise. Thus, in summary, patients with CHARGE/CHD7 mutations appear to have an immune deficiency which resembles that seen in the 22q11.2 deletion, i.e., diminished T cell counts with preservation of function. Given the high frequency of infection in these patients, screening for immune deficiency may well be warranted. Conversely, patients with this particular immune deficiency may benefit from CHD7 testing following normal 22q11.2 deletion studies.

759/F

X-linked Bilateral Abductor Vocal Cord Paralysis: A Case Report. *R. Veith[1], A. Khmour[2], D. Beste[1], P. Trapane[2].* 1) Children's Hospital of Wisconsin, Milwaukee, WI; 2) Medical College of Wisconsin, Milwaukee, WI.

Stridor in neonates may often have an underlying cause of vocal cord paralysis; however, familial vocal cord paralysis is much less common. Cases of isolated congenital adductor paralysis and isolated congenital abductor paralysis have both been reported. Families with congenital adductor paralysis have demonstrated autosomal dominant inheritance (OMIM 150270) whereas families with congenital abductor paralysis have demonstrated either autosomal dominant (OMIM 150260) or X-linked inheritance (OMIM 308850). Those with the X-linked form of congenital abductor paralysis are the rarest with only 4 pedigrees reported to date.

We report a family with a phenotype of vocal cord paralysis due to bilateral abductor paralysis that appears to be inherited in an X-linked fashion. The family contains 2 affected males and 1 affected female in two generations. The affected individuals are a male child and a female child of two sisters whose brother is also affected. A third male has a history of breathing difficulties for whom a diagnosis has not yet been confirmed. All affected individuals have had neonatal onset of stridor leading to the placement of a tracheotomy for adequate ventilation. Tracheotomy placement ranges from four days of age to two months of age. Dysmorphic features are not present in affected individuals. We believe that this family has the X-linked form of congenital abductor paralysis (OMIM 308850). X inactivation studies will be performed in addition to chromosome studies and DNA microarray analysis.

760/F

The Face of Feingold: Two Three-Generation Families with ODED Syndrome. *W. Al-Hertani[1], H. van Bokhoven[2], G.E. Graham[1]*. 1) Department of Genetics, Children's Hospital of Eastern Ontario & University of Ottawa, Ottawa, Ontario, Canada; 2) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Feingold (ODED) syndrome is an autosomal dominant condition characterized by microcephaly, variable cognitive disability, short stature, esophageal and duodenal atresias and hypoplasia, brachymesophalangy and syndactyly of the digits. The syndrome is caused by mutations in MYCN (2p24.1) and to date there is no evidence for genetic heterogeneity. Here we present six affected individuals (a daughter, mother and maternal grandmother in two unrelated families) with molecularly proven diagnoses. In family A the diagnosis was suggested by the presence of TEF in both mother and daughter and supported by the presence of microcephaly, facial findings and typical digital findings. In contrast, the diagnosis in family B was made on the basis of facial features without a history of intestinal atresia or typical limb findings in the proband. The salient facial characteristics in our patients include short, upslanting palpebral fissures, bilateral epicanthus (which may resolve with outgrowth of the nasal root) and an impression of hypotelorism in adulthood. The face of Feingold syndrome has not been emphasized in the literature despite its utility in establishing the diagnosis, particularly in the absence of gastrointestinal atresias and obvious extremity findings such as moderate or marked syndactyly. The women in our families also draw attention to the presence of tapered fingers and a large sandal gap in addition to the generalized brachymesophalangy, clinodactyly of the index finger and thumb hypoplasia already recognized as characteristic. Our families also confirm that the cognitive phenotype in Feingold syndrome can be normal, reinforcing a recent suggestion that "microcephaly-digital abnormalities-normal intelligence" is probably not a distinct condition. In both of our families cognition was normal in the grandmothers and sub-normal in the daughters and granddaughters, possibly reflecting ascertainment bias.

761/F

The Importance of Genetic Counseling and Multidisciplinary Approach to Rare Disease Report a case of Johanson-Blizzard Syndrome and review of Literature. *B. Bozorgmehr[1], A. Kariminejad[1], M. Zenker[2], M.H. Karimi-Nejad[1]*. 1) Clinical Genetics, Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran; 2) Institute of Human Genetics, Erlangen, Germany.

Proband is an eleven month old Iranian girl, third offspring of first cousin parents. Their first and second child died in the neonatal period without any diagnosis. The proband revealed growth and developmental delay, short statures, microcephaly, hypoplastic alae nasi, scar of repaired scalp defect, upsweeped hair, hypothyroidism, deafness and malabsorption, consistent with Johanson-Blizzard syndrome. She was admitted to hospital several times without any conclusive diagnosis. DNA samples of the proband and parents were sent to institute of Human Genetics, Dr. Zenker for molecular analysis of the UBR1 gene. A homozygous sequence alteration in intron 26 was detected in proband. She was homozygous for some known SNPS dispersed over the UBR1 gene. Heterozygous carrier state was detected in her parents.

762/F

Adams-Oliver Syndrome: Clinical Variability in a Four-Generation Family. *N. Brunetti-Pierri[1], J.T. Hecht[2], I. Van den Veyver[1], T. Eble[1], C.A. Bacino[1]*. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, University of Texas Medical School at Houston, Houston, Texas, USA.

Adams-Oliver syndrome (MIM 100300) is a rare disorder characterized by congenital scalp defects, terminal transverse limb defects, and cutis marmorata telangiectatica. Limb abnormalities are typically limb truncation defects affecting the distal phalanges, entire digits and/or distal limbs. Cardiac and vascular malformations have also been frequently reported in this disorder. Autosomal dominant inheritance is the most frequently reported mode of inheritance for Adams-Oliver syndrome, although autosomal recessive inheritance has also been suggested. We report a new family of Mexican ancestry with Adams-Oliver syndrome with multiple affected individuals in four generations that segregates in an autosomal dominant fashion. The affected members exhibit significant phenotypic variability ranging from distal phalangeal involvement to severe limb reduction defects. The absence of congenital scalp defects in some family member suggests that this feature is not an invariably finding in Adams-Oliver syndrome patients. The etiopathogenesis of this disorder remains unclear, but genes involved in vasculogenesis/angiogenesis during limb development have been proposed as possible candidates for this disorder. An Agilent 244K Whole Human Genome CGH Microarray analysis failed to reveal any significant copy number changes to suggest loss or gain of genetic material in affected patients of this family. Currently linkage studies on this large family are underway to identify the gene responsible for Adams-Oliver syndrome.

763/F

Novel clinical manifestations in Pallister-Killian Syndrome: Comprehensive evaluation of 18 affected individuals and review of all previously reported cases. *L.B. Campbell[1], K. Park[1], M. Jackson[1], A. Kostanecka[1], M. Pipan[1], P.D. Pallister[2], I.D. Krantz[1]*. 1) Department of Clinical Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Medical Genetics, Shodair Children's Hospital, Helena, MT.

Pallister-Killian Syndrome (PKS) is a multisystem developmental disorder caused by tetrasomy of chromosome 12p that exhibits tissue-specific mosaicism. The spectrum of clinical manifestations in PKS is wide and includes craniofacial dysmorphia, clefting, ophthalmologic, audiologic, cardiac, musculoskeletal, diaphragmatic, gastrointestinal, gentinourinary, and cutaneous anomalies in association with cognitive retardation and seizures. Growth parameters are often normal to elevated at birth with deceleration of growth postnatally. The prevalence of PKS has been estimated to be approximately 1 in 20,000 live births but is likely underascertained since tetrasomy 12p is often not present in the blood and requires fibroblast or other tissue sampling to identify. We report the clinical findings in 18 individuals with PKS who were all evaluated at the first family meeting of the PKS Foundation held at The Children's Hospital of Philadelphia in the summer of 2006. This meeting represented a unique opportunity to report on a large cohort of individuals who were comprehensively evaluated by clinicians trained in dysmorphology (including Dr. Pallister) as well as consistently performed developmental assessments. The findings in this cohort were compared to findings summarized from 145 previously reported cases described in the literature. Several novel clinical characteristics were consistently identified in this cohort and will be described. Reassertion of a mild variant is documented and underscores the need for careful physical examination and consideration of skin biopsy in higher functioning individuals with less striking clinical findings. This report expands the clinical manifestations of PKS and highlights the highly variable expressivity of this disorder with important implications for diagnosis and counseling.

764/F

Screening for dup7q11.23 in children with expressive language delay. *J.O. Cardy[1], M.J. Somerville[2], E.J. Young[3], S. Bamforth[2], M. Lilley[2], L.R. Osborne[3]*. 1) Comm Sciences and Disorders, University of Western Ontario, London, Ontario, Canada; 2) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 3) Medicine, University of Toronto, Toronto, Ontario, Canada.

The association of duplication of the Williams-Beuren syndrome region on chromosome 7q11.23 with deficits in expressive language in a few individuals led us to initiate a search for additional subjects. We decided to perform both a genetic and phenotypic screen. For the genetic screen, we recruited children with a primary diagnosis of expressive language delay or apraxia through speech-language pathologists and interactive web sites for families with apraxia. Informed consent was obtained, saliva collected, DNA extracted and duplication of the WBS region tested using real-time SYBR Green amplification of fragments from *GTF2I*, *ELN* and *BAZ1B* on an AB 7900 instrument. An initial screen of 150 subjects did not identify any with a duplication of 7q11.23. In conjunction, we have been carrying out a phenotypic screen of individuals attending genetics clinics, to identify patients who bear similar facial and/or clinical characteristics to the patient with dup7q112.3 that we originally reported in 2005. We identified a 5-year old boy with a diagnosis of severe receptive and expressive language delay. His younger sibling also had severe language delay, and both children were globally developmentally delayed. A third, older sibling had a mild speech impairment. All three children attended school with the help of teachers' aids. Real-time PCR analysis demonstrated that the two younger siblings had a 1.55 Mb duplication of the WBS region, but that the eldest sibling, who showed the mildest symptoms, did not. Their mother, who also had academic difficulties, showed a similar facial dysmorphism to our original dup7q11.23 patient but no DNA sample was available for analysis. We conclude that careful phenotypic screening of patients attending genetics clinics may identify more individuals with dup 7q11.23 than a more general genetic screen of subjects with speech and expressive language delay. This is likely due to the enormous genetic and environmental heterogeneity of speech and language impairment.

765/F

NEUROFIBROMATOSIS TYPE 1: NOVEL PHENOTYPES INVOLVING MINERALIZED CRANIOFACIAL TISSUES. *D.L. Domingo[1], J.L. Sloan[2], S.C. Mitchell[1], T.X. Wu[1], T.C. Hart[1], D.R. Stewart[2]*. 1) NIDCR/NIH, Bethesda, MD; 2) NHGRI/NIH, Bethesda, MD.

OBJECTIVE: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder (50% de novo mutations) involving the NF1 gene, characterized by cafe-au-lait spots and neurofibromas. Osseous features (i.e., macrocephaly) have been described. Oral features are poorly defined. Our prospective study identified abnormalities of oral & craniofacial mineralized tissues. METHODS: 50 postpubertal NF1 patients (range:16-73yrs, mean:34.1yrs; 20males,30females) received clinical, radiographic & cone-beam CT evaluations of the oral & craniofacial structures. Centile charts were constructed for occipitofrontal head circumferences (OFC) in relation to height. RESULTS: Using visual inspection & palpation, intraoral bony protruberances were found in 70%(n=35). To eliminate the ethnic divergences in previous reports, calculated prevalences in our study were limited to Caucasians (n=46;94%). Buccal exostoses - uni-/bilateral rows of asymptomatic bony nodules on the facial alveolar ridges - were observed in significantly higher numbers compared to historical norms: 26 Cauc NF1 patients (56%) manifested maxillary buccal exostoses (normal freq:17.3%;p=2.02x10-12) and 9(19.6%) manifested mandibular buccal exostoses (norm:5%;p=1x10-5). Mandibular tori - bony protuberances on the lingual mandible above the mylohyoid line - also occurred in significantly higher frequencies (n=6;14%;norm:5.9%;p=0.0398). Assessing all NF1 patients, occipital osteophytes were observed radiographically in 28(56%) and were significantly more common in males (n=17;60.7%) than females (n=11;39.3%)(p=0.0012). Age distribution were as follows: teens:33%; 20s:44%; 30s:33%; 40s:82%; >50yrs:100%. Among those with OFC <90th percentile, 68%(n=17) manifested occipital osteophytes. CONC: Tissue growth excess is common in NF1. Significant overgrowth of craniofacial mineralized tissues were demonstrated, suggesting that these new findings are part of the NF1 clinical spectrum. The age-related increased occurrences of occipital osteophytes suggest a possible secondary etiology; these also were more common in patients without macrocephaly. Our observations may shed additional insight on this highly variable disease.

766/F

Cutis Laxa Type II associated with craniosynostosis, joint contractures and corpus callosum agenesis. *R. Garcia, F. Suarez.* Inst de Genetica Humana, Pontificia Univ Javeriana, Bogota, D.C., Colombia.

We presented a new born of masculine sex with Cutis Laxa type II, associated with craniosynostosis, joint contractures and corpus callosum agenesis. Product of first pregnancy, mother of 25 year old, parents were not known to be related. He presents to the physical examination weight of 2800 grams, height of 48 centimeters, OFC of 41 centimeters. The skin is loose with redundant folds, with a slow return on stretching, but the facial skin were unaffected. Head: microcephaly, brachycephaly, wide anterior fontanel. Extremities: rhizomelic shortness of upper and lower limbs, elbow and wrist contractures, finger flexion contractures, bilateral equinovarus. Abdominal wall: bilateral inguinal hernia. Neurologic: Marcus Gunn phenomenon and hypotonia. Radiological images: widened metaphyses, congenital hip dislocation; cerebral MRI: corpus callosum agenesis and colpocephaly. Skin Biopsy showed a diminution of elastic fibers throughout the dermis. High resolution cytogenetic analysis showed no abnormality. No evidences of abnormalities in copper. The clinical characteristics, with facial skin unaffected, let us classifies the patient as an affected by Cutis Laxa type II with neurological abnormalities not described before.

767/F

Bilateral dysplastic kidneys as a feature in patients with duplication 17p11.2 syndrome. *E. Goh[1], M. Shago[2], M. Sgro[3], D. Chitayat[1], R. Mendoza-Londono[1].* 1) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Paediatric Lab Medicine, The Hospital for Sick Children; 3) Department of Paediatrics, St. Michael's Hospital, Toronto, ON.

The duplication 17p11.2 syndrome (MIM 610883) is a recently described clinical entity characterized by mild dysmorphic features, mild mental retardation, and behavioural difficulties. Its incidence has been predicted to be 1:20,000, which is an estimate derived from the frequency of its reciprocal deletion syndrome, Smith-Magenis. However, duplications may go undetected because the symptoms tend to be nonspecific and less well recognized than those seen in microdeletion syndromes. This poses difficulties in the accurate diagnosis, management and counselling for families of individuals with this disorder. We describe three cases of duplication 17p11.2 that were diagnosed by routine cytogenetic analysis with confirmation by interphase FISH. One of our patients presented at 19 months of age with bilateral dysplastic kidneys, patent ductus arteriosus, failure to thrive and developmental delay. Review of the literature revealed renal abnormalities are seen in at least one previous patient with 17p11.2 duplication. A thorough review of the phenotype in this condition illustrates the variability in the syndrome's clinical manifestation. The results have been compared to previous cases of known isolated dup(17)(p11.2p11.2) as well as a few cases with chromosomal or single gene disorders involving this region. This analysis suggests that hypotonia, seizure disorder, hearing impairment, ocular involvement, feeding difficulty, scoliosis, and renal abnormalities are features that should alert the clinician to the possibility of this diagnosis. The 17p11.2 region harbours genes known to be related to attention deficit-hyperactivity (ADHD2), deafness (MYO15A), and scoliosis (IS2). Understanding how the dosage of these genes contributes to the syndrome can aid in understanding the underlying molecular mechanism that leads to the phenotype. In addition, the presence of dysplastic kidneys in some of these patients suggests that renal ultrasound should be part of the initial assessment of these patients.

768/F

Crosstalk between the angiotensin II, TGFB and Wnt signaling cascades inhibits preadipocyte differentiation in Marfan syndrome. *E.C. Klein, R.D. Cohn, C. van Erp, T.M. Holm, J.P. Habashi, L. Myers, D.L. Huso, H.C. Dietz.* Inst Genet Med, Dept Comp Med, HHMI,Johns Hopkins Univ Sch Med, Baltimore, MD.

Marfan syndrome (MFS) is caused by a deficiency of the connective tissue protein fibrillin-1 and a subsequent increase in TGFβ signaling. Losartan, an angiotensin II type-1 receptor antagonist, attenuates TGFβ signaling and prevents these manifestations. Individuals with MFS lack the ability to deposit fat stores despite adequate nutrition. Here we show that mice heterozygous for a fibrillin-1 missense mutation showed a wider variation in adipocyte size than wild-type (WT) mice, with many tiny adipocytes. Given that TGFβ has been shown to inhibit preadipocyte differentiation *in vitro*, we hypothesized that increased TGFβ signaling may underlie the fat phenotype in MFS. We observed increased expression of thrombospondin-1 (TSP-1), an activator of TGFβ, increased phosphorylated Smad2 (pSmad2), an effector of TGFβ signaling, and increased expression of Pref-1, a marker of preadipocytes and an inhibitor of preadipocyte differentiation, in mutant fat. Wnt signaling has been shown to regulate adipocyte differentiation in culture systems, and defective signaling can contribute to obesity-related phenotypes. We now show that fibrillin-1 deficient mice show excessive canonical Wnt signaling, as evidenced by increased steady-state abundance of unphosphorylated β-Catenin. Remarkably, losartan normalizes the levels of TSP-1, pSmad2, Pref-1 and β-Catenin, and corrects the size distribution of adipocytes in mutant mice. Given that losartan had no effect on these parameters in WT mice, this suggests that TGFβ is not a significant physiologic regulator of preadipocyte differentiation in adult mice. Rather, unanticipated crosstalk between the angiotensin II, TGFβ and Wnt signaling cascades emerges as the predominant pathologic inhibitor of preadipocyte differentiation in MFS. In vivo treatment with TGFβ neutralizing antibody normalized Wnt signaling, defining AngII→TGFβ→Wnt as the order for these interactions. This pathogenic sequence may be related to other disease states associated with increased TGFβ signaling and reduced fat stores including scleroderma and Camurati-Engelmann disease.

769/F

The extended phenotypic spectrum of 9p deletion (partail monosomy 9p) syndrome. *M. Michelson[1], C. Vinkler[1,2], M. Yanoov-Sharav[1,2], T. Lerman-Sagie[2,3], D. Lev[1,3].* 1) Genetics Inst, Wolfson Medical Ctr, Holon, Israel; 2) Metabolic Neurogenetic clinic, Wolfson Medical Ctr, Holon, Israel; 3) Pediatric Neurology Unit, Wolfson Medical Ctr, Holon, Israel.

Structural aberration of chromosomes is associated with various syndromes. Partial deletion of short arm of chromosome 9(9p-)or partial monosomy 9p is a rare but specific clinical entity. Phenotypic presentation is variable. The clinical features include mental retardation, craniofacial malformations,short neck,heart defects and behavioral problems. Trigonocephaly and upward slanting palpepbral fissures are found virtually in all patients. Non ketotic hyperglycemia was described in some cases. Most of the cases are de novo deletions. Few cases are due to unbalanced rearrangements. We present a two and a half year-old girl with developmental delay, dysmorphic features, neonatal hypoglycemia, ventricular septal defect, cleft palate and severe congenital dislocation of hips. Karyotype in leucocytes was normal, 46 XX. Comparative genomic hybridization (A-CGH) analysis revealed deletion of 9 pter; with breakpoints between RP11-1036k24 and RP11-1057121. Parental studies are normal. Although most clinical features of the patient are consistent with monosomy 9p- syndrome, our patient presents with distinct malformations that have not been described previously: including severe neonatal hypoglycemia and congenital hip dislocation resistant to therapy.

770/F

Triple X Syndrome Accompanied by Aortic Coarctation. *L. Murrain[1], A.L. Shanske[2].* 1) Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY; 2) Center for Craniofacial Disorders, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY.

Triple X syndrome (47, XXX) is one of the most common sex chromosome abnormalities in females, with an incidence rate of approximately 1 per 1,000 female births. Triple X Syndrome is usually the result of non-disjunction in maternal meiosis I. We evaluated a 4-year-old Jamaican-American female referred to our pediatric genetic clinic for abnormal chromosome analysis, abnormal gait, and aortic coarctation. On exam she was noted to have a short broad neck, synophrys, and a well-healed left thoracotomy scar. She was the full-term product of a pregnancy complicated by advanced maternal age, thalassemia minor, and well-controlled gestational diabetes class A1. She was born via uncomplicated vaginal delivery with a birth weight of 3856 g. In the nursery she was diagnosed with aortic coarctation, and underwent surgical repair at 3 months. At three years of age she was noted to have an abnormal gait. Chromosome analysis revealed a 47, XXX karyotype. While triple X syndrome is associated with considerable phenotypic variability, the vast majority of patients will express a normal phenotype. Affected individuals may display tall stature, premature ovarian failure, developmental delays, learning disabilities, and behavior problems. More recently it has been suggested that genitourinary, gastrointestinal malformations, congenital adrenal hyperplasia, and pituitary tumor be added to the phenotypic spectrum. Several case reports in the literature have described triple X syndrome with Turner stigmata. Although our case lacks stigmata, she has the second most common congenital heart defect associated with Turner syndrome. Our case suggests that cardiac defects be considered as part of the clinical spectrum of 47, XXX, and cardiac assessment be included in the management of affected individuals.

771/F

Van Allen-Myhre Syndrome: Report of a New Case with Chondrodysplasia Punctata. *S. Parkash[1,2], S. Keating[3], E. Kolomietz[3], D. Chitayat[1,2].* 1) Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Department of Laboratory Medicine and Pathobiology, Mount Sinai Hospital, Toronto, Ontario, Canada.

Van Allen-Myhre Syndrome consists of ectopia cordis, exomphalos, ectrodactyly, oligodactyly, absent sacrum, radial hypoplasia, microphthalmia, hemifacial microsomia, and cutis aplasia, among other findings. The syndrome was first described in a female infant of Mexican-American first cousins (Van Allen and Myhre, 1991). A second case was reported by Zolotukhina et al (1993). A third case, though not labeled as having Van Allen-Myhre Syndrome, describes a fetus with many features of the syndrome (Pivnick et al, 1998). Hancock et al (2002) reported a case initially thought to be suggestive of Van Allen-Myhre Syndrome, however was later felt to have features of Goltz Syndrome (Hancock et al, 2002). Goltz Syndrome, a presumed X-linked dominant disorder linked to Xp22, is associated with defects in the skin, skeleton, soft tissues, and eyes. We report a fetus with ectopia cordis, omphalocele, hyperlordosis, scoliosis, and ectrodactyly. Karyotype and microarray analysis was normal, with no deletion in the Xp22 region. Xrays revealed changes consistent with chondrodysplasia punctata. To our knowledge, this is the 4th case of Van Allen-Myhre Syndrome reported in the literature, and the first case with chondrodysplasia punctata, which adds to the confusion regarding overlap between this condition and Goltz Syndrome.

References Hancock S et al. (2002) Probable Identity of Goltz Syndrome and Van Allen-Myhre Syndrome: Evidence From Phenotypic Evolution. Am J Med Gen 110: 370-379. Pivnick EK et al. (1998) Infant with Midline Thoracoabdominal Schisis and Limb Defects. Teratology 58: 205-208. Van Allen MI and Myhre S. (1991) New Multiple Congenital Anomalies Syndrome in a Stillborn Infant of Consanguinous Parents and a Prediabetic Pregnancy. Am J Med Gen 38: 523-528. Zolotukhina T et al. (1993) Unusual Complex of Congenital Malformations. Am J Med Gen 46: 728-729.

772/F

Noonan Syndrome Associated with Neuroblastoma. *J. Pierre-Louis[1], S. Viero[2], J. Kirsh[2], D. Chitayat[1,2].* 1) Mount Sinai Hosp, Prenatal Diagnosis Medical Genetics Program; 2) Hospital for Sick Children, Divisions of Pathology (SV), Cardiology (JK) and Clinical and Metabolic Genetics (DC), Toronto, Ontario, Canada.

Noonan syndrome (NS) is characterized by distinct facies, short stature, congenital heart defect; broad/webbed neck; unusual chest shape with superior pectus carinatum, inferior pectus excavatum, and low-set nipples, cryptorchidism and variable developmental delay. Associations between NS and an increased risk of some malignancies, notably leukemia and neuroblastoma, have been reported. Recent data indicate that somatic PTPN11 mutations occur in children with sporadic juvenile myelomonocytic leukemia, myelodysplasic syndrome, B-cell acute lymphoblastic leukemia, and acute myelogenous leukemia. We report an additional case of NS associated with a mutation in the PTPN11 gene who developed neuroblastoma. A term male infant born to 38 year old primigravida mother with a birth weight of 3.46kg and Apgar scores were 8 and 9 at 1 and 5 minutes respectively. After birth, he was found to have facial dysmorphism, pulmonary stenosis and supravalvular pulmonary stenosis and had an unsuccessful balloon dilatation at 2 months and thus has a surgical repair. His weight and height were always below 3rd centile despite good appetite and calorie intake. He had mild developmental delay. NS was suspected and DNA analysis for the PTPN11 gene showed that he was heterozygous for a single base change of A→G in exon 8 of the PTPN11 gene. This mutation is predicted to change the normal Asparagine residue (AAT) to an Aspartic acid residue (GAT) at position 308 of the SHP-2 protein. An abdominal ultrasound at 16 months showed a paravertebral mass posterior to the left kidney and a biopsy was consistent with nodular ganglioneuroblastoma with favorable histology and intermediate mitotic index and favorable biological features with n-MYC not amplified and chromosome 1p not deleted. This case adds to the growing body of literature suggesting potential link between NS and malignancies including neuroblastoma. The role of Shp2 in Ras activation and the frequent mutations of RAS in human tumors lend support the possibility that PTPN11 mutations play a broader role in carcinogenesis.

773/F

Importance of dental anomalies to the diagnosis of Smith-Magenis syndrome: description of two cases. *C.R.L. Silva[1], C.S. Teixeira[1], R.S. Honjo[1], D.R. Bertola[1], L.M.J. Albano[1], A.C.V.K. Santos[2], C. Rosenberg[2], C.A. Kim[1].* 1) Genetics Unit, Instituto da Criança, São Paulo, SP, Brazil; 2) Departamento de Genética e Biologia Evolutiva - USP, São Paulo, SP, Brazil.

Smith-Magenis syndrome (SMS) is a contiguous gene syndrome caused by interstitial microdeletion in 17p11.2. It is characterized by craniofacial dysmorphisms, characteristic behavioral abnormalities and sleep disturbances. Dental anomalies have been rarely reported. Here we describe two sporadic cases of SMS associated with the dental abnormalities. Case 1: male, 18 yo, first child of a non-consanguineous healthy couple, was referred at age 7 due to short stature and delayed neurocognitive development. Physical examination: short stature, peculiar craniofacial features (brachycephaly, broad forehead, upslanting palpebral fissures, thick lips, thick lobes of the ears, long eyelashes), pulmonary stenosis, hypoplastic nipples, cryptorchidism, brachydactyly, and congenital hip dislocation. Karyotype (G banding): 46, XY. Dental findings: caries, missing teeth (left upper and lower second premolars and right lower second premolar) and taurodontism. Case 2: male, 17 yo, second child of a non-consanguineous and healthy couple, presented with speech delay. Clinical findings at 10 yo: short stature, brachycephaly, broad forehead, midfacial hypoplasia, broad nasal bridge, elongated palpebral fissures, cupid's bow upper lip, hoarse voice, brachydactyly, clinodactyly of 5th fingers, persistence of fetal fingerpads, mental retardation, hyperactivity, high limiar to pain and polyembolokoilamania. Odontological examination: taurodontism. After a long clinical follow-up without a definitive diagnosis, just recently array-CGH became available and detected a microdeletion (1,6Mb in case 1 and 3,6Mb in case 2) suggestive of SMS, confirmed by FISH. Both patients did not present sleep disturbances. In the literature, dental anomalies in SMS include especially taurodontism and tooth agenesis. Our report reinforces that these dental findings are important ones, suggesting that they could be another clue in the diagnosis of SMS.

774/F

Cardiac rhabdomyoma diagnosed prenatally in a boy diagnosed after birth with Silver-Russell syndrome: a new form of tumor associated with the syndrome. *M. Sklansky[1], L.M. Randolph[2].* 1) Pediatric Cardiology, Childrens Hospital Los Angeles; 2) Pediatrics-Medical Genetics, Childrens Hospital Los Angeles, Los Angeles, CA.

K.O.'s 27-y.o. Hispanic mother and 29-y.o. non-consanguineous Hispanic father presented to the CHLA-USC Institute for Maternal-Fetal Health late in pregnancy for evaluation of a large left intracardiac mass. It was judged to likely be a left ventricular rhabdomyoma. No other ultrasound abnormalities were seen. They were counseled regarding the risk of tuberous sclerosis. K.O. was born full term, 2330 g, and MRIs of head, abdomen and chest were negative for masses. Cardiac mass measured 19 x 9 mm w/o hemodynamic significance, but he appeared to have failure to thrive and concern raised for tuberous sclerosis, so was referred to Genetics again. Development normal. Hearing, vision normal. Had pyloric stenosis repair at 2 mos age. Wt >2 S.D. below mean; ht 5th %ile; HC 2nd %ile. Triangular facies and prominent forehead. L-sided hemihypotrophy. No skin lesions using Woods lamp. Slightly blue sclerae. Left leg 3 cm shorter than right, and L arm 1 cm thinner than R arm. Diagnosed with Silver-Russell syndrome (SRS). F-u echo showed mass in heart shrinking. Uniparental disomy (UPD) chromosome 7 testing negative using markers D7S641, D7S493, D7S510, D7S630, D7S515 and D7S2423 but was not informative for segmental UPD. SRS is due to mat(UPD) in 10% of cases, and it can be partial or complete UPD. Craniopharyngioma, testicular seminoma, Wilms tumor and hepatocellular carcinoma have been reported in SRS. To our knowledge, cardiac rhabdomyoma has not been reported in SRS. Plans are to complete segmental UPD7 testing and to do H19 hypomethylation studies. He has been referred to Orthpedics and Endocrinology.

775/F

The common inversion of the Williams-Beuren syndrome region does not cause clinical symptoms. *E.J. Tam[1], E.J. Young[1], C.A. Morris[2], C.R. Marshall[3], S.W. Scherer[3], C.B. Mervis[4], L.R. Osborne[1].* 1) Medicine, University of Toronto, Toronto, Ontario, Canada; 2) Pediatrics, University of Nevada School of Medicine, Las Vegas, NV; 3) Genetics & Genomic Biology, SickKids, Toronto, Ontario, Canada; 4) Psychological and Brain Sciences, University of Louisville, Louisville, KY.

The 1.55 Mb Williams-Beuren syndrome (WBS) deletion is caused by meiotic recombination between highly similar flanking DNA segments. A common inversion of the region, WBSinv-1, also occurs through recombination between flanking repeats in opposite orientation to each other and exists as a polymorphism in the general population. WBSinv-1 was also found in individuals with general features associated with WBS (eg. mental retardation, ADHD, friendly personality) but no deletion, suggesting it could cause clinical symptoms. In order to investigate the possible role of WBSinv-1 in WBS symptoms, we performed a full clinical, developmental and genetic assessment of two previously reported atypical WBS patients with WBSinv-1. The phenotypes of these atypical patients did not show significant clinical or psychological overlap with those of individuals with WBS, suggesting that the presence of the WBS inv-1 chromosome and clinical symptoms in these patients is coincidental. In addition, a 1.3 Mb duplication of part of the velocardiofacial syndrome region on chromosome 22q11.2 was found in one patient, which may account for her symptoms. We also examined the expression of genes within the WBS region at 7q11.23 in unaffected carriers of WBSinv-1, but found no evidence of significantly altered expression of any of the genes tested, even in an individual who was homozygous for WBSinv-1. These results suggest that WBSinv-1 does not cause clinical symptoms. Caution should be taken when diagnosing patients with atypical presentation of rare syndromes and diagnosis should be carried out by health professionals with extensive experience in the specific syndrome, wherever possible. Whole genome analysis, which is becoming more routine in the clinical setting, may reveal previously unidentified copy number variants that could contribute to syndromic features.

776/F

Dental evaluation of Kabuki syndrome patients. *C.S. Teixeira, C.R.L. Silva, R.S. Honjo, D.R. Bertola, L.M.J. Albano, C.A. Kim.* Genetics Unit, Instituto da Criança, São Paulo, SP, Brazil.

Kabuki syndrome (KS) is a multiple congenital anomalies syndrome of unknown cause, first described in 1981 based on Japanese patients. It is characterized by a peculiar facies, postnatal growth deficiency, mild to moderate mental retardation, immunological deficiency, unusual dermatoglyphic patterns with persisting fingerpads, and various skeletal and visceral anomalies. The main features of the facial dysmorphisms are: arched eyebrows, with sparse or dispersed lateral third of the eyebrow, long palpebral fissures with eversion of the lateral portion of the lower eyelid, hypoplastic columella and prominent ears. Oral manifestations are commonly observed in KS (68% of the cases) and may comprise micrognathia, retrognathia, high-arched palate, cleft lip/palate, bifid tongue and uvula, widely spaced teeth, ectopic permanent first molars, delayed tooth eruption pattern, impacted teeth and other dental anomalies such as hypodontia, conical teeth, neonatal teeth, large pulp chamber and absence of incisors teeth. In this study, 10 patients with clinical diagnosis of KS were evaluated by dental examination and panoramic radiographic. Absence of the incisors teeth were found in 7 patients (70%), and lateral incisors were the most common absent teeth. Among these 7 patients, 2 presented dental absences in the both arches (upper and lower), 4 presented absences in only one arches and 1 patient presented associated absence of a superior canine. Because of the absent teeth, the patients had presented widely spaced teeth, and in these cases, orthodontic treatment was indicated. Caries were found in 50% of the patients, but it might be associated with mental retardation and bad hygiene. Although a large number of distinctive dental findings have been described in the literature, in our cohort teeth agenesis is the only and very prevalent feature. This specific abnormality may be helpful in establishing the diagnosis solely based on clinical grounds thus far.

777/F

CATSHL Syndrome: Report of Three New Families and Further Delineation of the Phenotype. *R. Toydemir[1], M. McMillin[2], P. Kezele[2], S. Felscher[2], D. Eunpu[3], K. Aleck[4], C. Marques[5], M. Bamshad[2].* 1) HHMI, Dept. of Human Genetics, University of Utah, Salt Lake City, UT, USA; 2) Dept. of Pediatrics, University of Washington, Seattle, WA, USA; 3) Nemours Children's Clinic, Jacksonville, FL, USA; 4) Section of Medical and Molecular Genetics, Dept. of Pediatrics, University of Arizona, Phoenix, AZ, USA; 5) University of Sao Paulo, Sao Paulo, Brazil.

CATSHL Syndrome (OMIM 610474) is a recently described skeletal dysplasia characterized by camptodactyly of the hands and feet, tall stature, and hearing loss. Less frequent clinical features include kyphoscoliosis, mental retardation, learning disabilities, and microcephaly. CATSHL is caused by substitution of a histidine for a highly conserved arginine residue in the kinase domain of the FGFR3 (p.R621H) that is predicted to result in loss of function. Accordingly, the clinical features of CATSHL recapitulate those found in the *Fgfr3* knockout mouse. To date, CATSHL syndrome has been reported in only a single large pedigree from the U.S. We now report the clinical characteristics of 3 additional families with CATSHL syndrome. The first case is a 7-month-old boy who has camptodactyly, tall stature (96%ile), and bilateral moderate sensorineural hearing loss. His head circumference is at the 25%ile. He also has scoliosis, dysplastic femurs, adducted left knee, and a valgus deformity of the left tibia with new periosteal bone formation. Neither parent is affected. The second case is a 12-year-old girl who has camptodactyly, height more than 95%ile for age, and bilateral sensorineural hearing loss. She has a large forehead and wide set eyes, and developmental delay. She has a 7-year-old brother, who also has camptodactyly and adducted thumbs. Their father reportedly has similar features but was unavailable for study. The third case is an 8-month-old girl born in Brazil who has camptodactyly and tall stature (97%ile). Her hearing appears to be normal. Her father is also affected and has camptodactyly, scoliosis, and tall stature. Phenotypic analysis of these 3 new families further delineates the clinical characteristics of CATSHL syndrome. Screening of *FGFR3* in these families is underway.

778/F

Oculo-Facio-Cardio-Dental (OFCD) syndrome : Somatic mosaicism of a large BCOR gene deletion in 2 monozygotic twins and three novel mutations. *S. Whalen[1], S. Manouvrier[2], O. Boute[2], F. Fellmann[3], F. Dastot-Le Moal[1], P. Bitoun[4], M-P. Cordier[5], I. Bailleul-Forestier[6], M. Goossens[1], A. Verloes[6], I. Giurgea[1].* 1) Genetique & INSERM U841,Hosp H.Mondor, Creteil, France; 2) Dpt génétique clinique, CHRU de Lille, France; 3) Dpt génétique médicale, CHU Vaudois, Lausanne, Switzerland; 4) Dpt génétique, Hosp J.Verdier, Bondy, France; 5) Dpt génétique médicale, Hosp E.Herriot, Lyon, France; 6) Dpt de génétique médicale, Hosp R.Debré, Paris, France.

Oculo-Facio-Cardio-Dental (OFCD) syndrome is a rare disorder associating congenital cataract, microphthalmia, characteristic dysmorphia, congenital heart defects, oligodontia, and radiculomegaly. OFCD syndrome results from mutations in the BCOR gene, located on Xp11.4, encoding a key transcriptional regulator during early embryogenesis. X-linked dominant inheritance is suggested, although a singular BCOR missense mutation, whose relevance remains controversial, was described in a male presenting with Lenz microphtalmia (microphtalmia, mental retardation, and other malformations). To further delineate the clinical spectrum of these disorders, we studied 7 females with OFCD syndrome and 4 males with Lenz microphtalmia, from 6 unrelated families. BCOR mutations were screened by direct sequencing, and deletions by QM-PSF and FISH analysis. Somatic mosaicism for a large deletion of BCOR (50% in peripheral leukocytes) was identified in 2 monozygotic twins presenting with typical OFCD syndrome. One twin transmitted this large deletion homogeneously to her daughter. In addition, 3 novel mutations were identified in 3 unrelated females: 2 frameshift (p.Pro288ArgfsX90 and p.Pro190ProfsX26), and one nonsense (p.Arg1480X) mutation. No mutation of BCOR was found in the patients with Lenz microphtalmia. In conclusion, we report 4 novel BCOR mutations in females with OFCD syndrome. These results broaden the clinical spectrum of OFCD syndrome, as 3 patients did not present heart defects, and one patient had mild mental retardation, thus suggesting that the condition is underdiagnosed. The first description of somatic mosaicism in OFCD syndrome is of particular relevance to genetic counselling.

779/F

A Japanese infant with ARC syndrome and tracheobronchomalacia. *H. Yoshihashi[1], K. Takamura[2], T. Yokoyama[2], N. Furuya[1], K. Kurosawa[1], K. Izumi[3], K. Kosaki[3].* 1) Division of Medical Genetics, Kanagawa Children's, Medical Center, Yokohama, Japan; 2) Division of Neonatology, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo, Japan; 3) Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan.

Arthrogryposis, renal dysfunction and cholestasis (ARC) syndrome (MIM 208085)is an autosomal recessive multisystem disorder that may be associated with germline VPS33B mutations. Other features variably reported include ichthyosis, mild dysmorphic signs, absent corpus callosum and recurrent infections, worsening nephrogenic diabetes insipidus (Gissen 2006). We describe a Japanese ARC infant with tracheobronchomalacia, which has not been reported. The subject was a girl infant born at term to non-consanguineous parents, with a weight of 2666g (-0.8SD), a length of 46cm (-1.4SD). At birth, there was generalized hypotonia and flexion contractures of the fingers and knees. At 9 days of life, she was evaluated for jaundice (serum TB,12.0mg/dl; DB,8.2mg/dl) and had a persistent cholestasis in infancy. At 3 weeks, she developed renal tubular acidosis with glycosuria, generalized aminoaciduria, hyper-β2-microglobulinuria and was diagnosed with Fanconi's syndrome. A peripheral lymphocyte karyotype was 46,XX. These findings were highly suggestive of ARC syndrome. Direct sequencing for VPS33B documented heterozygous splice donor site mutation (c.403+2T>A : maternal) within intron 6 and 143bp deletion (paternal) including whole sequences of exon12. Since 3 months of age, she has required mechanical ventilation because of progressive dyspnea and was diagnosed as having a tracheobronchomalacia, which had not observed as yet. In summary : As a common complications of ARC syndrome, the tracheobronchomalacia has not yet been reported. Recurrent infections described in most of previous reports may result from that by exclusion of other causes. For the early diagnosis, it would appear reasonable to repeat the urinary analysis if the neonate has arthrogryposis and persistent jaundice. There are only a few reports of ARC syndrome in Japan. Further cases may provide insight into the consideration of clinical information.

780/F

The Proximal Chromosome 14q Microdeletion Syndrome: Delineation of the Phenotype using High Resolution SNP Oligonucleotide Microarray Analysis (SOMA). *E. Torgyekes[1], K. Anyane-Yeboa[2], O. Nahum[3], S. Pirzadeh[4], V. Jobanputra[3], D. Warburton[5], A. Shanske[6], B. Levy[3].* 1) Dept Neurology and Pediatrics, Columbia Univ, New York, NY; 2) Dept Pediatrics-Clinical Genetics, Columbia Univ, New York, NY; 3) Dept Pathology, Columbia Univ, New York, NY; 4) New York-Presbyterian Hospital Columbia Medical Center, New York, NY; 5) Dept Genetics and Development, Columbia Univ, New York, NY; 6) Dept Pediatrics, Albert Einstein College of Medicine, Bronx, NY.

We report two cases with overlapping small interstitial deletions involving regions 14q12 to q13.3. Both children have severe developmental delay, failure to thrive, microcephaly and dysmorphic features. Brain MRI revealed partial agenesis of the corpus callosum in both cases. Patient 1 is 28 months old. Her additional clinical features include abnormal ears, hypertelorism, body asymmetry, bilateral hypoplastic optic nerves causing blindness, sinus arrhythmia, abnormal temperature regulation, apneic episodes, myoclonic jerks and opistotonus. Patient 2, an 11 month old male, was also diagnosed with lissencephaly by MR,I and in addition has EEG confirmed seizure disorder. Cytogenetic analysis revealed a normal karyotype in patient 1 and an apparently balanced three-way translocation in patient 2 involving chromosomes 4, 14 and 11. We were able to detect and characterize the deletion in both patients with extraordinary precision using high resolution SNP Oligonucleotide Microarray Analysis (SOMA). Furthermore, we review and compare published cases with a deletion involving the 14q12-13.3 chromosomal region. More exact characterization of these deletions will improve our ability to make phenotype-genotype correlations in this and other areas of the genome.

781/F

Moyamoya Disease: Identification of the First Gene for the Disease and Insight into the Genetic Basis. *S.J. Bourgeois, V.T. Tran-Fadulu, D. Guo, S.L. Swineford, E. Regalado, D.M. Milewicz.* Internal Medicine, Univ of Texas HSC Houston, Houston, TX.

Moyamoya disease (MMD) is a premature stroke disease due to occlusive lesions in the terminal portion of the internal carotid arteries. A genetic basis of MMD is well established; 10% of patients having a family history of MMD. Using families with multiple members with thoracic aortic aneurysms and dissections (TAAD), we identified that ACTA2 missense mutations cause of familial TAAD. ACTA2 encodes smooth muscle cell (SMC) specific α-actin, a contractile protein that is the most abundant protein in SMCs. Although we identified a spectrum of ACTA2 mutations causing FTAAD, 3 unrelated TAAD families with mutations altering ACTA2 R258 had the surprising finding of strokes under the age of 30 years in 6 mutation positive members, including 4 individuals diagnosed with MMD and therefore establishing ACTA2 as the first gene for MMD. Based on the fact that a single gene mutation can lead to both TAAD and MMD, we hypothesized that MMD is a systemic vascular disease, and patients may have a family history of MMD or other premature or rare vascular diseases. We obtained medical and family histories on 27 probands with MMD (86% Caucasian). There was a bimodal distribution of age of onset of MMD (36% with onset <10 yrs of age and 32% onset ages of 30-40 yrs). In the probands, 100% of those examined had livedo reticularis (purplish rash due to occlusion of dermal capillaries), 22% had migraines, 7% had renal artery stenosis, and 4% had a cerebral aneurysm. On family history (1st and 2nd degree relatives due to the young probands), 18% had a history of MMD, 18% had a history of only premature strokes (< 55 years, not MMD), 26% had a history of premature coronary artery disease (CAD < 55 years) and 22% both strokes and CAD. Therefore, we conclude that MMD is a manifestation of a systemic vascular disease and a family history of premature strokes and CAD is present in the majority of MMD patients (85%). These findings suggest that inherited genes leading to a spectrum of premature vascular diseases, with MMD at the severe end of the spectrum, may be more common than previously reported.

782/F

Confirmation that a three base pair deletion in the MITF gene results in Tietz Syndrome. *R.K. Basran[1], T. Maher[1], M. Ito[1,2], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Waardenburg syndrome (WS) is an autosomal dominant hearing-pigmentary disorder accounting for approximately 3% of congenitally deaf children. WS can be divided into four subtypes (I-IV) based on genetic and clinical criteria. The most common form, WS I, is characterized by the presence of congenital hearing loss, dystopia canthorum and pigmentary disturbances of the eyes, hair and skin. WS II can be distinguished from WS I by the absence of dystopia canthorum. A related hearing-pigmentary disorder with a similar clinical phenotype to WS II is Tietz syndrome (TS, MIM103500). TS is a rare autosomal dominant disorder characterized by congenital profound and completely penetrant hearing loss and pigmentary changes. Within WS II, the degree of depigmentation and hearing loss is more variable when compared to TS. Mutations within the MITF gene have been shown to cause both WS II and TS depending on their location. Mutations occurring in the basic region of the protein lead to TS whereas mutations interfering with the dimerization of the protein lead to WS II. A 1.5 year old boy with a clinical history of ocular albinism and hearing loss was referred to our laboratory for evaluation. Using automated DNA sequence analysis he was found to have a previously reported in-frame deletion of an arginine residue in exon 8. There have been a limited number of reports describing mutations within the MITF gene producing TS. The three base pair deletion of the arginine residue we describe here has been previously reported in one family with an albinism-deafness phenotype resembling the clinical picture of TS. Hence our case provides confirmation of this single amino acid deletion generating a phenotype of TS. Further clinical and genetic analysis of the proband's family will provide insight into the classification of the syndrome generating hearing loss in this pedigree. Identification of this mutation within the MITF gene in the proband will now allow tracking through the family, more accurate genetic counseling, and the opportunity for prenatal diagnosis, if desired.

783/F

Molecular Genetic Diagnosis of Haemophilia A from Jammu region of J&K State, India. *P. Kumar[1], V. Dogra[1], W.K. Balwan[1], M. Idris[2], G.R. Chandak[2], S. Gupta[1].* 1) HGRCC, Zoology, University of Jammu, Jammu, Jammu, India; 2) Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, Andhra Pradesh, India.

Hemophilia A is one of the most common X-linked Genetic disease caused by different mutations in the factor VIII gene. It is estimated that about 50% of severe haemophilia A cases are the result of an inversion in factor VIII gene. In the work we present an analysis of 33 haemophilia A patients and 35 family members (Mothers and Sisters) of the 33 patients by using both the direct and indirect mutation detection techniques. Direct mutation analysis of inversion Intron 22 has been carried out by Conventional Southern Hybridization technique, Of the 33 patients, 22 were severely affected by the disease haemophilia A. 9 of these 22 severely affected patients had inversion in intron 22 of distal type and 2 had inversion in intron 22 of Proximal type. Amongst the 10 moderately affected patients 3 had inversion in intron 22 of Distal type, while in the remaining 7 patients none of the two i.e. Distal or Proximal type of inversion could be detected. In a single case of mild type neither Distal nor Proximal type of inversion could be detected. Only one mother of moderately affected patient was found carrier for distal type of inversion mutation. For indirect mutation analysis, two intragenic markers BclI and XbaI located in intron 18 and 22 respectively were taken up. Specific regions of factor VIII gene were amplified followed by restriction digestion in order to find out the informativeness of the marker. BclI marker intron 18 RFLP was informative for 24/51 X-chromosomes and allele frequency was 47.05%, whereas the heterozygosity rate was 9/16 (56.25%) for women studied and XbaI marker on intron 22 RFLP was informative for 14/51 individuals and allele frequency was 27. 45%.the heterozygosity rate was 6/16 (37.50%) for females studied.

784/F

SSADH deficiency - an underdiagnosed cause of mental retardation and behavioral problems. *L. Mehta[1], S. Ramanathan[1], J. Maytal[2], J.A. Neidich[3], D.Z. Salazar[3], C. Jakobs[4], K.M. Gibson[5], P.L. Pearl[6].* 1) Medical Genetics &; 2) Pediatric Neurology, Schneider Children's Hospital, NY; 3) Biochemical Genetics, Quest Diagnostics Nichols Institute, CA; 4) Clinical Chemistry, VU University Medical Center, Amsterdam; 5) Pediatrics & Pathology, Univ of Pittsburgh School of Medicine, PA; 6) Neurology, Children's Natl Med Center, Washington DC.

A 12 y.o. girl was evaluated for hypotonia, learning disabilities and attention deficit disorder with recent episodes of unresponsiveness in school. Baseline EEG and telemetry were normal. Family history was significant for a sister who died at 20. She had learning disabilities, seizures, self-injurious behavior, hallucinations and a diagnosis of depression. There was cognitive regression and memory loss following seizures. No definitive diagnosis was made and cause of death remained unknown. Parents were consanguineous. Our patient had normal chromosomes, subtelomeric FISH, plasma amino acids and fragile X testing. Urine organic acids showed elevated 4-hydroxybutyrate (γ-hydroxybutyrate or GHB) suggestive of succinic semialdehyde dehydrogenase deficiency (SSADHD). Measurement of SSADH activity in lymphocytes confirmed the deficiency. The patient developed overt seizures and is treated with oxcarbazepine with good control. SSADHD is a rarely diagnosed cause of developmental delays, particularly in expressive language, and neuropsychiatric abnormalities, including hallucinations and seizures. The pathophysiology is related to accumulation of GHB, an agonist of GABA receptors in the brain. GHB is neuropharmacologically active and acts as a sedative. The non-specific nature of symptoms often leaves SSADHD unsuspected. GHB accumulates in urine, plasma, and CSF. Urine organic acid analysis is a good initial diagnostic test, if done in a lab that reports GHB elevations. Some labs do not do so because GHB, like other sedating drugs, is a restricted substance. Urine organic acids are perceived to have a low diagnostic yield in patients with learning disabilities and MR but testing is appropriate in such situations. Many clinicians are not aware of this diagnosis, hence further elaboration of the natural history and pathophysiology will be helpful.

785/F

A *COL2A1* mutation in a patient with unknown skeletal dysplasia: unclassified type II collagenopathies. *A. Sathienkijkanchai[1], N.H. Robin[1,2].* 1) Dept Genetics,; 2) Dept Pediatrics, Univ Alabama at Birmingham, Birmingham, AL.

The skeletal dysplasias are a group of more than 250 disorders characterized by abnormal formation of the skeleton because of intrinsic derangement of the growth, development, and/or differentiation. Disease-causing genes have been identified in more than 150 diseases such as *FGFR3*, *COL2A1*, and *COMP* gene. The type II collagenopathies are a heterogeneous group of disorders resulting from mutations in the collagen 2 gene. There is a wide spectrum in severity in this group ranging from invariably lethal (achondrogenesis II/hypochondrogenesis) through spondyloepiphyseal dysplasia congenital (SEDC), Kniest dysplasia, and Strudwich type SE(M)D to more mildly affected Stickler dysplasia. The unifying findings in type II collagenopathies are, in common, involvement of the spine (platyspondyly) and epiphyseal of the long bones ("spondylo-epiphyseal" pattern).

Here, we present a patient with skeletal dysplasia. The patient is a seven-year-old Caucasian female presenting at birth with severe short stature and respiratory distress. Additionally, she was found to have cleft palate, bilateral clubfeet, large PDA with ASD, and hearing loss. Initially, her skeletal survey was interpreted as hypochondrogenesis which is the condition that most patients do not survive the first 6 months of life. However, finally, the diagnosis of unclassified type II collagenopathies has been raised after her recent skeletal survey was reviewed and demonstrated platyspondyly, metaphyseal flaring, absence of the capital femoral epiphyses, unossified pubic bones with relatively normal bones of hands and feet. The diagnosis has been confirmed by finding a mutation in *COL2A1* gene which is c.1421G>A. So, she has carried a Gly471Glu substitution. Therefore, identification of disease gene (molecular diagnosis) on this patient would help us to provide better care and treatment as well as improving understanding of the disease and making this distinction important for counseling the family.

786/F

Maternal uniparental disomy 14 detected in patients suspected to have Prader-Willi syndrome. *S. Saitoh, K. Hosoki.* Dept Pediatrics, Hokkaido Univ Sch Medicine, Sapporo, Japan.

Maternal uniparental disomy 14 [upd(14)mat] is characterized by intrauterine growth retardation, neonatal hypotonia, precocious puberty, and truncal obesity. The phenotypes of upd(14)-mat resemble those of Prader-Willi syndrome (PWS) which is characterized by neonatal hypotonia, small hands and feet, mental retardation, and hyperphagia resulting in obesity beyond the infancy. Mitter et al. (2006) recently reported that upd(14)mat was detected in 4 out of 33 patients who were suspected to have PWS, and raised the question that upd(14)mat could be underestimated in patients with features resembling PWS. However, other studies have failed to detect upd(14)mat in cases resembling but lacking PWS. Therefore, we examined fifty eight Japanese patients initially suspected to have PWS based on clinical features, but for whom normal results of the *SNURF-SNRPN* DNA methylation test excluded the diagnosis of PWS. Using these samples, we examined DNA methylation status at the promoter region of the imprinted *MEG3* gene, located in 14q32.2. If aberrant DNA methylation was identified, we carried out a microsatellite polymorphism study and determined the parental origin of each chromosome 14. We identified abnormal hypomethylation at the *MEG3* promoter in 3 out of 58 patients. An almost complete lack of methylation was found in 2 patients, but 1 patient demonstrated a faint methylated signal. The polymorphism study demonstrated that the 2 patients lacking *MEG3* promoter methylation had complete upd(14)mat, whereas the patient with partial hypomethylation was mosaic for upd(14)mat. All 3 patients with upd(14)mat had intrauterine growth retardation, neonatal hypotonia and feeding difficulty, with PWS suspected during infancy. Our results further support the resemblance of upd(14)mat and PWS phenotypes, particularly during infancy, and demonstrate the significance of *MEG3* methylation testing for PWS-like patients in whom PWS is excluded.

787/W

Literacy, numeracy, and the development of an Individualized Risk Information System (IRIS): A genetic counseling tool for BRCA+ breast cancer patients. *S. Brown[2], J. Culver[1], D. MacDonald[1], K. Metcalfe[3], H. Burke[4], M. Robson[5], S. Sand[1], A. Thornton[1], M. Grant[1], K. Osann[2], J. Weitzel[1].* 1) City of Hope, Duarte, CA; 2) University of California, Irvine, Irvine, CA; 3) University of Toronto, Toronto, Ontario, Canada; 4) George Washington University, Washington, DC; 5) Memorial Sloan Kettering CA Ctr., New York, NY.

BRCA+ breast cancer (BC) patients face markedly elevated risks of second primary tumors, and clinicians must communicate complex information about these risks and risk-reducing strategies. To enhance patient decision-making, we developed IRIS, a computerized genetic counseling communication tool that calculates and conveys individualized risk predictions associated with various risk reduction scenarios, based on a predictive model from a large cohort of BRCA carriers. IRIS' graphical output includes: 1)the absolute risk of second primary BC 2)disease-specific mortality and 3)the effect of risk-reducing mastectomy and/or oophorectomy. An expert panel of genetic counselors, nurses, and physicians evaluated the perceived utility of IRIS as a genetic counseling tool, and lay focus groups of BRCA+ women demonstrated preferences for IRIS' graphical representations. As health literacy and numeracy (numerical ability) may be critical to understanding and informed decision-making, 120 women at high-risk for breast cancer were recruited via the FORCE website (www.facingourrisk.org) to interpret graphical breast cancer risk, make hypothetical treatment decisions, and rate each graph using a six-point Likert scale. Health literacy was estimated using the validated instrument, Rapid Estimate of Adult Literacy in Medicine (REALM), and numeracy was estimated using a six-question test. The mean score was 4 out of 6 correct answers. The Pearson correlation between numeracy and graph interpretation was .627, p<.0005. Multiple linear regressions showed that 42% of the graphical interpretation variance was explained by numeracy alone, with little additional impact of education level. Based on this input, we are now revising IRIS' graphical output as part of an integrated decision support component to facilitate decision-making about risk reduction options.

788/W

Validity of gene testing for neurofibromatosis 1 mutations by direct DNA sequencing. *J.L. Hatfield, R. Rojas, S.M. Purandure, J.J. Mulvihill, S. Li.* Department of Pediatrics, University of Oklahoma, Oklahoma City, OK.

Neurofibromatosis (NF1) is one of the most common dominant neurogenetic disorders, affecting 1 in every 3500 individuals worldwide. The NF1 gene spans 350 kb of genomic DNA, containing 60 exons and encoding 12 kb of mRNA. Detection of NF1 mutations is a challenge because of the diversity of genetic mutations, the absence of mutational clustering, the size of the gene, and the existence of numerous pseudogenes. Currently, detection of NF1 mutations is approached by FISH, protein truncation test, and DHPLC followed by DNA sequencing. We used a whole NF1 cDNA screening methodology to study 84 individuals, including 45 definite clinical patients (both familial and sporadic by NIH criteria), 16 normal individuals as controls, and 23 patients with some clinical suspicion of NF1. After informed consent, RNA from peripheral blood was obtained from each individual using the total RNA purification kit (Qiagen). The RNA was reverse transcribed using Superscript II reverse transcriptase (Gibco, BRL). The entire NF1 cDNA was amplified in 15 overlapping fragments, ranging from 562 to 982 nucleotides. The size of the PCR products was verified by electrophoresis in 2% agarose gels before sequencing. Different types of novel and known mutations have been identified in the patient group and, as expected, no pathogenic mutation has been found in the control group. Of the 23 suspected patients, 12 had mutations, most considered pathogenic. Gene testing, especially in young or sporadic cases, performs well in resolving diagnostic uncertainty.

789/W

The need for continuing care: Patients with BRCA mutations desire follow-up genetic counseling. *J.Gamm Ruschman[1], E. Miller[1], K. Theobald[2], S. Knapke[1].* 1) Div Human Genetics, Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH; 2) St. Elizabeth Medical Ctr, Edgewood, KY.

Patients seen at CCHMC for genetic counseling related to BRCA1/2 testing are seen by a genetic counselor and a geneticist. Traditionally, patients receive pre- and post- test counseling. After BRCA1/2 results are given, patients are referred to their original provider for management. This clinical service has been provided since 1996, with no routine follow-up of mutation carriers. We assessed patients' interest in follow-up genetic counseling using a survey designed as a center-specific patient needs assessment. It was sent to 212 patients with positive BRCA test results. We received 89 responses (42%). Of those that responded, 39 (43%) indicated they were interested in a follow-up genetic counseling appointment. The main reasons patients cited for interest in follow-up genetic counseling were: to learn about risks for other family members (33%), to learn about new research related to genetic test result (44%), or to discuss screening or prevention options (15%). We used several Likert scale questions to measure the patient's current emotions about her genetic testing. 65% of patients that reported that they had been upset about their testing in the last week were interested in follow-up as compared to 40% of those that did not indicate they were upset (p=0.064). Also, 59% of those that reported they had strong feelings about their genetic testing indicated an interest in follow-up genetic counseling as compared to 38% of those that did not report strong feelings(p=0.066). The results indicate that at our center many patients desire continued follow-up genetic counseling services, especially related to discussing further testing within the family, to learn about new research related to their genetic testing results, or discuss additional management options. Additionally, a trend suggests that the patients seeking these services may be more likely to be having strong emotions about their results, and psychosocial genetic counseling will likely be a very important component of these follow-up sessions.

790/W

Changes in an Inherited Ring (22) as a Result of Meiotic Recombination; Implications for Counseling. *V. Jobanputra, E. Ash, K. Anyane-Yeboa, A. Sobrino, O. Nahum, B. Levy, D. Warburton.* Columbia University Medical Center, New York, NY.

We describe a case of a 21 month old child with developmental delay, microcephaly (<3rd %), coarse hair, epicanthic folds & hypotonia. Language & any intentional sounds were absent & there was lack of eye contact. Walking was unsteady with a wide-based gait. Brain MRI showed enlarged ventricles without hydrocephalus. Chromosome analysis revealed a maternally inherited ring(22). Cytogenetic studies on the mother showed the r(22) to be present in about 10% of her lymphocytes. FISH on the mother indicated that the 22qter probe was adjacent to the centromere in the r(22). Her ring was also ARSA+ & D22S75+. Thus, no long arm material appeared to be missing & the maternal karyotype describing the ring could be written as 46,XX,r(22)(p11.2q13.3).ish r(22)(D22S75+, ARSA+, qter+). Cytogenetic analysis of the child revealed a non-mosaic ring chromosome that was larger & had a different morphology than that observed in the mother. There was a non-staining gap next to the centromere that was acro-p positive by FISH analysis. The r(22) was also positive for D22S75 but negative for ARSA and 22qter. While the child appeared to have inherited the same ring from the mother, it was in fact different having gained (short arm including the 2ndary constriction) and lost (distal 22q) material. SNP Oligonucleotide Microarray Analysis (SOMA) confirmed the child's deletion and showed it was 3.6 Mb in size. The patient's karyotype was thus 46,XX,r(22)(p13q13.31).ish r(22)(acrop+, D22S75+, ARSA-, qter-), oligo arr q13.31qter(45,979,243-49,580,000)x1. These changes are not consistent with the rearrangements expected to occur in mitosis within the inherited ring. Rather they suggest that meiotic exchange occurred between the ring and the normal 22 in the mother, thus introducing the short arm material. To our knowledge this is the first case suggesting that such exchanges can occur. For counseling purposes it is important to note that an apparently benign ring in a parent can undergo rearrangements in meiosis to produce an unbalanced chromosome associated with developmental abnormalities.

791/W

Assessing the Use and Impact of Information Sheets and Patient Letters Given Prior to and Post Genetic Counseling. *S. Armel, A. Buchanan, K. Rajamanikkom, R. Demsky, B. Rosen.* Familial Breast and Ovarian Cancer Clinic, Princess Margaret Hospital, Toronto, ON, Canada.

Many patients overestimate their personal risk of developing breast and ovarian cancer prior to genetic counseling. Similarly, there may be confusion concerning genetic counseling and the basis for the referral. In this study we address this issue by evaluating the effectiveness of providing patients with written information before and after genetic counseling. At the Familial Breast and Ovarian Cancer Clinic (FBOCC) at Princess Margaret Hospital in Toronto, Canada, patients are sent an information sheet prior to their appointment outlining basic cancer genetics, risks, genetic testing, and screening methods. Patients are also sent a summary letter 1-2 weeks after counseling outlining decisions and recommendations made during the appointment. 182 patients who underwent genetic counseling at the FBOCC in 2006 were recruited immediately following counseling to complete a questionnaire pertaining to the information sheet. A second questionnaire was sent 1-2 weeks after the summary letter to evaluate its effectiveness. Of 182 patients participating, 118 completed the second questionnaire. 99% of patients found the information sheet helpful, specifically in obtaining general information about cancer (70%), information about genetic counseling and genetic testing (42%), understanding the nature of the appointment (51%), and in answering questions (53%). The majority of patients (99%) agreed we should continue to provide the information sheet, and do so prior to the appointment (91%). Results for the summary letter were similar, with 99% appreciating the letter and 98% agreeing we should continue to provide it. Between the information sheet and the summary letter 92% preferred to receive both. The results were fairly consistent among patients who sought counseling based on a familial mutation, patients with cancer, and patients without cancer. The results from this study illustrate the benefit of providing written information to patients prior to genetic counseling in addition to providing a summary letter of their session 1-2 weeks following.

792/W

An Evolving Model for Malignant Hyperthermia Genetic Testing. *D. Steele[1], B.W. Brandom[2], E.E. Smith[1], J.A. Kant[3].* 1) Center for Medical Genetics, Magee-Womens Hosp, Pittsburgh, PA; 2) Dept of Anesthesiology, Univ of Pittsburgh, Pittsburgh, PA; 3) Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA.

Background and Methods: Malignant hyperthermia (MH) is an autosomal dominant disorder associated with mutations in the RYR1 gene which predispose patients to often fatal reactions during anesthesia. The validated test that confirms MH susceptibility is an expensive bioassay of viable muscle. Associated with the introduction of an *RYR1* screening panel, we designated a genetic counselor to provide information and support to patients and physicians with medical backup from an experienced anesthesiologist. Goals included education that only 50% of patients with clinically proven MH (positive muscle biopsy) have mutations in the *RYR1* gene, the percentage is even lower in patients with a family history or possible MH episodes, the screening panel would not detect all mutations in this 106 exon gene and thus negative genetic testing does not exclude MH susceptibility. We also developed a form for use by a healthcare professional to collect data for transmission to the MHAUS Patient Registry from patients seeking genetic testing. **Results**: 74 patients have been referred for MH testing. 45 spoke with the genetic counselor and 20 of those underwent genetic testing. 25 additional samples were submitted directly to the laboratory. Of 6 patients with *RYR1* gene mutations, only one did not have contact with the counselor. One positive patient had targeted testing following discovery of a novel mutation in a family member. Samples have been submitted from medical examiners and institutions for medicolegal issues. The counseling framework facilitates testing of appropriate family members, the avoidance of unnecessary testing and its costs, and referral for confirmatory muscle testing, if needed. The coordination of laboratory results with the MHAUS Registry facilitates the identification of new disease-associated mutations as well as genotype/phenotype correlations. **Conclusions**: Integration of genetic counseling and data collection from patients seeking genetic testing for malignant hyperthermia (MH) facilitates test utilization. The counselor is a valuable resource for patient and physician.

793/W

Recruitment Approaches for a Cancer Registry-Based Study of BRCA1/2 Mutations among Young African American Breast Cancer Patients. *S.T. Vadaparampil[1,2], J. Weber[2], J.A. Betts[2], T. Pal[1,2].* 1) Dept. of Interdisciplinary Oncology, University of South Florida College of Medicine, Tampa, FL; 2) Div. of Cancer Prevention and Control, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

The prevalence and penetrance of BRCA1/2 mutations in the African American (AA) community is largely unknown. This lack of knowledge is perpetuated by low rates of participation by AA women in clinical and research BRCA1/2 genetic counseling and testing. This abstract describes approaches, materials, and procedures developed to promote participation in a population-based study of BRCA1/2 mutations among young AA women with early onset breast cancer recruited through the Florida Cancer Data System (FCDS). Study recruitment involves 2 mailed contacts followed by a phone call from the study team. A multi-step approach to optimize recruitment at each of these contact points was implemented using: (1) a Community Advisory Panel (CAP) to develop and refine the study recruitment brochure, phone script, questionnaire, and procedures; (2) pilot testing of materials with the target audience; and (3) review of pilot testing results with the CAP. Based on this process, the study brochure was modified to include personal vignettes and photos of AA women, the terms 'Women of Color' and 'Black Women', and the legacy that BRCA1/2 testing may leave for the family and AA community as a whole. The recruitment phone script was modified to highlight information about the relationship of BRCA1/2 genes and early onset breast cancer in AA women. The study questionnaire now includes lay explanations of medical terms that were difficult for participants to understand and preparatory suggestions to shorten interview time. Finally, study procedures now include the option of telephone-based genetic counseling and an in-home blood collection process to minimize participant time and travel burdens. By utilizing these methods, the study team hopes to increase participation among young AA breast cancer patients. The lessons learned from the current study can be applied to other genetics studies recruiting individuals from a variety of cultural and ethnic backgrounds.

794/W

Diagnostic testing for Duchenne/Becker Muscular dystrophy using Dual Priming Oligonucleotide (DPO) system. *J.H. Kim[1], G.H. Kim[1,2], J.J. Lee[2], D.H. Lee[3], J.K. Kim[3], H.W. Yoo[1,2,4].* 1) Genome Research Center for Birthe Defects and Genetic Disorders, Asan Institute for Life Sciences,; 2) Medical Genetics Clinic and Laboratory, Asan Medical Center; 3) Seegene Institute of Life Science; 4) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Duchenne and Becker type muscular dystrophy (DMD and BMD, OMIM #310200, 30076) are common X-linked heritable muscle diseases in children caused by mutations in the dystrophin gene. Large exon deletions in the gene are found in about 60% of DMD patients. A serial set of multiplex PCR has been employed to detect the deletion mutation, which frequently generates noise PCR products due to the presence of multiple primers in single test tube as well as the stringency of PCR conditions. This often leads to a false-negative or false-positive result. To address this problematic issue, we introduced the Dual Primer Oligonucleotide (DPO) system. DPO contains two separate priming regions joined by a polydeoxyinosine linker, which serve high PCR specificity even under less optimal PCR conditions. We tested 50 healthy controls, 50 patients with deletion mutation as deletion-positive patient controls, and 20 patients with no deletions as deletion-negative patient controls using DPO-multiplex PCR. Both the presence and extent of deletion were verified by simplex PCR spanning promoter region(PM) and 18 exons including exons 3-4, 6, 8, 12-13, 17, 19, 43-48, 50-52, 60 in all 120 controls. DPO-multiplex PCR showed 100% of both sensitivity and specificity for the detection of presence of deletion. However, it shows 71.2 % of sensitivity and 100% of specificity in determining the extent of deletions. In conclusion, DPO-multiplex PCR method is another useful molecular testing modality for the diagnosis of patients with DMD/BMD since it is easy to perform, fast and cost-effective as well as the result shows relatively high analytical validities.

795/W

Development of a Comprehensive and Efficient Molecular Diagnostic Assay for the Autosomal Dominant Polycystic Kidney Disease (ADPKD) genes, PKD1 and PKD2. *Y. Tan[1], J. Blumenfeld[1,2,3], S. Donahue[2,3], R. Belenkaya[1,2,3], T. Parker[1,2,3], D. Levine[1,2,3], H. Rennert[1,2,3].* 1) Weill Cornell Medical College; 2) The Rogosin Institute; 3) Rockefeller University, New York, NY.

ADPKD is one of the most common hereditary disorders affecting about 1 in 500 people. ADPKD is genetically heterogeneous with PKD1 and PKD2 accounting for 85% and 15% of mutations, respectively. Diagnosis of ADPKD is mainly performed by renal imaging, but genetic testing plays an important role, particularly in young, asymptomatic individuals, or those without a family history, where the imaging studies may be inconclusive. Genetic analysis of PKD1 has proven extremely difficult because of the large transcript and complex reiterated gene region. We have developed a comprehensive, rapid and efficient molecular assay for detecting mutations in PKD1 and PKD2, using SURVEYOR Nuclease and the WAVE NA High Sensitivity System (Transgenomic), and compared the analysis results to sequencing results reported by a commercial reference laboratory for 25 patient samples. Mutation analysis revealed a total of 90 sequence variants including all 82 changes reported by the reference laboratory (100% sensitivity). 76 variations (84.4%) were in PKD1 and the reminder 14 (15.6%) were in PKD2. Of the 90 variants, 14 were pathogenic mutations, 6 from PKD1 and 8 from PKD2, consisting of 7 nonsense, 4 truncating mutations and 3 splicing defects. The remaining 76 variants included 26 missense, 33 silent and 17 intronic changes, 8 of which were not previously reported by the reference laboratory. Moreover, of the 14 pathogenic mutations, 2 nonsense mutations were incorrectly determined by the reference laboratory to be homozygous mutations. The pathogenic potential of the missense variants was evaluated by evolutionary conservation and Grantham score for chemical difference software. Of the 26 missense variants, 4 were scored as probably pathogenic by all software applications. Overall, pathogenic or probably pathogenic mutations were detected in 21of the 25 (84%) patient samples. Taken together, these results demonstrate that this method is highly accurate and reliable for identifying sequence variations in ADPKD genes.

796/W

Comparison of Hexosaminidase A enzyme assay and mutation testing: Is enzyme assay still necessary in Tay-Sachs population screening? *A. Schneider[1], R. Keep[1], D. Dorsainville[1], T. Bardakjian[1], D. Finegold[3], W. Sun[2], A.M Roe[2], J. Lebow[1], S. Nakagawa[2], J. Zhan[2], S. Gross[2].* 1) Dept Genetics, Albert Einstein Med Ctr, Philadelphia, PA; 2) Albert Einstein College of Medicine, Bronx, NY; 3) Univ Pittsburgh,Childrens Hosp Dept Pediatrics, Pittsburgh, PA.

Background: The Victor Center for Jewish Genetic Diseases was established with the mission of education, screening and counseling for the disorders that occur more frequently in the Ashkenazi Jewish(AJ)population. The program provides free screenings on college campuses and for newlywed couples. While Tay-Sachs(TS)carrier screening historically has relied on biochemical enzymatic assays, molecular analyses for the common founder mutations is now also commonly performed. Aim: The goal of this present study was to clarify actual detection rates for these two methodologies in a community-based, non-selected Jewish population and thereby determine whether there is additional benefit to the continued use of the TS enzyme assay for carrier detection. Methods: During the period from March 2006 to March 2007, 632 people were screened for Tay-Sachs disease(college students and newlyweds). A single laboratory was used for all assays. All samples were tested for the common founder mutations, as well as the 2 pseudodeficiency alleles. Serum Hexosaminidase A(Hex A)assay was performed on all samples. Platelet assays were performed on inconclusive samples(68). Results: All of the carriers were detected by enzyme assay. Two of the 24 carriers(8%)were negative for the common AJ mutations by DNA testing. One of these individuals was adopted and the other reported mixed ancestry. Conclusion: As the AJ population diversifies with intermarriage and adoption, DNA testing for the common AJ mutations will invariably miss Tay-Sachs carriers. While molecular testing may be appropriate for cascade screening of family members of known carriers and for individuals with well delineated Ashkenazi Jewish background, these preliminary results strongly suggest the continued use of biochemical methodologies in the more genetically diverse, self-identified young adult Jewish population. **Background:Aim:Methods:Results:Conclusion:**.

797/W

Prevention of Homozygous b-thalassemia by Carrier Screening and Prenatal Diagnosis in India. *Sarita. Agarwal, M. Pradhan.* Genetics, SGPGIMS, Lucknow, UP, India.

We report here results of a 3-year pilot voluntary screening program coupled with prenatal diagnosis directed to the prospective prevention of homozygous b-thalassemia in India. The screening program took two approaches: testing of the extended family members of the high risk couples and secondly screening of all referral anemia cases to rule out thalassemia carrier status for premarital genetic counseling in unmarried carriers while married were educated for prenatal diagnosis. The screening of extended family members of high risk couple was very effective as, out of 200 couples, in 113 cases the procedures were performed as both the partners were carrier and women were pregnant at the time of screening. The DNA diagnosis revealed 28% affected fetuses, 61% carriers and 24% fetuses as normal. On screening of 2287 cases, 291 cases were identified as b-thalassemia, 56 as b-thalassemia with structural hemoglobin variants [Eb, Sb & Db]. Genotype-phenotype correlation confirmed 243 cases as thalassemia heterozygous & 48 cases as thalassemia homozygous. In 50 cases where both the partners were carrier of b-thalassemia of which only 20 couples could opt for prenatal diagnosis after counseling since pregnancy was positive. 20 CVs samples of this screening group revealed, 5 affected fetuses. In total 33 affected fetuses were terminated from high-risk couples [133]. Prenatal detection was achieved by DNA analysis on chorionic villus samples obtained by ultrasound guided trans abdominal or trans vaginal procedures. The ARMS-PCR and sequencing methods were used for the identification of mutations. The PND program through carrier screening and extended family screening program has prevented the birth of thalassemia homozygous by 1.33% [33/2487] in India. By introducing PND program in the country it is possible to prevent the disease in large to avoid mental & physical trauma to the family and financial burden on medical & health services in the country. For PND program the idea of molecular spectrum of the disease in different parts of the India needed where endogamy and consanguinity in various ethnic groups is quiet common.

798/W

Evaluation of Genetic Services in Ontario: Patient Satisfaction and Health Care Utilization. *M. Cappelli[1], N. Barrowman[1], J. Carroll[2], N. Carson[1], C. Gilpin[1], F. Miller[3], M. Mullen[1], L. Velsher[4], B. Wilson[5].* 1) Dept Psychology, Children's Hosp Eastern Ont, Ottawa, ON, Canada; 2) Univ of Toronto, Toronto, Canada; 3) MacMaster Univ, Toronto, Canada; 4) North York Gen Hosp, Toronto, Canada; 5) Univ of Ottawa, Ottawa, Canada.

In 2000, publicly funded clinics were established in Ontario to provide comprehensive cancer genetic counseling services. Considerable interest in genetic testing, coupled with increasing knowledge of the availability of these services has to led to greater demand on these services. This ongoing study, which began in 2003, evaluates cancer genetic services in Ontario for hereditary breast, ovarian and colorectal cancers. Emphasis is on patient satisfaction with these services, and patient knowledge and practices following genetic counseling. A prospective, repeated measures design was used. Patients were recruited from five regional sites across Ontario. All participants complete a self-report survey (Time 1) following their genetic counseling appointment. Patients who underwent genetic testing completed two additional surveys: a telephone interview at Time 2 (4-6 weeks after receiving genetic test results), and a mail-out survey at Time 3 (one year after receiving genetic test results). To date, 640 surveys (Time 1) have been mailed and 489 returned (response rate = 76.4%). Preliminary data demonstrate that 95.7% of patients were satisfied with the manner in which they were received by the staff at the genetic clinic. As well, 93% felt that they were given the information they wanted about the risks and benefits of genetic testing. A notable area showing decreased satisfaction is communication between the genetic counselor and the physician about genetic test results (72.2%) as well as recommended screening (62.1%). By identifying current deficiencies in the services provided, cancer genetics services can improve and evolve which, in light of anticipated future demands, is critical for sustainability. These results have implications for policy formulation regarding cancer genetics programs in Ontario and elsewhere.

799/W

An Expanded Ashkenazi Jewish Prenatal Carrier Screening Panel - 16 Diseases. *L. Edelmann, S.A. Scott, L. Liu, Y. Wang, R.J. Desnick, R. Kornreich.* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York, 10029.

Since the 1970s, when prenatal carrier testing for Tay-Sachs disease began, we have performed prenatal carrier testing and counseling for the Ashkenazi Jewish (AJ) population. Due to selection and/or genetic drift, the AJ population are at increased risk for certain severely debilitating and/or fatal autosomal recessive diseases in which specific mutations are present in the majority of affected patients. Previously, our disease panel included eleven disorders: Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher's disease, glycogen storage disease Ia, maple syrup urine disease, mucolipidosis type IV, Niemann-Pick disease type A, and Tay-Sachs disease. These have carrier frequencies that range from 0.070 (1 in 15) to 0.008 (1 in 125) and have 94 to 99.5% detectability in the AJ population. Recently, we added four disorders (lipoamide dehydrogenase deficiency (E3), Usher type III (USHIII), familial hyperinsulinism (HI), and nemaline myopathy (NM)) to the panel with detectabilities of 90 to 95%. Their AJ carrier frequencies range from 0.012 (1 in 85) to 0.008 (1 in 120) and were determined by screening 1000 anonymous unrelated AJ individuals from the greater New York Metropolitan area using a recently developed multiplex PCR/allele-specific primer extension Luminex® FlexMAP bead-based assay. In addition, we also determined the Usher type I (USHI) carrier frequency in this AJ cohort to be 0.006 (1 in 170). Given this disorder has a detectability of ≥75%, residual risk counseling is required. The cumulative carrier frequency for E3, USHIII, HI and NM mutations in our AJ cohort was 1 in 26 (1 in 23 when including USHI), highlighting the potential benefit in including these disorders in an AJ screening panel. With the clinically approved expanded panel of 15 disorders (16 including USHI), approximately 1 in 4 AJ will be a carrier for at least one of these diseases.

800/W

Failure to detect a DM1 expansion using triplet repeat-primed PCR. *J.S. Parboosingh, R.J. Klock, P.J. Bridge.* Dept Medical Genetics, Alberta Children's Hosp, Calgary, AB, Canada.

Recently, the CTG repeat expansion in the DMPK gene causing myotonic dystrophy failed to be detected in two patients by triplet repeat-primed PCR (tpPCR) using frequently cited primers. In recent years, this method has been applied to a number of trinucleotide repeat disorders as it has several advantages over the traditional Southern blot analysis. Triplet-primed PCR allows for a rapid turnaround time on a small amount of DNA particularly important for diagnostic confirmation in hypotonic babies and prenatal cases; however, it does not allow one to estimate the size.

TpPCR has been the method of choice in the Molecular Diagnostic lab for 20 months. During this time 40 screens have been performed for a variety of reasons including: confirmation of diagnosis, and prenatal testing. A two step approach is taken: 1) primers flanking the repeat are used to determine the number of repeats within the normal range and up to approximately 100 repeats; and 2) tpPCR is performed on all samples with a single repeat from step 1 to exclude the presence of a large expansion and thus confirm homozygosity. We have designed tpPCR assays utilizing both DNA strands (using both a CTG and CAG repeat primer) allowing for bidirectional tpPCR. We have detected 17 patients with expansions. Two of the 17 expansions were not detectable using the frequently cited P1 primer with the CTG repeat primer but were detectable using the complementary CAG repeat primer and the opposite flanking primer (other strand). Utilization of tpPCR in only one direction would have led to a false negative result for these patients. This has led to the identification in the SNP database of a C>T substitution within the P1 primer binding site. We will present these findings as well as a frequency for this polymorphism.

801/W

Real-time multiplex allele-specific PCR for 35delG genotyping based on SYBR Green I fluorescence. *E.L. Sartorato[1], C.A. Oliveira[1], J.Jr. Pedrazzoli[2], M.L. Ribeiro[2].* 1) Lab de Genetica Molecular Humana/CBMEG, Universidade Estadual de Campinas, Campinas, SP, Brasil; 2) UNIFAG,Universidade Sao Francisco, USF, Braganca Paulista, SP, Brasil.

In developed countries approximately 1 in 1000 children is born with a hearing loss severe enough to require special education services, and about 60% of the cases of isolated deafness have a genetic origin. Although doctors know about more than 100 genes for hearing at the moment, only a few are routinely tested for. The main genetic test being offered at present is a test to screen the gene for the connexin 26 protein. Mutations in the connexin 26 (GJB2) gene are the most commonly known cause of nonsyndromic recessive deafness (NSRD). One specific mutation, a deletion of G, in a sequence of six Gs (35delG), accounting for approximately two thirds of GJB2 alleles from persons with NSRD. The prevalence of heterozygous 35delG carriers among hearing population is high (2-4%) in several countries where this mutation analysis was performed. Thus, the aim of the present study was to develop a single-step and single-tube method for 35delG genotyping by real-time multiplex allele-specific PCR and melting curve analysis. The preliminary results obtained from 10 samples showed a high accuracy compared to those obtained with a conventional allele-specific PCR. Although this method requires expensive equipment, it is inexpensive in terms of consumables. It is also very rapid, reliable and suitable for large-scale screening. This method also would be useful when combined to other diagnostic methods for early diagnosis, and associate to multidisciplinary services to set the main objective of rehabilitation.

802/W

Development of a MLPA assay for Norrie disease gene (NDP) deletion testing. *Y. Shen[1,3], H. Zhu[3], W. Xin[1,3], S. Smith[2], J. Gusella[3], K. Sims[1,3].* 1) Neurogenetics DNA Diagnostic Laboratory, Massachusetts General Hospital, Boston, MA; 2) Division of Genetics at Children's Hospital Boston and HPCGG at BWH and MGH, Harvard medical School, Boston, USA; 3) Center for Human Genetic Research,Massachusetts General Hospital, Boston, MA.

Norrie disease (OMIM #310600) is a rare X-linked recessive neurologic disorder. It is characterized by congenital bilateral blindness, and variable clinical features. In our clinical molecular DNA diagnostic lab, PCR-based exon sequencing technique is able to effectively detect small mutations in the Norrie disease gene (NDP). Large deletions including one or more exons were suspected when one or more exons failed to amplify after using a second set of primers. Carrier status of female family members in whom deletion is presumed could not be tested by direct sequencing. To improve female analysis in these deletion families for counseling purpose, we developed an easy, fast and reliable method using MLPA (multiple ligation-dependent probe amplification) technique. This method allows detection of deletion involving any or all three exons of the NDP gene in Norrie male patients and their female relatives. The MLPA based assay, using synthetic NDP exon specific probes, confirmed all suspected deletion cases (affected males) and confirmed the same deletion patterns in their respective carrier female family members. Patients with large deletion account for 23.4% (30/128) of all ND patients in whom we have identified pathogenic mutations in our lab. The deletion mutations were further confirmed by mapping of the deletion breakpoint. Furthermore,this assay confirmed the deletion carrier status in all available mother samples (n=14), suggesting that de novo deletion in NDP gene is not a frequent event in Norrie disease patients. Thus, we have demonstrated that the MLPA method is highly sensitive, specific and reliable. The MLPA assay is now a routine NDP mutation detection procedure in our clinical molecular testing lab and it is a valuable tool for diagnosis of male detection cases, genotype-phenotype correlation analysis of NDP deletion patients and female carrier status assessment.

803/W

The National Ophthalmic Disease Genotyping Network: eyeGENE™. *S.J. Tumminia[1], A. Nezhuvingal[1], D. Scheim[2], N. Smaoui[1], D. Blain[1], H. Chin[1], B.P. Brooks[1].* 1) NEI/NIH, Bethesda, MD; 2) Private Systems Specialist, Blacksburg, VA.

Purpose: Create a National Ophthalmic Disease Genotyping Network (eyeGENE™) to facilitate inherited ophthalmic disease research. **Methods:** Individuals are recruited into eyeGENE™ from academic centers and private clinical practices. Clinicians complete a registration process, obtain informed consent and assure that genetic counseling is provided. Blood and DNA are processed in a CLIA-certified fashion for molecular diagnostic testing. Remaining DNA is stored at the eyeGENE™ Repository. The clinician submits phenotypic information via a secure eyeGENE™ database which also contains genotype results. Upon approval, investigators may access the database, DNA samples for research or recruit individuals for participation in a clinical study. **Results:** We created eyeGENE™ which includes a Network of CLIA labs, a DNA repository and a database that couples anonymous genotype-phenotype data. An external Steering Committee provides opinions regarding scientific, ethical, and management issues. CLIA laboratories provide testing for over 40 disease genes including macular diseases (e.g., Best disease, Stargardt disease), other retinal diseases (e.g., retinitis pigmentosa, choroideremia), strabismus (e.g., congenital fibrosis of the extra-ocular muscles), glaucoma (e.g., dominant juvenile glaucoma, Axenfeld-Rieger syndrome), and corneal dystrophies (e.g., Reis Bucklers', granular dystrophies). Minimal phenotypic criteria have been established for each disease tested. On September 20, 2006 the first patient sample was received through eyeGENE™. At the time of abstract submission, 90 samples are being analyzed. **Conclusions:** A National Ophthalmic Disease Network was created to manage molecular diagnosis and clinical data for use in research. eyeGENE™ also provides CLIA-certified molecular diagnosis to patients. eyeGENE™ will eventually facilitate the discovery of new ophthalmic disease genes, identify genetic modifiers of disease; aid in the analysis of genotype:phenotype correlations and enhance recruitment for clinical trials. eyeGENE™ will be a resource for the vision community and benefit medical care for a broad patient population with inherited eye disease.

804/W

Genetic Testing and Genetic Counseling for Severe Male Factor Infertility Prior to Intracytoplasmic Sperm Injection (ICSI). *M. Wick[1], D. Morbeck[2].* 1) Dept of OB/GYN, Mayo Clinic and Foundation, Rochester, MN; 2) Dept of OB/GYN, Division of Reproductive Endocrinology, Mayo Clinic and Foundation, Rochester, MN.

Infertility affects 10-15% of couples. Twenty percent of cases are due solely to male factor. Genetic alterations including CF,chromosomal abnormalities and Y microdeletion account for 15-30% of severe male factor, i.e., azoospermia and severe oligospermia. Intracytoplasmic sperm injection(ICSI)enables couples with severe male factor to achieve pregnancy. However, ICSI offspring have a higher incidence of sex chromosome abnormalities than the general IVF population. Additionally,genetic causes of infertility in the male will be passed on to male offspring. Thus,the American Society for Reproductive Medicine and the American Urological Society have provided recommendations regarding counseling and genetic screening for male factor infertility(Fertil Steril 2006;86:S202-9). The guidelines recommend that couples planning ICSI for male factor infertility(<5-10 million sperm/ml;non-obstructive azoospermia)should be counseled about potential genetic risks. To establish a baseline for these recommendations at our institution,we reviewed records of all couples needing ICSI for male factory infertility from 1998-2006. Only histories of those consented for research studies were reviewed. Complete records were available for 469 couples. 126(27%)males had significant male factor infertility warranting informed risk and genetic testing under the new recommendations. Of the 126 reviewed, 7(5.5%) were informed of risk and offered genetic testing, 16 (12.7%) were offered testing but declined, 27(21%) were referred to Urology/Endocrinology, 20(15.9%) received testing and referred to Urol/Endo, 21(17%) were referred to Genetics, 2(1.6%) were referred to genetics for another genetic disease, and 33(26%) received no testing/counseling. These results indicate that at least a quarter of couples with severe male factor infertility were not fully aware of possible genetic risks associated with ICSI. Increased communication between Genetics and REI and adoption of the new guidelines should facilitate appropriate testing and counseling of these couples.

805/W

GENETIC TESTING AND COUNSELING FOR FSHD- THE WOLFSON EXPERIENCE 2000-2006. *M. Yanoov-Sharav[1,2], E. Leshinsky-Silver[2,3], C. Vinkler[1,2], M. Michelson[1,2], S. Cohen[3], T. Lerman-Sagie[2], M. Ginzberg[2], M. Sadeh[4], D. Lev[1,2].* 1) Institue of Medical Genetics, Wolfson Medical Center, Holon, Israel; 2) Metabolic Neurogenetic Clinic Wolfson Medical Center, Holon, Israel; 3) Molecular Genetics Laboratory Wolfson Medical Center, Holon, Israel; 4) Department of Neurology Wolfson Medical Center, Holon, Israel.

FSHD, is a dominantly inherited, late onset, progressive disease. At present, no treatment or prevention of symptoms are available. There is considerable clinical variability, even within families. There is clinical overlap between FSHD and other limb girdle muscular dystrophies. The gene causing FSHD has not been identified, but molecular diagnosis can be made by analyzing the length of the D4Z4 repeat area on chromosome 4q35. Results of DNA analysis can support or rule out the clinical diagnosis of FSHD, but there may also be non- conclusive, "gray zone" results. Prenatal diagnosis, PGD and pregnancy termination in cases of fetuses with the "FSHD genotype" are ethically controversial, and are offered only in a few centers in the world. METHODS: 66 individuals were tested for D4Z4 repeat number. 59 patients were referred due to clinical suspicion, 7 were asymptomatic and had a first-degree relative with FSHD. RESULTS: In 77% the results were conclusive. In 23% the results were in the gray zone (1 asymptomatic). 19 individuals (29%) received genetic counseling from our medical geneticists. Cognitive involvement was rare. One family exhibited genetic anticipation - a rare finding in FSHD. CONCLUSIONS: Only 77% of the cases allowed for unequivocal support or ruling out of this diagnosis. Maximal utilization of the existing molecular test for FSHD demands detailed clinical and family pedigree information. We recommend that genetic counseling by medical geneticists be given before and after molecular testing for FSHD, in addition to the neurological follow-up. Presymptomatic testing should only be offered after detailed counseling of the person to be tested and should not be recommended for young adults, still at high risk for becoming symptomatic.

806/W

Use of dried blood spots in ELISA detection of IL-7. *K. Chen[1], S.A. McGhee[1], E.R.B. McCabe[1,2].* 1) Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; 2) Human Genetics, David Geffen School of Medicine at UCLA, and Department of Bioengineering, Henry Samueli School of Engineering and Applied Science, UCLA, Los Angeles, CA, USA.

IL-7 is a 25 kDa cytokine with a nonredundant role in T cell homeostasis. Due to abnormal T-cell development, patients with untreated severe combined immunodeficiency (SCID) have elevated levels of IL-7. Patients with SCID benefit substantially from hematopoietic stem cell transplantation in the first month of life. We proposed to determine if ELISA detection of IL-7 in dried blood spots would be useful in the development of a newborn screening test for SCID. Blood samples were obtained from healthy adults. Using commercially available ELISA kits (R&D Systems), IL-7 levels were measured in (1) plasma, (2) dried plasma spots, and (3) dried blood spots. As positive controls, samples were spiked with recombinant human IL-7. IL-7 levels in dried plasma spots correlated with levels detected in fresh frozen plasma. Using Western blot analysis, IL-7 antibody was able to detect immunoreactive proteins in each sample type. We identified a low molecular weight protein that contributes to a high background in ELISA detection of IL-7 in dried blood spots. We examined a variety of strategies to reduce the high background by removing the low molecular weight protein prior to analysis, and by using different antibodies to detect IL-7. IL-7 can be recovered from dried plasma spots. Because IL-7 is present in picogram levels in the blood, there are multiple factors that can interfere with highly sensitive ELISA detection in dried blood spots. Reduction of these interfering factors will be required for use of dried blood spots for IL-7 detection as a newborn screening method for SCID.

807/W

Tay-Sachs Carrier Testing By Hexosaminidase A Assay In Serum And Platelets And By Mutation Analysis. *S. Nakagawa[1,7], J. Zhan[7], W. Sun[7], A.M. Roe[1], A. Schneider[2], D. Finegold[3], J. Charrow[4], K. Aleck[5], S. Minkoff[6], J.D. Hoffman[6], A. Spencer[1], S. Apfelroth[1,7], N. Schreiber-Agus[1], S.J. Gross[1,7].* 1) Albert Einstein College of Medicine, Bronx, NY; 2) Albert Einstein Med Ctr, Philadelphia, PA; 3) Children's Hospital of Pittsburgh, Pittsburgh, PA; 4) Chicago Center for Jewish Genetic Disorders, Chicago IL; 5) Jewish Genetic Diseases Center of Greater Phoenix, Phoenix, AZ; 6) Tufts-NEMC, Boston, MA; 7) Jacobi Medical Center, Bronx, NY.

BACKGROUND: Different methodologies have been used for Tay-Sachs Disease (TSD) carrier screening in the Jewish population. Serum analysis can be inaccurate due to pregnancy or oral contraceptive use and has a high rate of inconclusive results. Therefore, other biochemical assays that measure Hexosaminidase A (HexA) in cells have been developed to overcome this limitation. **AIM**:To determine the screening characteristics of the HexA isoenzyme platelet assay for carrier testing in the Jewish population. **RESULTS**:Carrier testing of 1036 self-identified Jewish individuals from various communities showed the following results:

Assay Method	Non-Carrier	Carrier(%)	Inconclusive(%)
Platelet	997	35 (3.4)	4 (0.4)
Serum	838	29 (2.8)	169 (16)
DNA	1005	31(3.0)	not applicable

Of the 4 inconclusive platelet assays, 2 had identifiable mutations. No DNA mutations were detected in the 997 platelet assay non-carriers. Of the 4 positive platelet assays that were not confirmed by DNA, all were of unknown or mixed ethnicity. **CONCLUSION**: We have demonstrated that the platelet Hex A assay is an excellent option for TSD carrier screening that is simple, accurate, and has a very low inconclusive rate (0.4%).

808/W

Evaluation of the Vyent Cystic Fibrosis Kit, IUO* and NanoChip® 400 System for cystic fibrosis newborn screening using dried blood specimens. *G. Hoffman, G. Kopish.* Newborn Screening Lab, Wisconsin State Laboratory of Hygiene, Univ. of WI, Madison, WI.

Purpose: The Wisconsin (WI) protocol for CF newborn screening consists of a two tier screen where the highest 4% of the daily immunoreactive trypsinogen (IRT) specimens tested are followed with a DNA test for the 23 mutation panel recommended by ACOG & ACMG. Currently, WI uses the reverse dot blot linear array (Roche, Inc) for DNA testing. The lab evaluated the Vyent Cystic Fibrosis Kit with the automated NanoChip 400 System (Nanogen, Inc) as an alternative CFTR DNA test. The evaluation assessed (1) fit into a routine screening environment, (2) ability to correctly genotype samples, (3) work flow, (4) robustness for newborn screening. **Methods:** DNA extracts were analyzed side-by-side with the linear array and the NanoChip 400. A total of 500 specimens were analyzed in 25 assays over eight weeks. Specimens with known mutations were rotated in each run so all 23 mutations detected with the kit were tested. Reproducibility was assessed by analyzing a standard set of specimens in each run. **Results:** The NanoChip 400 is an automated allele detection system that fits on a standard bench top. The system software and operation were easy to learn and transferable between staff. Concordance for all markers between methods was 100%; (n= 6,003) for 261 specimens and controls analyzed. There were no "low signals" or "indeterminate" calls made during the study. Results of the specimen set repeated in each run showed no discrepancies and there was no deterioration in assay performance (e.g. fluorescent signal), showing the assay to be robust and reproducible. The average hands-on time with the linear array was about 5 hours per run while the automated NanoChip 400 reduced the time to about 30 minutes per run excluding DNA preparation (same for both methods). **Conclusions:** The Vyent Cystic Fibrosis Kit when used with the NanoChip 400 is a robust, accurate, highly automated method that significantly reduces the hands-on time for detecting CFTR mutations in dried blood spot specimens for newborn screening. *Investigational Use Only. Performance characteristics have not been established.

809/W

Sequencing of the *CFTR* coding regions is required to optimize molecular diagnosis of cystic fibrosis in patients with clinical features and one identified disease-causing mutation. *M.B. Sheridan, N. Wang, P. Mogayzel, G.R. Cutting.* Johns Hopkins University, Baltimore, MD.

Patients with cystic fibrosis (CF) manifest symptoms in the respiratory tract, GI tract, male reproductive tract and sweat gland due to mutations in *CFTR*. Non-classic CF patients have disease in a subset of these organ systems. Most non-classic CF patients have two mutations in *CFTR*. A subset of patients have only one CF-causing mutation after screening for a panel of common CF-causing mutations or following mutation scanning of the coding region of *CFTR*. These patients present a diagnostic dilemma and a challenge for genetic counseling. We evaluated 9 patients with only one CF-causing mutation identified after a screen of 97 *CFTR* mutations (3 patients) or scanning of the coding region of *CFTR* (6 patients). Eight patients, including one set of siblings, have non-classic CF with borderline or elevated sweat [Cl^-] and lung disease. One patient has classic CF. Each of these patients have features that are consistent with CFTR dysfunction including a CF-like nasal potential difference, P. aeruginosa infection, or congenital bilateral absence of the vas deferens, suggesting that they have a 2nd *CFTR* mutation. Mutations that are not detected by screening include insertions or deletions, mutations outside of the *CFTR* coding region that affect RNA splicing or expression, or mutations in the coding region of *CFTR* that were missed by screening. To exclude the 3rd possibility, the 27 exons and flanking introns of *CFTR* were sequenced. A 2nd mutation was identified in the coding region of CFTR in 6 of 9 patients; 3 had screening for 97 known *CFTR* mutations, while the other 3 had comprehensive scanning of the coding region of *CFTR* by modified TGGE. Each of the mutations has been previously described in CF patients and is predicted to cause CFTR dysfunction. These results demonstrate that patients with one *CFTR* mutation and features of CF are likely to have a 2nd mutation in the coding region of *CFTR*. Thus, sequencing of *CFTR* in patients with only one mutation after screening should reduce unnecessary clinical and molecular evaluations in patients being evaluated for a CF diagnosis.

810/W

Detection of large rearrangements in the CFTR gene by multiplex ligation-dependent probe amplification (MLPA) assay in cases where sequencing fails to detect two disease-causing mutations. *A.M. Svensson[1, 2], L.S. Chou[2], C. Miller[3], J. Robles[3], I. Sinitsyna[3], K.V. Voelkerding[1, 2], R. Mao[1, 2], E. Lyon[1, 2].* 1) Department of Pathology, University of Utah, Salt Lake City, UT; 2) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 3) ARUP Laboratories, Salt Lake City, UT.

Over 1,300 mutations have been identified in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Most of these are point mutations or small deletions/insertions which may be detected by sequencing. Large gene rearrangements in CPTR have recently been reported. Purpose: The CFTR sequencing protocol at ARUP Laboratories interrogates the entire 27 exons and partial intronic regions of the gene. The present study was undertaken to determine whether testing for large gene rearrangements could improve the mutation detection rate. Methods: Nine cases with abnormal quantitative pilocarpine iontophoresis sweat chloride (SC) values (>60 mEq/L) and 20 cases with borderline SC levels (40-60 mEq/L) with only one or no mutations detected by the ACMG panel followed by sequencing, were tested using a multiplex ligation-dependent probe amplification (MLPA) assay (MRC-Holland, Amsterdam, The Netherlands). Forty-three probe pairs tagged with universal primer sequences hybridize to adjacent target sequences and are then joined by thermostable ligase. The probe products are amplified by multiplexed PCR using a FAM fluorescent dye-labeled consensus primer pair. The amplicons are then separated by capillary electrophoresis. Peak profiles for each amplicon are normalized and compared to a control sample. The calculated relative peak height is then used to determine the copy number of each target sequence. Results: One deletion was detected among the 9 cases with high SC values. None of the cases with borderline SC levels showed rearrangements. Conclusion: MLPA was able to identify one deletion among 9 patients with SC>60, that had previously been tested with sequencing. We conclude that the MLPA assay for detection of large rearrangements is of value in the laboratory workup of CF cases where one or no mutations have been identified by sequencing.

811/W

Surfactant-based Rapid DNA Extraction from Archived Blood Spots on Filter Paper for Molecular Analysis. *U. Bhardwaj[1], F. Mashayekhi[2], D.T. Kamei[2], E.R.B. McCabe[1,2,3].* 1) Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; 2) Bioengineering, Henry Samueli School of Engineering and Applied Science, UCLA, Los Angeles, CA, USA; 3) Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Dried blood spots (DBS) are widely used in neonatal screening for metabolic and genetic diseases in the United States and elsewhere. DBS on filter paper facilitate the collection, transport, and storage of blood samples for laboratory use. We present a new surfactant-based method to extract DNA from DBS that had been stored for over 10 years. Specifically, we tested the performance of the nonionic surfactants Triton X-100 and Triton X-114, which are comprised of a hydrophilic polyethylene oxide chain followed by a hydrophobic 4-(1,1,3,3-tetramethylbutyl)phenyl group. Various Triton X-100 and 114 solutions were compared with the commonly used Chelex method regarding their ability to improve detection with polymerase chain reaction (PCR) primers for dystrophin exon 20. In our method, DNA was extracted from the 3 mm filter paper punch samples with solutions containing different concentrations of the two surfactants, and subjected to PCR amplification. The 5% Triton X-100 and 7% Triton X-114 solutions increased the yield of DNA relative to the Chelex-100 method. These results suggest that the surfactants enhance the ability to remove DNA from the paper by improving the wetting properties of the solution through altering surface and interfacial tensions. This rapid, simple, and inexpensive extraction method generated superior results from archived specimens compared with the standard method and may represent a useful tool in newborn screening and molecular epidemiologic studies.

812/W

Newborn screening for cystic fibrosis in the Czech Republic: systematic utilization of prenatal diagnosis since 1990 has decreased incidence of the disease. *M. Macek[1], M. Balascakova[1], A. Holubova[1], V. Skalicka[2], V. Vavrova[2], D. Zemkova[2], P. Kracmar[3], J. Camajova[1], T. Piskackova[1], F. Votava[3].* 1) Department of Medical Genetics, Charles University -UH Motol, Prague, Czech Republic; 2) Department of Pediatrics, Charles University - UH Motol, Prague, Czech Republic; 3) Department of Pediatrics, Charles University - UH Kral. Vinohrady, Prague, Czech Republic.

An early diagnosis of cystic fibrois (CF) is a favorable prognostic factor. Unless meconium ileus is present at birth, CF can be misdiagnosed by practitioners. Increase of the age at dg. (ADG; before 1998 /median 0.58 years/; 1999-2005 /1.2 years/; p = 0.036), due to the devolution of healthcare since 1993, led us to initiate a pilot CF newborn screening project (NBS; IRT/DNA/IRT; II/2005-XI/2006), covering ~62% of all newborns. Concentrations of immunoreactive trypsinogen (IRT) were measured in 76,438 Guthrie cards and their levels above the arbitrary cut off (75ng/ml) were found in 799 cases (1.05%). Positives were subjected to DNA testing using population specific *CFTR* panel with a ~84% detection rate. In total, 12 CF cases were identified and the median ADG was 37 days (range 26-54). Interestingly, we also diagnosed previously unrecognised CF in 3 older sibs. Fifty three IRT positive newborns with only 1 *CFTR* allele, were subjected to follow-up sweat testing. Thus far, 45 cases were negative (ie. unaffected heterozygotes), while in one instance a borderline result requires long-term monitoring. When using NBS data alone the incidence of CF was rather low (1: 6,369), compared to the previously (from early sixties) epidemiologically established value of 1:2,700. However, when prenatal diagnosis data from within the study period were taken into account incidence of CF has increased to 1:3,900. Overall, our study proved that NBS is a feasible and efficacious tool for uniform and early diagnosis of CF. The current lower incidence of the disease reflects systematic, population wide application of prenatal diagnosis (via compulsory ultrasound screening for fetal hyperechogenic bowel) since 1990. Supported by VZFNM00064203(6112).

813/W

Use of the genetic test for Factor V Leiden in practice and impact on patient management. *A.M. Laberge[1], B. Psaty[2], L. Hindorff[2], W. Burke[1,3].* 1) Institute for Public Health Genetics; 2) Cardiovascular Health Research Unit; 3) Dept of Medical History and Ethics; Univ. of Washington, Seattle, WA.

Genetic testing for disease predisposition is perceived as one of the potential benefits of the Human Genome Project. Factor V Leiden (FVL) is a common genetic variant associated with a predisposition for venous thromboembolism (VTE) and adverse pregnancy outcomes. The American College of Medical Genetics (ACMG) and the College of American Pathologists (CAP) have issued recommendations on who should be tested for FVL. This study describes the use of the genetic test for FVL in a large health system (Group Health). The medical charts of all 272 individuals tested for FVL in 2003 were reviewed. Preliminary results on 164 individuals show that the median age of individuals tested for FVL is 49 years (range 17-85 yrs). Male:female ratio is 1:2.6. Heterozygote status was identified in 21% of subjects; no homozygotes were identified. Most (77%) were tested as outpatients, whereas 20% were tested in acute settings. Family practitioners requested the test most frequently (39%), followed by general internists (18%), obstetricians (10%), hematologists (8%), neurologists (7%), and pulmonary specialists (4%). The test was done at the patient's request in 7%. Post-test counseling was done for only 2%. Pre-test counseling was not done. Testing was performed in the context of VTE in 42% of subjects, family history of VTE or FVL in 15% respectively, arterial thrombosis in 12%, and pregnancy outcomes (including fetal loss) in 15% of women subjects. When comparing test use in practice to the recommended clinical indications for testing, we observed that practice was in agreement with ACMG recommendations for 65% of subjects and with CAP recommendations for 51%. Patient management was modified after FVL testing in 20% of subjects. Modifications were considered but not implemented in an additional 11%. Modifications included length of treatment, use of prophylaxis, and management of other risk factors. These findings suggest that the uptake of a test for genetic predisposition in practice does not necessarily follow available recommendations or initiate changes in patient management.

814/W

Impact of Life Experiences of Individuals with Osteogenesis Imperfecta (OI) on Reproductive Decision Making. *M. Szybowska, C. Shuman, D. Chitayat, R. Mendoza-Londono.* Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, ON CANADA.

Osteogenesis imperfecta (OI) is characterized by decreased bone mineral density leading to fractures resulting from minimal trauma. The purpose of this study was to identify the factors that influence reproductive decision making in individuals with OI, their attitudes toward prenatal testing, and the extent of their knowledge about genetic counselling services. Adults with OI (N=174) were recruited via the Osteogenesis Imperfecta Foundation and completed a 26 item open/close-ended online survey and the Ferrans and Powers Quality of Life Index. 43% of participants stated they had children and 95% were diagnosed with OI prior to having their first child. The majority of individuals specified that their children were conceived naturally and 55% of pregnancies were planned. Participants stated that they did not want to transmit OI as the main reason for choosing not to have children. The most influential factors affecting the decision to have or not have children were: desire for children(57%), marital status(46%) risk of transmitting OI(46%), personal state of health(43%), and information from genetics health care professionals(HCPs)(21%). 54% of individuals stated that if they were pregnant today it would be important for them to know prenatally if the baby had OI, stating preparation(70%) as the main reason for this choice. There was no statistical difference between the total quality of life (QOL) scores of individuals with OI who had children versus those who had not. However, individuals with children had a statistically higher QOL on the family subscale ($p < 0.0001$). Also, those diagnosed in adolescence appeared to have a lower QOL. Surprisingly, only 39% of the participants had received genetic counselling. The results of this study highlight the need for HCPs to support both the physical and psychosocial needs of individuals with OI undergoing reproductive decision making. As well, care needs to be taken to ensure that these individuals receive accurate information about OI early in life so that they are equipped to make informed reproductive choices as adults.

815/W

Consumers' attitudes towards current and prospective reproductive genetic testing. *F.M. Hathaway[1, 2], E. Burns[3], H. Ostrer[1].* 1) Human Genetics Program, New York University School of Medicine, New York, NY; 2) New York University Clinical Cancer Center, New York, NY; 3) Stern College for Women at Yeshiva University, New York, NY.

Purpose: As our knowledge and abilities in molecular genetics continues to expand, so does our ability to prenatally detect certain conditions and traits. It is, however, unknown if this increase in knowledge and ability will be accepted by the consumers of genetic services. Our study gauges the consumers' opinion towards reproductive testing for diseases and enhancements. **Methods:** From the period of July 2006 until February 2007, every patient that came to the NYU Human Genetics Program for prenatal counseling was asked to participate in the survey prior to their initial visit with a genetic counselor. A total of 999 surveys were collected. Consumers were asked to indicate traits and conditions for which they would want reproductive testing. **Results:** The majority of respondents would elect to have genetic testing for mental retardation (75%), deafness (54%), blindness (56%), heart disease (52%), and cancer (51%). We found consistency in respondent's reaction to testing depending on the degree to which the condition was life-altering. Of those who would want testing for heart disease, approximately 88% would also want testing for cancer ($p \leq 0.001$). Similarly, of those that wanted testing for blindness, 99% would also have testing for deafness ($p \leq 0.001$). A small group indicated interest in prenatal genetic testing for enhancements, such as superior athletic ability (10%) and superior intelligence (12%). Although most respondents did not desire testing for enhancements, few respondents were able to identify what specific restraints on genetic testing should be put in place. **Conclusion:** Our study suggests that consumers, in one medical genetics practice, desire more reproductive genetic testing than what is currently offered. Their selection of tests, however, suggests self-imposed limits on testing for diseases and enhancements. In addition, this study found that the majority of consumers would continue with reproductive testing knowing that it might reveal information about themselves.

816/W

Molecular Diagnostic Testing for Retinal Diseases. *A.J. Karoukis[1], K. Branham[1], L. Chen[1], R. Aaatre-Keshavamurthy[1], K. Downs[1], R. Caruso[2], J.R. Heckenlively[1], R. Ayyagari[1].* 1) Ophthalmology , University of Michigan, Ann Arbor, MI; 2) National Eye Institute, National Institutes of Health, Bethesda, MD.

Retinal dystrophies are a phenotypically and genotypically heterogeneous group of diseases that are inherited in autosomal dominant, recessive, X-linked, mitochondrial and complex modes. The phenotype of these diseases covers a broad and overlapping spectrum of clinical signs and symptoms. We have provided molecular diagnostic testing to patients with retinal conditions associated with mutations in the genes ABCA4, ELOVL4, EFEMP1, RDS, Bestrophin, TIMP3, CRX, CTRP5 and RPE 65. Mutation analysis was carried out by sequencing all coding exons and flanking intronic sequence. A total of 501 diagnostic tests were performed and the molecular basis of disease was identified in 227, while the disease associated mutations were not detected in the rest. Segregation of mutations was confirmed by analyzing 49 samples of parents and/or siblings. Part of this data was presented in an earlier publication. Ophthalmic molecular diagnostic testing is still in its infancy. We will present case studies to illustrate benefits and limitations of diagnostic testing including the value of genetic counseling and patient education prior to ordering testing.

817/W

Genetics Home Reference Information Rx Program. *S.M.M. Selmer[1], S.C. Calvo[2], M.L. Cheh[2], J.A. Mitchell[3].* 1) Lockheed Martin Corp., Rockville, MD; 2) Lister Hill National Center for Biomedical Communications, National Library of Medicine, Bethesda, MD; 3) University of Utah, Salt Lake City, UT.

Genetics Home Reference (GHR) is a consumer-friendly web site (http://ghr.nlm.nih.gov/) from the National Institutes of Health that seeks to educate patients, health professionals, and the general public about human genetics. Since 2003, this online resource has provided reliable information about genetic conditions and the gene or chromosome variations that contribute to those conditions. The public is increasingly looking for health information online, and GHR continues to address consumers' need for clear, easy-to-understand information about the expanding field of human genetics. GHR's latest initiative, the Information Rx program, helps healthcare professionals point their patients to accurate, dependable health information on the Internet. Under this program, free Information Rx pads enable doctors and nurses to write "prescriptions" that direct patients to the GHR web site for an explanation of genetic disorders and related topics. Healthcare professionals can visit the Information Rx Store (http://www.informationrx.org/) to request supplies at no cost. The GHR Information Rx program is particularly relevant for families of infants undergoing newborn screening. GHR has recently added specific information about each of the 29 conditions recommended for newborn screening by the Health Resources and Services Administration (HRSA). The GHR Information Rx program can link new and prospective parents with consumer-friendly information about any of these conditions. Since the program's inception in late 2006, healthcare professionals have ordered more than 1,100 Information Rx pads to educate their patients about genetics topics. Evaluation of the GHR Information Rx program is planned with the families of patients diagnosed through newborn screening.

818/W

Development of a customized genetic information manual. *K. Christensen[1,2], K. White[1], S. Haga[3], B.C. Burke[4], K. Zonno[4], L. Tuttle[5], L. Wise[1], S.F. Terry[1].* 1) Genetic Alliance, Washington, DC; 2) Univ. of Michigan School of Public Health; 3) Institute for Genome Sciences and Policy, Duke University; 4) New England Public Health Genetics Education Collaborative; 5) NERGG, Inc.

As genetics is increasingly integrated into healthcare, it is important that healthcare providers understand basic genetics, newborn screening, genetic diseases, and genetic services and be able to present these topics to their patients clearly. This information is most useful if it is customized for the users' state or region: they should have information about community-specific resources to allow rapid and accurate referrals. "Understanding Genetics: A Guide for Patients and Health Professionals," is a manual about genetic services customizable for any state or region. Given the need to refer individuals, the guide includes culturally sensitive information on local newborn screening programs, patient stories, and local resources. The first version of the manual was customized for the needs of underserved populations in Washington, D.C. To begin, the authors reviewed genetics materials from a broad range of organizations. The next step consisted of an informal needs assessment of area healthcare providers. We also conducted focus groups at Bread for the City, a nonprofit organization that provides vulnerable residents of Washington, DC with comprehensive services, about what they knew or thought about genetics and the types of materials they found helpful. The manual was created and critiqued by an Advisory Council consisting of leaders in medical genetics, public health, consumer advocacy and health education. The DC Dept. of Health distributed the manual to area health providers in DC community health clinics. The six member states of the New England Public Health Genetics Education Collaborative (a subcommittee of NERGG, Inc.) worked together and with Genetic Alliance on a customized version of the guide that incorporated information on newborn screening, community health resources, consumer fact sheets, and personal health stories from all six states. This version appears on the websites of Genetic Alliance, NERGG, and the departments of health of the six states, as well as in print.

819/W

Wikigenetics. *A.M. Chappelle[1], Y. Konno[1], K. White[1], P.F. Terry[2,3], K. Battle[4], K. Christensen[1,5], S.F. Terry[1].* 1) Genetic Alliance, Washington, DC; 2) Genomic Health, Inc; 3) PXE International; 4) Tucker Capital; 5) Univ. of Michigan School of Public Health.

The public needs credible and up-to-date information on human genetics. It is difficult for any one entity to provide a comprehensive overview of genetics that retains currency and is understandable by the lay public. In addition, though entities such as Wikipedia have tried to provide a comprehensive overview of scientific topics, they have found that the culture of professional science does not encourage collaboration and sharing of information in an open access forum. WikiGenetics (wikigenetics.org), a web-based encyclopedia of human genetics, allows the lay public to obtain quality, current information on human genetics. A professional advisory committee comprised of experts in genetics, genomics, policy, education, and advocacy developed the policies, principles and structure for WikiGenetics, with the dual purpose of maintaining its quality and keeping its literacy level appropriate for the lay public. These committee members have encouraged their fellows, students, and/or staff to create and edit articles. The information from the lay publication, Understanding Genetics: A Guide for Patients and Health Professionals, was used as a basis for the Wiki, since it was well vetted and its literacy level was appropriate for the lay public. Additionally, experts are invited to author various articles in order to keep the content areas balanced. Similar to other Wikis, both invited and self-identified volunteers proctor WikiGenetics to keep its quality high. The 10,000 organizations in Genetic Alliance's network were notified about and encouraged to use WikiGenetics. WikiGenetics is linked to related articles in Wikipedia so that people looking for information on genetics can easily find WikiGenetics. By recognizing users as stakeholders, WikiGenetics will generate collaboration among all members of the genetics community to result in a current, accurate, and accessible resource.

820/W

The Geneticist-Educator Network of Alliances (GENA) Project: An NSF-sponsored Math and Science Partnership Grant to ASHG. *K. Shaw[1], K. Van Horne[1], D. Marsland[2], H. Milne[2], T. Horn[3].* 1) ASHG, Bethesda, MD; 2) National Science Resources Center, Washington, DC; 3) National Association of Biology Teachers, Reston, VA.

The Geneticist-Educator Network of Alliances (GENA) Project will provide the partnering scientific societies involved with tools to instruct, facilitate and measure the meaningful engagement of science, technology, engineering and mathematics (STEM) faculty members in secondary science education. The GENA Project is exploring ways that an ASHG-sponsored secondary science education outreach effort can play a positive role in the career development of both junior (pre-tenure) and senior (post-tenure) level genetics faculty. Exemplary inquiry-based educational materials in genetics will be utilized to design methods to facilitate meaningful interactions between scientists and their local education community. Development of a network of geneticist-educator alliances will be used to design teaching strategies relating to standards and misconceptions in genetics that can to decrease time required for scientists to prepare for outreach, thus maximizing the effective and meaningful interaction between the geneticists and students. To date, 13 geneticist-educator alliances have been selected and trained as part of this program. Over the next two years the GENA project will recruit another 80 geneticists to participate and assist in the development of this model program that will become an integral part of the strategic development plan for the education efforts of both ASHG and GSA, thus making K-12 education outreach a truly systemic aspect of society activities.

821/W

Development, Evaluation, and Use of a Genetic Literacy Concept Inventory for Undergraduates. *B.V. Bowling, E.E. Acra, C.A. Huether.* Dept of Biological Sciences, Univ Cincinnati, Cincinnati, OH.

There is continued emphasis on increasing and improving genetics education for grades K-12, medical professionals, and the general public. An additional critical audience is the undergraduate student in introductory biology and genetics courses. There has been little effort to assess these students' understanding of genetics concepts and their level of genetic literacy (i.e. genetics knowledge as it relates to and impacts their lives). We have developed, evaluated, and used a new survey instrument to assess the genetic literacy of undergraduate students taking introductory biology or genetics courses. The Genetic Literacy Concept Inventory (GLCI) is a 31-item multiple choice test that addresses 17 concepts identified as central to genetic literacy by a team of ASHG professional geneticists. The items were selected and modified based upon reviews by 25 genetic professionals and educators. The inventory underwent additional review in student focus groups and pilot testing. Analysis was carried out on content validity, discriminate validity, internal consistency, and stability of the inventory, with results indicating it is reasonably valid and reliable. The GLCI has been utilized pre-course and post-course in six introductory non-major biology and genetics courses, with over 350 students taking the inventory. Current data from students in introductory biology courses show a pre-course average of 41% correct. Post-course scores increased only modestly to an average of 48% in these courses which emphasized genetics to varying degrees. Even in an introductory genetics course the pre-course average of 54% increased to only 59%. These results are consistent with similar studies in physics and chemistry where concept inventories have been implemented in courses using more traditional teaching methods. This study directly enhances genetics education research by providing a valid and reliable instrument for assessing genetic literacy in undergraduate students. It also begins to look critically at current genetics education at the undergraduate level and suggests that to achieve genetic literacy, adjustments in the way we teach genetics may be necessary.

822/W

Health educators' likelihood of practicing public health genomics. *L.S. Chen, P. Goodson.* Department of Health & Kinesiology, Texas A&M University , College Station, TX.

Introduction: With the completion of the Human Genome Project, a new field, Public Health Genomics, which addresses the application of genomic discoveries into population health, emerges. While health educators are responsible for conducting genomic education and increasing genetic literacy for lay communities, it is unknown whether they are ready to be involved in this new field. Therefore, the purpose of this study is to examine health educators' likelihood to incorporate genomic information and technologies into their practice. Methods: We surveyed a nationwide sample of health educators regarding their likelihood of practicing public health genomics. A theoretical model, developed to predict their likelihood, was tested with the survey data utilizing Structural Equation Modeling analytical techniques. Results: From 1,607 surveys included in the final analysis, our sample is not very likely to practice public health genomics. The proposed model fit the survey data well (CFI = 0.961, RMSEA = 0.066), and suggested participants' genomic knowledge, attitudes, and self-efficacy were significantly and positively correlated to their likelihood of adopting genomic competencies. Conclusion: Although professional groups have advocated for the practice of public health genomics in recent years, health educators in our sample still exhibited little likelihood of incorporating this innovation. As genomic knowledge, attitudes, and self-efficacy were associated with intention to practice public health genomics, education efforts may successfully increase health educators' involvement in public health genomics.

823/W

Felix the Double Helix: Teaching Elementary Students about DNA. *H.D. Edwards[1], W.J. Introne[1], A.M. Garcia[1], T.C. Markello[1], H.M. Dorward[1], M.A. Kayser[1], D.M. Krasnewich[1], G.A. Gahl[1,2], M.A. Merideth[1,2].* 1) NHGRI, NIH, Bethesda, MD; 2) Intramural ORD, NIH, Bethesda, MD.

Recent evidence supports the theory that early science education in children improves their natural scientific and math abilities (1). Given the paucity of curriculum material for genetics education of elementary-age children, we have designed an interactive educational project to teach kindergarten through second grade students about DNA through the use of a life-size costume: Felix the Double Helix. The main goals of this community outreach project are to introduce elementary students to "science in action," and promote an interest in science The presentation, which lasts 30 minutes, incorporates the use of songs, a game and audience participation to meet 4 main teaching objectives: 1) What is DNA? 2) Where can we find DNA? 3) Why does Felix the Double Helix look the way he does? 4) How can we protect our DNA? The presentation is given in both English and Spanish to meet the needs of the primarily Spanish-speaking student population. Ultraviolet light beaded bracelets are distributed at the end of the program to reinforce the message about protecting DNA from sun damage by using sunscreen. Evaluation forms are given to the teachers and reviewed by the team to adjust the presentation based on their feedback . Continued development of curriculum to educate elementary school children will assist in meeting the goal of promoting science and math. Future plans for this project include finding optimal tools to assess the comprehension level of the children and expanding the program presentation materials to higher elementary school grade learning levels. 1) Gallenstein, NL. Engaging young children in science and mathematics. Journal of Elementary Science Education, 9/22/05.

824/W

Perceptions from Undergraduate Nursing Students Regarding Nurses' Competencies in Genetics and Genomics. *M. Floria-Santos[1], E.M.M. Santos[2], L.C. Nascimento[1], L.M. Alvarenga[1], M.F. Prearo[1], C.M. Cenzi[1], K.A. Calzone[3].* 1) University of São Paulo at Ribeirão Preto College of Nursing, Department of Maternal-Child Nursing and Public Health, SP, Brazil; 2) Cancer Hospital A.C. Camargo, SP, Brazil; 3) National Institutes of Health, National Cancer Institute, Center for Cancer, Bethesda, MD, USA.

Introduction: International health organizations have emphasized the importance of integrating genetics/genomics content into nursing curricula to prepare the nursing workforce now and for the future. This research aimed to increase Brazilian undergraduate nursing students' awareness about that importance, and to assess their perceptions regarding "Essential Nursing Competencies and Curricula Guidelines for Genetics and Genomics". Methods: This is a descriptive exploratory study with a quantitative approach. Competencies were translated into Portuguese, and a 6 point Likert scale was applied to each. Data were collected between March-October/2006. Students answered sociodemographic questions and scored each competence. Results: 221 responded, 33.7% first year students, 36.7% second; and 30.3% fourth; mean age was 21.38 yo; 94.1% women; 97.3% single, and 87.8% Caucasian. The 62% students who knew the meaning of genomics showed highest levels of concordance with the competencies. First year students reported more agreement with the competencies compared with fourth year, (25/28 competencies showed statistical differences). The most scored competencies related to knowledge and technology incorporation into nurse practice, and last was insurance providers/payers. Discussion: Differences among years can be attributed to nursing curriculum changes. Concordance level probably has cultural influences. Future research is needed to compare perceptions and identify needs among nurses worldwide.

825/W

Genetic medicine and physician assistants: Development of a web-based, case-driven educational program. *C.M. Goldgar[1], E. Harvey[2], C. Wolpert[3], K. Healy[4], K. Clarke[5], J.D. McInerney[2].* 1) University of Utah, Salt Lake City, UT; 2) NCHPEG, Lutherville, MD; 3) University of North Carolina, Greensboro, NC; 4) Midwestern University, Downers Grove, IL; 5) Towson, MD.

The explosion of information in genetic medicine holds immediate and future implications for all healthcare providers. Many physician assistants (PAs) already feel the impact of genetics in practice, but lack adequate training to apply genetic information effectively or to answer patient questions appropriately. In 2006, a group of PA educators received a grant from the National Coalition for Health Professional Education in Genetics (NCHPEG) to develop an interactive, case-driven, educational website for use by PAs, PA educators, and PA students.

Key learning objectives of the website include: 1) collecting/recording a family history in pedigree format, 2) recognizing "red flags" that signal a genetic contribution to disease, 3) accessing valid genetic resources, and 4) referring appropriately to genetic professionals.

Three interactive cases, all in primary care settings, illustrate common conditions that have a genetic component and are designed to reinforce basic genetic principles. PAs work thorough the cases as they would a typical patient encounter, with an emphasis on "thinking genetically." Additional components of the site complement the cases, but also introduce stand-alone genetic competencies-e.g., genetics primer, family history exercises, genetic testing modules, and teaching tools.

A pretest/post-test will collect data from clinical PAs who register for CME hours, and from PA students who pilot the program. The presentation will focus on the development and pre-testing of the project's genetic curriculum, with the expectation that the curriculum may be useful for genetics educators working in diverse settings. The website will be available to all PAs in fall 2007.

.

826/W

Identifying Gaps in Residency Training: Distressing Results from the "Dictation Station". *G.E. Graham[1], S. Langlois[2].* 1) Children's Hospital of Eastern Ontario and University of Ottawa, ON; 2) BC Women and Children's Hospital and University of British Columbia, Vancouver, BC.

Like most specialist physicians, Clinical Geneticists rely almost exclusively on letters to communicate our assessment, treatment and follow-up plans to other health care providers. Since we often write directly to patients to summarize complex and crucial information (an unusual practice in other medical disciplines), we depend on letters to a greater extent than most. Despite this, letter-writing skills are rarely "taught" in a formal sense and dictation skills are almost never taught. While there has been some attention drawn to written communication since the introduction of the RCPSC CanMEDS 2000 framework and the explicit identification of the Communicator role, most Canadian Medical Genetics residents are not being taught the principles of good consult letter writing and to our knowledge none are being taught effective dictation skills. In the context of an OSCE exam and with advance consent from participants, we asked 10 residents to dictate the physical examination on a fictitious patient with a recognizable syndrome using clinical measurements and a facial photograph. The tapes were transcribed verbatim and the transcripts scored by a clinical geneticist who was blinded to the identity and training level of the dictating resident. A senior clinical geneticist who was unaware of the study completed the same dictation to help establish criteria by which the resident's transcripts could be evaluated. We found the majority of residents to have poor dictation skills as measured by clarity, verbal fluency, organization, use of punctuation and efficiency (words/sec playback). There was only a loose correlation between the quality of the dictation as judged by these indicators and the training level of the resident, suggesting that residents do not acquire sufficient skills by practice alone. This pilot project, while performed in an artificial circumstance with a small number of residents, has convinced us that a formal study of resident dictation skills that includes a teaching intervention and pre-/post-intervention evaluation is warranted.

827/W

Methods of Educating the Next Generation in Genetics and Genomics Science. *S.E. Harding, V.L. Bonham, C.L. Easter, D.H. Lea, J. Witherly.* Education and Community Involv, National Human Genome Research Institute/NIH, Bethesda, MD.

The overall goal of this presentation is to describe two genomic science education programs developed by the National Human Genome Research Institute (NHGRI) for students and faculty. The NHGRI Education and Community Involvement Branch (ECIB), created in 2003, serves as a liaison between NHGRI and the public to inform the public of the latest advances in genomics. One of ECIB's main strategies is to reach out to high school and college faculty who have shown an interest in genetics and genomics but who have not yet integrated these topics into their curricula, as well as high school and college students who have shown an interest in science and genetics but have not yet determined their career path. To that end NHGRI established a Current Topics in Genomic Research Short Course in 1997 to engage students and faculty from underrepresented minority institutions to incorporate genomics into the curriculum and to expose students to genomic research careers. Over the past 10 years, 300 faculty and students from underrepresented minority and rural institutions have participated in the Short Course. ECIB also reaches out to students across the country with National DNA Day, a nationally recognized science education program aimed at high school students. NHGRI partners with ASHG, the Genetic Alliance and the National Society of Genetic Counselors to connect genetics professionals with science classrooms around the country. Through the use of educational materials, online resources and speakers, students learn about the latest advances in genetics, as well as ways they might get involved in the field. Beginning in 2005, high school students across the nation have been invited to take part in a live, on-line Chatroom staffed by NHGRI. In 2007 NHGRI staff received a 52 percent increase in questions from 2006 and responded to a total of 648 questions answered in the 10 hour period. In this presentation, the two programs will be described including the number of students and faculty reached; the number and type of institutions participating; results of evaluations indicating how information has been used by participants; and how these programs can be adapted.

828/W

The development and evaluation of a genetics concept inventory. *A.M. Hott.* Department of Biology, Southern Connecticut State University, New Haven, CT.

Modern science education reform includes the development of standards and recommendations for content as well as the development and evaluation of pedagogy, but demonstrates limited assessment of student knowledge. Student knowledge assessment is an important factor in measuring the scientific literacy of current students. Concept inventories have been developed and used for the past fourteen years to assess non-science major student conceptual understanding of a content area. Inventories have been developed in the fields of physics, astronomy, chemistry and biology. The development and evaluation of a Genetics Concept Inventory (GCI) based on the ASHG genetics content recommendations for non-science majors is presented here. A study of 130 tracked students of introductory biology for the non-science major resulted in a reliability estimate of 0.62 that is supported by a respected panel of genetics educators' revisions, no significant gender bias, and the ability of junior and senior biology majors to outperform the non-science majors. Pretest/Posttest comparisons show a significant increase in five of six genetics content areas as well as a 9% increase on the overall percent score for the instrument.

829/W

Impact of an Undergraduate Genetics Education Workshop on Faculty Participants and Their Students. *C.L. Moskalik, C.A. Huether.* Biological Sciences, University of Cincinnati, Cincinnati, OH.

The American Society of Human Genetics (ASHG) hosted a first-annual, full-day Undergraduate Genetics Education workshop at the 2006 Annual meeting in New Orleans, LA. Biology faculty at undergraduate institutions surrounding the New Orleans area and ASHG members whose primary role is self-reported as teaching were recruited to attend. The workshop presented teaching techniques and genetic information that can contribute to effective teaching of genetics to undergraduate students. Assessment of the workshop's immediate and long-term impact was an important goal. This was accomplished by 1) collecting anonymous feedback from participants at the end of the workshop and 2) collecting pre- and post-workshop survey data from those who attended. Pre-workshop data were obtained from 47 pre-registrants. Only 30 (64%) of these pre-registrants attended, and of these, 26 (87%) completed an anonymous paper-based survey following the workshop, which assessed its immediate impact. Eighty-one % (n=21) reported that their expectations for the workshop were met and 92% (n=24) reported they will implement some aspect of the workshop in their future teaching plans. Post-workshop data were collected at the end of the 2006-07 academic year to determine the degree of implementation of workshop material. Only 14 participants (47%) completed both the pre- and post-workshop surveys and these data are currently being analyzed to assess its longer-term impact. Additionally, data regarding its impact on student learning using a recently developed Genetics Literacy Concept Inventory have been collected from classes taught by four of the workshop participants.

Based on immediate, anonymous participant feedback and preliminary data analyses from the pre- and post-workshop surveys, the workshop was successful; it met participant expectations and nearly all participants reported intent to use some of the material in their own teaching. This is important for how the teaching of genetics and student learning might be improved, and as encouragement for future workshops. However, further analysis and additional data will be needed to fully assess the longer-term value.

830/W

Training Students to be the Teachers: Using Peer Led Team Learning to Instruct Undergraduate Students as Science Museum Docents. *T.C. Rosser[1], R.E. Pyatt[2], K.R. Powell[3].* 1) Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Dept of Pathology, Ohio State University, Columbus, OH; 3) Center for Behavioral Neuroscience, Georgia State University, Atlanta, GA.

From June 2004 through January of 2005, the Fernbank Museum of Natural History in Atlanta hosted the traveling exhibit The Genomic Revolution described as the most comprehensive presentation on the complex subject of genomics at that time. The museum is typically self-guided but because of the complexity of the topic and a functional lab within the exhibit, it was decided to staff The Genomic Revolution with a team of paid undergraduate docents. A docent (derived from the latin word docere meaning to teach) serves as a bridge between the museum and the attendees, acting as the face and voice of the collection. Our challenge was to create a training program covering genetic principles along with the communication and leadership techniques needed for their interpretation in a museum setting. While the course content was organized around the physical arrangement of the exhibit, the basic structure was modeled on Peer Led Team Learning (PLTL). The PLTL system uses small group sessions to allow students to workshop challenging questions as a unit outside of direct instructor intervention. Each group has a student leader who demonstrates knowledge of the material, has shown outstanding leadership, and possesses good communication skills. Instruction began with a general review of concepts presented in a lecture format. All further training consisted of a short introduction for each section by an instructor followed by a PLTL workshop on that subject. Evaluation of the training program was conducted through student self assessment after two weeks working on the exhibit floor. Students reported a gain of knowledge in most of the areas covered such as Genetically Modified Organisms or Cloning and a majority felt the PLTL discussions on these subjects were "very helpful" in their training as a docent. PLTL successfully served as a framework in which to instruct students in the scientific content and promote the synthesis of that information which are both necessary as a museum docent. .

831/W

Knowledge on heredity and genetics among Japanese. *A. Sakurai, Y. Yamanouchi, Y. Mori, R. Kawamura, T. Kosho, K. Wakui, T. Wada, Y. Sekijima, Y. Fukushima.* Dept Med Genet, Shinshu Univ, Matsumoto, Japan.

Thanks to our improvement of understanding on human genome, genome-based personalized (tailor-made) medicine, which provides idealized treatment and drug choice for each patient, is becoming realistic. In order to appropriately utilize such new medical technology, it is required that a meaning of genetic information is correctly recognized and handled by both medical professionals and general public. However, in Japan, education of human genetics has been pointed out to be insufficient both qualitatively and quantitatively. Even in a medical school, education of human genetics is not fully established. We performed questionnaire-based surveys to evaluate knowledge and impression of heredity and genetics among various population and professional groups such as general physicians, nurses, community health nurses, medical students, non-medical college students and elderly (mostly over 60 yrs) citizens who are not engaged in medical services. When asked knowledge of genetics-related terms such as "DNA", "gene" and "chromosome", percentage who answered "understand and can explain what it is" or "roughly understand what it is" were, ~50% among nurses, ~60-70% among newly-enrolled medical students, ~40-50% among newly-enrolled non-medical students, and ~30% among elderly citizens. The term "genome" was not well recognized and only 10% of nurses, 10% of non-medical students and 6% of elderly citizens answered that they understand what this word stands for. Terms related genetic medicine such as "genetic test" and "gene therapy" were further less recognized; 60-70% of non-medical college students and 60-80% of elderly citizens answered "have not heard such word" or "have heard but do not know what it means". Medical students well recognized those words but their conception was not always correct. For instance, about half of medical students thought gene therapy can prevent transmission of mutant gene from affected parent to offspring. In general, knowledge on human genetics is apparently insufficient among Japanese population even in medical professionals. It is urgently asked to establish standardized education of human genetics and improve genome literacy.

832/W

Developing genetic competency in undergraduate nursing students: Follow-up to a 2006 study. *L. Tribble.* Education Division, Greenwood Genetic Center, Greenwood, SC.

Background: The largest group of healthcare providers is registered nurses whose work allows a unique and holistic view of patients. In the 21st century of genomic medicine, nurses need to understand basic genetic concepts, to identify patients in need of genetic services through the collection of family histories, and to provide information regarding genetic testing.

Objective: To determine if the instructional content from a 3 hour nursing elective in human genetics for third year students was useful and encountered in students' senior level coursework and clinical experiences.

Methods:A six question survey was developed and posted on electronic Blackboard for access by 35 students who had completed a semester elective in human genetics in early 2006. Questions were designed to assess which instructional topics were identified by students as being encountered in their senior coursework and clinical rotations.

Results: Eleven of the 35 (31.4%) students completed the survey.

1. Students identified the topics of newborn screening, prenatal testing and family history as being the most useful in their current classes and rotations.

2. Students reported that genetic information proved useful in senior level courses, specifically obstetrics and gynecology, pediatrics, maternal/newborn nursing, medical-surgical nursing,and in clinical work.

3. Participants indicated an opinion that genetics will play an important role in modern healthcare and expressed confidence in their ability to locate genetic services and information for patients.

Conclusion: Based on survey results, it is suggested that a specific course in human genetics at the undergraduate level is both needed and beneficial in professional nursing preparation. Curriculum should emphasize relevancy and application to nurses' work and attention should be noted to emphasize specific topics of genetic instruction.

833/W

Essay Contest Reveals Misconceptions of High School Students in Genetics Content. *K. Van Horne, K. Shaw.* American Society of Human Genetics, Bethesda, MD.

Multiple national educational organizations have called upon scientists to become involved in K-12 education reform. Whether involvement consists of sporadic interaction with students or more sustained partnerships with teachers, the engagement of scientists can take many forms. In this case, scientists from the American Society of Human Genetics, the Genetics Society of America and the National Association of Genetic Counselors have partnered together to organize an essay contest for seventh through twelfth graders as part of the activities surrounding National DNA Day. In addition to promoting genetics education in our middle and high schools and awarding students and teachers who excel in the life sciences, this contest has achieved a secondary goal of identifying student misconceptions in genetics, which may assist K-16 educators actively trying to identify potential barriers to student learning in their own classroom. Through analysis of the critical writings of almost 2,500 essays we have identified a variety of topics where there are large gaps in student understanding, including the broad categories of patterns of inheritance and genetic engineering. We have integrated this data into the Geneticist-Educator Network of Alliances (GENA) Project in order to develop learning cycles that address these specific misconceptions and potentially provide opportunities for successful interventions that will rectify student misconceptions in these areas. This work serves as a resource for others who are considering the use of an essay contest as a teaching or evaluation tool for students in their own discipline as well as a model for others to become engaged in science education reform.

834/W

Assessing Knowledge of Genetics by the United States Medical Licensing Examination (USMLE). *D.J. Waggoner[1], M.G. Blitzer[2], G. Feldman[3], M.S. Watson[4], R.E. Pyeritz[5].* 1) Univ Chicago; 2) Univ Maryland; 3) Wayne State Univ; 4) Am Coll Med Genetics; 5) Univ Pennsylvania.

American medical students and recent graduates take 3 Steps of the USMLE. Step 1 assesses understanding and application of sciences basic to the practice of medicine; Step 2 assesses patient care under supervision; and Step 3 assesses unsupervised medical practice. Four times in the past 12 years, most recently in May, 2007, representatives of the APHMG, ACMG & ASHG worked with the National Board of Medical Examiners (NBME), which creates and administers the USMLE, to assess the focus on genetics in each Step. Exams in 1995 had few questions that assessed knowledge of genetics, and most (2/3) were in Step 1. Genetics societies counseled the NBME, and medical geneticists volunteered and were selected for item-writing committees. Subsequent audits documented gradual progress in incorporating genetics questions. Currently, questions that address basic genetic principles or knowledge of hereditary disorders and congenital malformations were more frequent on all Steps, with the greatest increases on Steps 2 and 3. Importantly, even when a genetic term or disease was the incorrect answer (a 'distractor', which did not qualify the question as 'genetic'), it was much more relevant to the sense of the question compared to previous audits. NBME identifies in Step 1 'genetic' questions to report an average 'genetic score' for students at each medical schools. We independently confirmed the validity of all questions NBME classed as genetic, but also identified additional questions that could have been so classified. Assisting the NBME in classifying questions will improve the reliability and utility of the genetics performance report. When the content of the genetic questions was evaluated in reference to 2001 APHMG & ASHG curricular criteria for medical schools, certain areas were overrepresented (e.g., specific facts about diseases), and other areas were not assessed. We also identified needed revisions to the existing curricular guidelines. The NBME remains committed to working with genetics societies to improve the assessment of genetics education for medical students and interns.

835/W

The Charger Products Program, Practical Commercial Experience for Graduate Students in Biotechnology. *R.J. Zahorchak[1], L. Boyd[2], R. DuBreuil[3], T. Moore[3], H. Zappe[4].* 1) Partnership for Biotechnology Research, Huntsville, AL; 2) University of Alabama in Huntsville, Huntsville, AL; 3) Open Biosystems, Inc., Huntsville, AL; 4) Nektar Therapeutics, Huntsville, AL.

The Charger Products program is a unique, experientially-driven, educational program currently designed to offer an alternative funding opportunity for select Ph.D. graduate students and provide them with hands-on experience in the varied aspects of biotechnology product development, manufacturing, and marketing in a challenging, supportive business environment. The program is sponsored by the Partnership for Biotechnology Research (PBR) and seed funding has been obtained through grants from the Hudson Alpha Institute for Biotechnology and the Alpha Foundation. The program is currently a partnership between PBR, the University of Alabama in Huntsville and Open Biosystems, Inc. Students in the program deal directly with the challenges of converting "proof of concept" technologies into viable products in the biotechnology marketplace. All individuals who participate in the program are part of an application-friendly educational experience that will expand their realm of future employment opportunities and facilitate their more rapid integration into future employment situations in the commercial sector. Most of the products in development fill needs in genetics and cell development research or education. The revenues of the products manufactured and sold by Charger Products will feed back into the program to help support existing and new assistantships. Charger Products was established approximately two years ago and already has developed and launched one product, the Leopard Transfection Array (LTA v1.0) which is sold through Open Biosystems, Inc. Improvement of this product is ongoing. Two other products in development involve the development of stable shRNA cells line arrays for cancer and cell development research and educational kits for middle school, high school and college genetics and biotechnology laboratories.

836/W

Did They Start The Conversation? An Evaluation of the NSW Family Health History Campaign. *K.K. Barlow-Stewart, K. Dunlop.* Centre for Genetics Education, NSW Health, Sydney, NSW, Australia.

Internationally campaigns promote collection of family health history (FHH) by patients. The NSW Health FHH campaign, August 2006, encouraged the community to discuss their FHH with their family and take the recorded information to their doctor. Development included community poll surveys, consumer and General Practitioner (GP) consultations, website development and general media preparation. GPs throughout NSW were informed prior to the campaign through their professional affiliations and articles in the medical media. Posters, pads with tips for collecting FHH and a simple chart Your Family Health History Record were distributed to all NSW GP practices. The limited, small budget media campaign targeted women 40-60 years. It included newspaper and magazine articles, radio interviews, a television interview on Australia's most popular breakfast show and promotion of the website. Evaluation methodologies: mail survey of GPs (response 135/606); poll survey (400 community members) and analysis of enquiries, website and media coverage. 28% (112) of the community randomly polled had seen or heard of the campaign; of these 48% reported discussing FHH with family members as a result; 35% had initiated a discussion with their doctor about their FHH. Website activity peaked immediately post the campaign (August: 324 visits) with particular interest in the FHH Record. 30% of responding GPs had heard of the campaign; of these 66% reported an increase in the number of patients who told them about their family history since the campaign. GPs reported the importance of patients finding out about their own FHH information stating that "I feel much more confident about the information I am being given when I ask about their family history" and "It made me realise that they may not know when I ask them about their FHH". GPs further commented on the benefits of the simplicity of the FHH record and some had made referrals as a result. While awareness of the campaign itself was limited, for those who were aware, the message had a significant impact. The findings support moving towards a national FHH campaign working with both GPs and the community.

837/W

Development of a community-based education initiative for genomic medicine. *V.C. Henrich[1], C. Christianson[1], K. Potter-Powell[1], S. Estabrooks Hahn[2], L. Evans[1], D. Bartz[1], T. Roxbury[1], S. Blanton[2], P. Lietz[3], J. Vance[2], M. Pericak-Vance[2].* 1) Ctr for Biotech, Genomics, and Health Research, University of North Carolina-Greensboro, Greensboro, NC 27402; 2) Miami Institute for Human Genomics, University of Miami, Miami FL 33136; 3) Moses Cone Health System, Greensboro, NC 27401.

The success of utilizing genomic medicine in the community health setting will depend on an informed interaction between the patient and their health care provider followed by appropriate interventions, as needed. This requires the understanding by these two groups that family history is essential for risk assessment, this risk can be reduced through medical intervention and lifestyle changes, and genetic testing is appropriate for a subset of people. As the basis for developing a community education program surrounding these concepts, the Guilford Genomic Medicine Initiative (GGMI) conducted 3 physician focus groups and 13 community focus groups to ascertain gaps in knowledge, perceptions, and attitudes related to genomic medicine. Physicians reported that they collect some family history information, but it is often incomplete. They also expressed concerns about the lack of guidelines for follow-up of at-risk patients and genetic services. To further assess community knowledge, attitudes, and awareness, the findings from the community focus groups were used to produce a telephone survey for residents in Guilford County, NC. Collectively, the focus groups and survey identified gaps in knowledge and misconceptions that highlighted the need for complementary learning objectives for each of the target audiences. For genomic medicine to be successfully integrated into health care, community members must be educated in basic genetics, understand the importance of family history and know what information to collect, patient education materials must address misconceptions and concerns, and physicians must have clear recommendations for risk assessment and evidence-based guidelines regarding follow-up.

838/W

Disease InfoSearch includes a Portal to National Library of Medicine Databases. *H. Ferguson[1], K. Puchir[1], K. White[1], J. Ostell[2], S. McDaniel[2], J. Coleman[2], L. Forman Neall[2], C. Falco[1], A. Krokosky[1], H. Travers[1], S.F. Terry[1].* 1) Genetic Alliance, Washington, DC; 2) NCBI, NLM, Bethesda, MD.

Accessing accurate information about genetic conditions has become increasingly difficult. Patients and their families are often unfamiliar with medical terminology and scientific literature. It may be difficult to ascertain what constitutes genetic disease-specific expertise. Healthcare providers may find medical and scientific information lacking reliability, either because it is based on out-dated information or because the available case studies do not conform to the higher ARHQ standards of evidence. Further, time constraints limit the abilities of healthcare providers to do extensive searches. Genetic Alliance's Disease InfoSearch (DIS)is an online search tool providing links to clinical information for many genetic conditions. Each entry includes detailed information about advocacy and support groups and provides updates on management and treatment when available. All of the information is deposited into DIS by the condition-specific advocacy organization. Aggregating information vetted by their professional advisory boards creates links to accurate information. These experts review the literature for their condition and recommend the most current quality information. In collaboration with the National Center for Biotechnology Information (NCBI), Genetic Alliance expanded DIS to include a portal to the National Library of Medicine (NLM) databases. A customized search targeting the user's condition directly from DIS uses backend filters to display NLM resources on a portal page. This portal organizes information so that a broad spectrum of users can drill directly into the NLM databases at the most appropriate level. A feature, MyNCBI, which allows users to track emerging information over time through a notification system, is highlighted for DIS users on the portal. The combined expertise of Genetic Alliance's advocacy organizations members and NCBI's staff make DIS an invaluable source of information for newly diagnosed individuals, their caregivers, and healthcare professionals, thereby improving the lives of those with genetic disorders.

839/W

Leveraging community resources through a repository. *K. White, K. Puchir, L. Wise, S.F. Terry.* Genetic Alliance, Washington, DC.

High quality, well-vetted resources are valuable to advocates, professionals, policymakers and federal agency officials. Finding useful resources in a timely manner and centralizing them for open access can be a difficult endeavor. Genetic Alliance launched a robust document repository service (www.resourcerepository.org) in 2007 that aggregates the combined resources of advocates, healthcare professionals, government agencies, think tanks, and other contributors. These resources cover a wide range of topics such as fundraising, FDA genetic testing guidances, advocacy at the state and federal level, media strategies, and clinical trials. The Repository contains all of the common file formats, including PowerPoint presentations, Word documents, and PDFs. Examples of shared resources include meeting presentations, how-to-guides, case studies, monographs, white papers, and position papers. The Repository allows visitors to browse collections in categories such as genetics services; ethical, legal, and social issues; organization development; public policy; and communications. Visitors can also track new content tailored to specific interests on a daily, weekly, or monthly basis; view the newest resource added to the Repository, and access the most frequently downloaded resources. The software allows individuals to contribute documents in a few simple steps. Documents are held and reviewed by an editorial team consisting of experts in genetics, electronic document storage, education and information archiving and retrieval. Documents are published, tagged with keywords, and a succinct abstract. Metrics are available for download.

840/T

Significant Locus Heterogeneity in Turkish Families with Autosomal Recessive Nonsyndromic Sensorineural Hearing Loss. *M. Tekin[1], H. Ozdag[2], A. Sirmaci[1], F.B. Cengiz[1], I. Aslan[1], S. Tasir-Yilmaz[2], D. Duman[1], B. Ozturk-Hismi[1], Z.S. Arici[1], A. Incesulu[3], S. Erbek[4], I. Yilmaz[5].* 1) Division of Clinical Molecular Pathology and Genetics, Department of Pediatrics, Ankara University School of Medicine, Ankara, Turkey; 2) Biotechnology Institute of Ankara University, Ankara, Turkey; 3) Department of Otorhinolaryngology, Osmangazi University School of Medicine, Eskisehir, Turkey; 4) Department of Otorhinolaryngology, Baskent University Hospital, Konya, Turkey; 5) Department of Otorhinolaryngology, Baskent University Hospital, Adana, Turkey.

Ninety-five percent of individuals with early-onset genetic deafness demonstrate autosomal recessive transmission in Turkey. Although mutations in GJB2 are responsible in 22% of all deaf families, their frequencies are much lower in affected subjects with consanguineous parents. In this study, we screened 5 large families having parental consanguinity with nonsyndromic sensorineural hearing loss for known autosomal recessive deafness loci. All families were tested and found to be negative for GJB2 mutations. Affymetrix GeneChip 10K or 50K arrays were used for genotyping. Homozygous genotype blocks flanking the known recessive deafness genes were explored in affected subjects. Additional microsatellite markers were also used. Two and multipoint linkage analyses using easyLinkage software package were later performed. Genomic loci for TMIE, CDH23, and MYO15A genes were shown to be linked in single families. A previously reported p.R84W (c.250C>T) mutation in TMIE, and a novel, c.3595-13C>T, mutation in CDH23 were demonstrated to be co-segregating with deafness as a completely penetrant autosomal recessive phenotype in each family. The c.3595-13C>T mutation in CDH23 was predicted to alter the binding of a splicing enhancer protein and was not found in 125 healthy Turkish controls. All known deafness genes were excluded in the remaining 2 families based on heterozygous genotypes of flanking SNPs or microsatellites in affected subjects. These results demonstrate that the etiology of autosomal recessive deafness is remarkably heterogeneous in Turkish families with parental consanguinity.

841/T

Characterization of Pathogenic Huntingtin Fragments. *L.R Smith[1,2], S.H Li[1], X.J Li[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA.

Huntington's Disease (HD) is a dominantly inherited, late-onset neurodegenerative disorder characterized by the expansion of a polyglutamine (polyQ) repeat located in the N-terminal region of the huntingtin (htt) protein. Wild-type htt consists of less than 36 glutamine repeats whereas the mutant version of the protein has an expanded polyQ repeat of greater than 37 glutamines. Mutant htt shows nuclear accumulation and affects gene transcription whereas wild type htt largely remains cytoplasmic. It is evident that full-length htt is cleaved to a number of N-terminal fragments containing the polyQ domain. Previous studies have shown that small N-terminal htt fragments enter the nucleus more easily than full length htt. The size of the N-terminus of the pathogenic fragments that are able to accumulate in the nucleus, however, is unknown. To address this issue, we have generated various truncated mutant and wild type htt constructs with varying N-terminal lengths (212, 300 and 500 amino acids) that are tagged at the C-terminus with an HA tag. Using immunocytochemistry and nuclear fractionation methods, we have analyzed the localization of these various constructs as compared to the shorter N-terminal exon 1 fragment consisting of 67 amino acids. We have found that the small N-terminal htt fragments less than 212 amino acids are likely to accumulate in the nuclei. Characterization of the nuclear localization of these htt fragments will elucidate how the size of htt fragments influences their nuclear localization and nuclear effect on gene transcription.

Supported by NIH grants NS 045016 and NS41669.

842/T

Genome-wide screen for Aicardi-Goutieres-like microcephaly syndrome suggests a molecular etiology distinct from Aicardi-Goutieres syndrome. *K.A. Aldinger[1], A. Rajab[3], M.E. Ross[4], W.B. Dobyns[2].* 1) Committee on Neurobiology and; 2) Departments of Human Genetics, Neurology and Pediatrics, The University of Chicago, Chicago, IL; 3) Genetic Unit, DGHA, Ministry of Health, Muscat, Sultanate of Oman; 4) Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, NY.

Background: Several autosomal recessive syndromes with intracranial calcifications in children are known including Aicardi-Goutieres (AGS), Cree encephalitis, Coats-plus and pseudo-TORCH syndromes. All four share early onset leukoencephalopathy, basal ganglia and white matter calcifications, and brain atrophy mimicking a CNS infection. Four AGS loci have previously been identified on 3p21 (AGS1), 13q14-21 (AGS2), 11q13 (AGS3), and 19p13 (AGS4), with Cree encephalitis allelic to AGS1. We have identified an AGS-like microcephaly syndrome (AGSMIC) that consists of microcephaly, short stature, variable mental retardation, and progressive motor deterioration in late childhood. Testing found diffuse basal ganglia and white matter calcifications, osteoporosis, normal fundi and normal TORCH titers; CSF has not been examined. **Objective:** To test the hypothesis that AGSMIC and AGS share similar genetic etiologies. **Methods:** We performed a genome-wide screen in a three-generation Omani family with a consanguineous first cousin marriage and four children affected with AGSMIC using the Affymetrix 50K XbaI SNP chip, followed by homozygosity mapping. We used WebQTL to determine whether expression of AGS-causative genes (*Trex1, Rnaseh2a, Rnaseh2c*) in mouse whole brain and striatum correlates with expression of genes within our 2q locus. Finally, we sequenced candidate genes to identify causative mutations. **Results:** Our results demonstrate the most significant evidence for linkage under an autosomal recessive model for AGSMIC on chromosome 2q35-36.3 (LOD = 3.01 at 215-229 cM), and moderate evidence for linkage at a second locus on 12q15-21.3 (LOD = 2.02 at 90-91 cM). Homozygosity mapping restricted the 2q locus to 9.5 Mb containing 93 known genes, and the 12q locus to 14 Mb containing 38 known genes. The AGS1-4 loci were excluded. Using WebQTL we identified 5 genes on 2q with correlated expression level of AGS-causative genes, but no causative coding mutations were found. **Conclusion:** We report a novel AGS-like phenotype and the first evidence for linkage suggesting that this disorder is genetically distinct from AGS.

843/T

ATTCT repeat interruptions in Brazilian patients with SCA10. *I. Alonso[1], T. Almeida[1], L.B. Jardim[2], O. Artigalas[2], M.L. Saraiva-Pereira[2], T. Matsuura[3], J. Sequeiros[1,4], I. Silveira[1].* 1) UnIGENe - IBMC, University of Porto, Porto, Portugal; 2) Hosp. Clínicas de Porto Alegre, Porto Alegre, Brasil; 3) Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan; 4) ICBAS, University of Porto, Porto, Portugal.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant neurodegenerative disorder caused by the expansion of an $(ATTCT)_n$ located in intron 9 of the *ATXN10*, a gene of still unknown function. SCA10 was first described in Mexican families presenting with cerebellar ataxia and seizures. Afterwards, Brazilian families were also described presenting spinocerebellar ataxia, but without seizures. We have previously described the presence of an expansion of the polymorphic ATTCT repeat, responsible for SCA10, in two Brazilian families presenting spinocerebellar ataxia without seizures. In these families we detected reduced penetrance alleles of 360-370 repeats, in elderly asymptomatic subjects. To investigate a previous hypothesis of interruptions in the $(ATTCT)_n$ tract functioning as a disease modifier, we assessed the interruption motif in an additional family with ataxia and seizures. By a modified PCR technique an abnormal discontinuous ladder, exceeding the range observed for normal alleles, was detected in this Brazilian family, presenting progressive cerebellar ataxia with associated seizures and onset during or after the 3rd decade of life. This suggested the presence of interruptions within the ATTCT expansion. Comparison of the expanded ladder pattern detected by modified PCR with the previously described suggests that this interruption is located more close to the 5' end of the repeat expansion, probably having 40-50 bp, followed by an additional stretch of ATTCT motif and a new interruption. We are now cloning the larger PCR products, obtained by the modified PCR technique for sequencing of the interrupted alleles and identification of the interruption motif present in this family.

844/T

Homozygous silencing of the T-box transcription factor TBR2/EOMES locus results in a microcephaly syndrome with polymicrogyria and corpus callosum agenesis. *L. Baala[1,2], S. Briault[3], H.C. Etchevers[2], F. Laumonnier[3], A. Natiq[1], J. Amiel[2], N. Boddaert[4], C. Picard[5], A. Sbiti[1], A. Asermouh[6], T. Attié-Bitach[2,7], F. Encha-Razavi[2,7], A. Munnich[2,7], A. Sefiani[1], S. Lyonnet[2,7].* 1) Département de génétique médicale, INH Rabat, Morocco; 2) Genetique INSERM U781, Hosp Necker, Paris 15, France; 3) INSERM U-619 Faculté de Médecine, Tours, France; 4) Service de Radiologie Pédiatrique, Hôpital Necker-Enfants Malades (AP-HP), Paris, France; 5) Centre d'étude des Déficits Immunitaires, Hôpital Necker-Enfants Malades (AP-HP), Paris, France; 6) Hôpital d'Enfants Avicenne, Rabat, Maroc; 7) Université René Descartes - Paris 5, Paris, France.

Mechanisms regulating brain size during neurogenesis include the regulation of neural progenitor proliferation and migration. We report a large consanguineous Moroccan family with a marked prenatal-onset microcephaly (mean occipito-frontal circumference at birth -4 SD) and severe motor delay with hypotonia in 4 affected children. Early lethality was observed in 3 children (death at 15-18 months of age), due to respiratory distress following chronic infections. The surviving child has had a persistent fever since birth. Recurrent infections have been noted. This autosomal recessive microcephaly syndrome was co-segregating with a homozygous balanced translocation between chromosomes 3p and 10q. The translocation was found at the homozygous status in all affected individuals (46,XY,t(3;10)(p24;q23)2x), while unaffected parents were heterozygous. We established a fine physical mapping and cloned the breakpoints on 3p24 and 10q23. Interestingly, neither of the two translocation breakpoints disrupted a known or predicted gene coding sequence. However, we showed that a position effect at the breakpoint on chromosome 3 silences the Tbox-brain2/Eomesodermin (TBR2/EOMES) transcript. Together with its expression pattern in the developing human brain, our data suggest an involvement of TBR2/EOMES in neuronal division and/or migration. Thus, mutations in not only mitotic and apoptotic proteins but also transcription factors may be responsible for malformative microcephaly syndromes.

845/T

Role of the polyproline region in aggregate size and subcellular localization of mutant huntingtin. *J.W. Bradford[1,2], J.Y. Shin[3], S.H. Li[1], X.J. Li[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; 2) Graduate Program in Genetics and Molecular Biology, Emory University School of Medicine; 3) Department of Neurology, UCSF, San Francisco, CA 94158.

Huntington's disease is the most common disease in a family of dominantly inherited neurodegenerative disorders caused by an expanded CAG/glutamine tract. An expansion of over 37 glutamines in the disease protein huntingtin results in the late onset of Huntington disease symptoms, including movement disorders, cognitive deficits, and eventually death. Polyglutamine expansion also causes huntingtin to misfold, aggregate, and abnormally accumulate in the nucleus. Following the polyglutamine tract in the N-terminus of huntingtin are two polymorphic polyproline regions. Polyproline regions have been characterized in many proteins. Proteins containing SH3 domains are found to interact with repeated proline regions. Thus, it is interesting to know whether the polyproline stretches play a role in the function of huntingtin. We have generated transfection vectors that express normal (23Q) and expanded (103Q) N-terminal human huntingtin with and without the polyproline regions. Upon transient transfection of HEK 293, PC12, and primary neuronal cell lines, we see a dramatic difference in aggregate size and subcellular localization between huntingtin with and without the proline regions. This finding suggests that the polyproline region may regulate huntingtin conformation to influence its aggregation and subcellular localization. Supported by NIH grants NS045016 and NS41669.

846/T

Examination of Candidate Genes for an Autosomal Recessive Syndrome of Epilepsy, Ataxia and Tremors. *A. Buhr[1], A. Daoud[2], A. Saadoon[2], S. Chen[1], R. Spiegel[1], H. El-Shanti[1].* 1) University of Iowa, Iowa City, IA; 2) Jordan University of Science and Technology, Irbid, Jordan.

STATEMENT OF PURPOSE: Gene discovery in epilepsy has been progressing rapidly in the past decade, but mutations are often found in single families with autosomal dominant epilepsy with very limited application of this knowledge to the sporadic forms. Autosomal recessive epilepsy has traditionally been resistant to gene mapping and identification because families are usually small and are not sufficient for linkage analysis. However, gene discovery in autosomal recessive epilepsy may provide insight into a new class of genes that play a role in sporadic idiopathic generalized epilepsy. We have identified a large inbred family with an autosomal recessive syndrome of ataxia, epilepsy and tremors. We mapped the gene to the pericentromeric region of chromosome 12 by homozygosity mapping. Within this region, we identified 10 to 15 candidate genes, but were able to exclude most of them by direct sequencing. METHODS Mutation detection in candidate genes was approached by direct sequencing of gene exons and splice sites and evaluation of identified variants by calculating population allele frequency of variants. We are currently exploring the role of mutational mechanisms other than point mutations in these candidate genes. RESULTS We selected the following outstanding candidate genes for our preliminary pass based on their function or their expression pattern: *ASB8, LRRK2, SLC38A2, SLC2A13, NELL2, FLJ20489, GLT8D3, ALG10, ALG10B*. Of the possible 117 exons to sequence, all but 16 were thoroughly completed. We found no etiologic mutations in those exons. We also examined *LRRK2* for exonic deletions or duplication. CONCLUSIONS We were able to preliminarily exclude the previously mentioned candidate genes from being responsible for the neurologic disorder in our family. These genes were evaluated for point mutations and small deletions or duplications, which we did not find. We are currently expanding our candidate gene list and exploring other mechanisms beyond point mutations and small deletions or duplications in these and other genes.

847/T

Coding sequence mutations of the GFAP gene, and variants of flanking regulatory regions, in Alexander disease patients. *I. Ceccherini[1], T. Bachetti[1], F. Caroli[1], R. Biancheri[2], D. Pareyson[3], R. Fancellu[3], L. Farina[3], G. Uziel[3], M. Savoiardo[3], M. Filocamo[2].* 1) Lab Molecular Genetics, G. Gaslini Inst, Genoa, Italy; 2) DPPM Lab & Dept Neurosciences, G. Gaslini Inst, Genoa, Italy; 3) Neurological Inst C. Besta Foundation, Milan, Italy.

Alexander disease is a progressive devastating leukoencephalopathy characterized by presence of Rosenthal fibers, which contain glial fibrillary acidic protein (*GFAP*), *a*B-crystallin and heat shock protein 27. Infantile, juvenile and adult forms of the disease, showing a progressively decreasing severity of the disease and more restricted MRI and pathologic abnormalities, are all associated with heterozygous mutations of the *GFAP* gene. It has been shown that *GFAP* overexpression may lead to intracellular inclusions, resembling Rosenthal fibers, and interfere with the proteasome-mediated degradation, thus suggesting that GFAP accumulation plays a crucial role in Alexander disease pathogenesis. We analyzed a panel of individuals, recruited according to MRI and clinical findings consistent with Alexander disease, for the 9 GFAP coding exons, finding a total of 22 mutations, either already reported in the literature or detected in our laboratory for the first time, in 21 unrelated patients half of whom affected by the adult form of the disease. In addition, we found a synonymous SNP allele (p.P47P) in 5 of these patients (11%), a proportion much higher than that reported in the general population (2.6%), likely reflecting a functional association with the disease development. To enlarge the mutational spectrum of *GFAP* in Alexander disease, we are focusing our investigations on a few individuals with suggestive phenotypes, mostly affected by adult and juvenile forms of the disease but carrying no *GFAP* mutations. In particular, both the 5' regulatory and the 3' untranslated regions are being sequenced to identify nucleotide variations that could enhance *GFAP* expression by interfering with either transcriptional regulatory pathways or *GFAP* messenger stability. Results obtained so far have only demonstrated heterozygosity for SNPs located upstream of the *GFAP* transcription start site (-986T>C, -1312T>C, -504T>A and -1926G>A).

848/T

Molecular and functional analysis of paraplegin gene (SPG7) mutations in patients with familial and sporadic spastic paraplegia. *D. Di Bella[1], C. Mariotti[1], M. Plumari[1], C. Gellera[1], F. Lazzaro[2], M. Muzi-Falconi[2], V. Fracasso[1], R. Fancellu[1], S. DiDonato[1], D. Pareyson[1], S. Baratta[1], F. Taroni[1].* 1) Div Biochem & Genetics, Fondazione IRCCS Istituto Neurologico C. Besta, Milan, Italy; 2) Dept of Biomol Sci & Biotechnol, University of Milan, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders. SPG7 mutations are responsible for autosomal recessive (AR) HSP with both "pure" and "complex" phenotypes. This gene encodes paraplegin, a component of mitochondrial mAAA metalloprotease. SPG7 was sequenced in 81 unrelated HSP patients [58 sporadic (S) and 23 AR cases]. A further group of 51 HSP patients (38 S and 13 AR) were screened for reduced paraplegin protein levels in lymphocytes. SPG7 gene was sequenced in the 7 patients exhibiting absence or severe reduction of paraplegin protein. The majority of patients presented a "complex" phenotype characterized by spastic gait and clinical and MRI signs of cerebellar involvement. Overall, pathogenic mutations (11 nonsense and 10 missense including A510V) were found in 19 sporadic patients and in 4 of the 36 familial cases but in none of 200 controls. Thirteen patients in the sporadic group (13.5%) and 4 in the familial group (11.1%) had mutations on both alleles. The remaining 6 sporadic patients carried a mutation on a single allele. Our study showed that: 1) analysis of paraplegin levels in lymphocytes from HSP patients is an efficient method for identifying mutated patients; 2) SPG7 mutations account for at least 13% (17/132) of HSP patients, an overall frequency higher than previously reported; 3) Western blot analysis demonstrated absence of paraplegin in patients carrying two null alleles and a severe reduction in patients with missense mutations including the A510V; 4) functional analysis of the mutant protein in a yeast cell system in which the human m-AAA is functionally reconstituted clearly indicated that the A510V, previously described as a polymorphism and more recently identified in numerous HSP patients (including 10 in our series) is a disease-causing mutation. [Partly supported by Telethon GUP04009 and Fondazione Mariani R0544 to FT].

849/T

A novel locus for an autosomal recessive form of hereditary spastic paraplegia (SPG35) maps to chromosome 16q21-q23. *K.J. Dick[1], R. Al-Mjeni[2], W. Baskir[2], R. Koul[2], M.A. Simpson[1], M.A. Patton[1], S. Raeburn[2], A.H. Crosby[1].* 1) Medical Genetics, St. Georges, University of London, London, United Kingdom; 2) Sultan Qaboos University Hospital, Muscat, Oman.

The hereditary spastic paraplegias (HSPs) are a group of clinically and genetically heterogeneous neurodegenerative disorders in which the cardinal pathological feature is upper motor neuron degeneration leading to progressive spasticity and weakness of the lower limbs. To date, 14 autosomal recessive HSP loci have been mapped. We have identified a large consanguineous Omani family in which an autosomal recessive form of HSP is segregating. The age of onset of the condition varies from 6-11yrs and is associated with seizures in two individuals. Following exclusion of known ARHSP loci, we performed 250K gene chip SNP analysis of all affected individuals. All affected individuals shared a 20.4Mb (3.25cM) region of homozygosity located on chromosome 16q21-q23.1, defined by SNP markers rs149428 and rs9929635 (peak multipoint LOD score of 4.86) designated SPG35. Two candidate genes, dynein, cytoplasmic 1, light intermediate chain 2 (DYNC1LI2) and vacuolar protein sorting 4 homolog A (VPS4A) were sequenced but no disease causing mutations were identified.

850/T

Deletion of *Mecp2* in hypothalamic neurons results in obese, anxious and aggressive mice. *S.L. Fyffe[1], J.L. Neul[1], R.C. Samaco[1], H.T. Chao[1], S. Ben-Shachar[1], E.H. Goulding[3], E. Sullivan[3], L.H. Tecott[3], H.Y. Zoghbi[1,2].* 1) Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) University of California San Francisco, San Francisco, CA.

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). *Mecp2* null mice are hypoactive, tremulous, have weight abnormalities and die by 10 weeks of age. Mice bearing a truncated *Mecp2* allele (*Mecp2*^{308}) survive up to 15 months and display many neurological features of RTT including weight abnormalities, increased anxiety-like behavior and an abnormal stress response. Furthermore, *Mecp2*^{308} mice have increased levels of the MeCP2 target gene, corticotropin-releasing hormone, in the paraventricular nucleus. This led us to propose that MeCP2 regulates the expression of neuron-specific genes and that dysfunction of specific neurons causes a subset of RTT features. To test this hypothesis, we removed *Mecp2* from the hypothalamic neurons of the paraventricular and supraoptic nuclei by crossing mice carrying a conditional *Mecp2* allele (*Mecp2*^{flox}) to mice that carry a *Sim1*-cre recombinase transgene. Eight neurobehavioral tests were performed on 16 mice from each of the 4 possible genotypes. Open field analysis revealed that the conditional knockout mice (CKO) have a lower center to total distance ratio than control littermates ($p<0.01$) suggesting that they experience elevated levels of anxiety. Resident intruder analysis demonstrated that CKO mice engage in more aggressive behaviors such as tail rattling and attacking ($p<0.01$) than their control littermates. Finally, CKO mice are heavier than wild type littermates ($p<0.001$) beginning at 7 weeks of age. We investigated the cause of this weight gain and found that CKO mice exhibit an increase in daily food intake ($p<0.0001$) without significant changes in activity level or resting metabolic rate. These findings suggest that MeCP2 is critical for the regulation of pathways involved in food intake, stress response and social behavior. Furthermore, they suggest that the loss of MeCP2 in specific hypothalamic neurons is sufficient to reproduce a subset of the features seen in RTT.

851/T

Clinical feature of autosomal dominat spinocerebellar ataxia linked to 16q22.1. *Y. Ichikawa, S. Tsuji, J. Goto.* Dept Neurology, Univ Tokyo, Tokyo, Japan.

Autosomal dominant cerebellar ataxias (ADCAs) are heterogeneous neurodegenerative diseases characterized by progressive cerebellar ataxia occasionally accompanied with other findings. A single nucleotide substitution (-16C>T) in the 5' UTR of the puratrophin-1 gene has recently been identified to be tightly associated with patients of families linked to chromosome 16q22.1 (16q-ADCA). In the 294 Japanese ADCA families analyzed in our laboratory on the referral basis, Machado-Joseph disease(MJD) / spinocerebellar ataxia type 3 (SCA3) was the most common ADCA, followed by dentatorubral-pallidoluysian atrophy (DRPLA) and SCA 6. We examined the possibility of 16q-ADCA about the 87 Japanese ADCA families excluded from SCA1, 2, 6, 7, 8, 12, 17, MJD/SCA3 and DRPLA. As a result, 16q-ADCA families comprised a substantial proportion (8.2%) among the Japanese ADCA families. Accordingly, 16q-ADCA was the fourth common Japanese ADCA. It has been reported that the characteristic clinical feature of the 16q-ADCA was slowly progressive pure cerebellar ataxia similarly to SCA6. We compared the clinical findings of 27 16q-ADCA patients whose clinical information was available with those of 46 SCA6 patients. The average onset age of 16q-ADCA patients was 56.3±7.4 years (40-68 years), which was higher than that of SCA6: 49.9±12.2 (23-76 years)($P = 0.02$ t-test). All the patients of 16q-ADCA showed ataxic gait or dysarthria as their initial symptoms (ataxia: 85.2%, dysarthria: 14.8%). Gaze nystagmus was more frequent in SCA6 patients (90.6%) than 16q-ADCA patients (65%). SCA6 patients showed higher percentage of hyperactive deep tendon reflexes than 16q-ADCA at 57.5% as opposed to 44% irrespective of the disease duration. Diplopia was observed in two 16q-ADCA patients, whose disease duration were 8 and 26 years. Subjective hearing impairment was observed in only one 16qADCA patient, and none in the SCA 6 patients irrespective of the disease duration. In summary, all 16q-ADCA patients showed cerebellar ataxia as to initial symptoms. The average onset age of 16q-ADCA was higher than that of SCA6, and the other neurological findings besides cerebellar ataxia tend to be fewer in 16qADCA than SCA6 patients.

852/T

Translation of *SOX10* 3' untranslated region causes a complex severe neurocristopathy by generation of a deleterious functional domain. *K. Inoue[1], T. Ohyama[2], Y. Sakuragi[1], Y. Lihua[1], R. Yamamoto[1], Y. Goto[1], M. Wegner[3], J.R. Lupski[2].* 1) Dept MR & BD Res, Natl Inst Neurosci, NCNP, Tokyo, Japan; 2) Dept Mol Hum Genet, Baylor Coll Med, Houston, TX; 3) Institut für Biochemie, Emil-Fischer-Zentrum, Universität Erlangen, Erlangen, Germany.

Peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome and Hirschsprung disease (PCWH) is a complex neurocristopathy caused by *SOX10* mutations. Most PCWH-associated *SOX10* mutations result in premature termination codons (PTCs), for which the molecular mechanism has recently been delineated. However the first mutation reported to cause PCWH was not a PTC. It was a disruption of the native stop codon that by conceptual translation extends the protein into the 3' untranslated region (3'UTR) for an additional 82 residues. Molecular pathoetiology for this extension mutation remains largely unknown. In this study, we sought to determine the functional properties of the SOX10 extension mutation using in vitro functional assays. Despite the wild type SOX10 coding sequence remaining intact, the extension mutation led to severely diminished transcription and DNA binding activities. Nevertheless, it showed no dominant-negative interference with wild type SOX10. Within the 82-amino acid tail, an 11 amino acid region (termed the WR domain) was responsible primarily for the deleterious properties of the extension. The WR domain, presumably forming an a-helix structure, dramatically inhibited SOX10 transcription activities from different positions within the SOX10 protein. The WR domain can also affect the other transcription factors in cis with graded effect, suggesting that it probably elicits a toxic functional activity by itself. Together, molecular pathology for the SOX10 extension mutation is distinct from that of more common PTC mutations. Failure to properly terminate SOX10 translation causes the generation of a deleterious functional domain within the 3'UTR that causes a severe neurological disease.

853/T

Redefining candidate region of, and identification of specific genetic changes for the chromosome 16q22.1-linked autosomal dominant cerebellar ataxia. *K. Ishikawa[1], T. Amino[1], N. Sato[1], K. Kobayashi[2], S. Asakawa[3], T. Toda[2], H. Mizusawa[1].* 1) Dept Neurology and Neurological Sciences, Graduate School, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Dept Human Genetics, Graduate School, Osaka University, Osaka, Japan; 3) Dept Molecular Biology, Keio University, Tokyo, Japan.

The chromosome 16q22.1-linked autosomal dominant cerebellar ataxia (16q22.1-ADCA; OMIM #117210) is the third frequent ADCA in Japan, and also shares responsible chromosome region with SCA4 found in Utah and Northern Germany. Clinically, 16q22.1-ADCA is characterized by late age-of-onset (average: 55.5 years) and purely cerebellar ataxia. Corresponding to the clinical features, the Purkinje cell in the cerebellum undergoes predominant degenerations in 16q22.1-ADCA. While the cause(s) of 16q22.1-ADCA and SCA4 had been elusive, a single-nucleotide -16C>T substitution in the *puratrophin-1* gene was identified specific for the 16q22.1-linked ADCA (Ishikawa et al. *Am J Hum Genet* 2005). However, one affected individual without the *puratrophin-1* genetic change was found subsequently in a new family, in which all other affected members harbored the -16C>T *puratrophin-1* genetic change. This would suggest the presence of causative gene instead of the *puratrophin-1* gene. To identify the real cause of 16q22.1-linked ADCA, we collected 65 families from diverse regions of Japan, and typed with densely mapped microsatellite and SNP markers in and around chromosome 16q22.1. We identified a new critical region where groups of markers showed identical alleles in all families including the aforementioned exceptional family. We also found a family in which 4 members had homozygous, two identical disease haplotypes, while two others harbored heterozygous, disease and normal haplotypes. These homozygotes had earlier age of onset than the heterozygotes. We have also found several genetic changes specific for all patients, since such changes were not seen in 600 control Japanese chromosomes. We are focusing some of these changes which may explain the cause of the 16q22.1-linked ADCA.

854/T

Truncations in the Carboxyl-terminus of Human 3'-5' DNA Exonuclease TREX1 Cause Autosomal Dominant Retinal Vasculopathy with Cerebral Leukodystrophy. *J.C. Jen[1], A.M.J.M. van den Maagdenberg[2,3], A. Richards[4], D. Kavanagh[4], P. Bertram[4], D. Spitzer[4], M.K. Liszewski[4], M. Barilla-LaBarca[4], G.M. Terwindt[3], Y. Kasai[5], K.R.J. Vanmolkot[3], B. de Vries[2], J. Wan[1], M.J. Kane[1], H. Mamsa[1], S.F. Nelson[6], R.R. Frants[2], R.W. Baloh[1], M.D. Ferrari[3], J.P. Atkinson[4].* 1) UCLA Neurology; 2) Human Genetics, Leiden U Med Ctr, The Netherlands; 3) Neurology, Leiden U Med Ctr; 4) Medicine/Rheumatology, Washington University, St. Louis, MO; 5) Genome Sequencing Center, Washington Uiversity; 6) UCLA Human Genetics.

Retinal vasculopathy with cerebral leukodystrophy (RVCL,MIM192315) is an autosomal dominant vascular syndrome of middle age onset due to a systemic microvascular endotheliopathy with an unusual ultrastructural appearance of multilaminated subendothelial basement membrane. We identified a large American family with cerebroretinal vasculopathy (CRV), characterized by retinal vasculopathy reminiscent of diabetic retinopathy and white matter brain lesions often mistaken for neoplasm or demyelination. The disease locus of CRV mapped to 3p21 that is shared by a Dutch family with hereditary vascular retinopathy (HVR) and a Chinese American family with hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS). These allelic disorders were henceforth designated RVCL. A collaborative effort led to the discovery of heterozygous carboxyl-terminal frameshift mutations in TREX1, a ubiquitously expressed 3'-5' repair exonuclease whose physiological function is largely unknown. Nonfunctional TREX1 mutations were recently demonstrated to cause Aicardi-Goutières syndrome (AGS1 [MIM225750] & AGS5 [MIM610905]) as well as chilblain lupus (MIM610448), suggesting a role for TREX1 in clearing altered DNA to prevent destructive autoimmune response. The involvement of TREX1 in the maintenance of systemic vascular integrity has not been previously recognized. The RVCL-causing truncated TREX1 proteins retain exonuclease activity but lose normal perinuclear localization, suggesting a toxic gain of function. Understanding the pathogenesis of RVCL may provide insight to stroke and vascular cognitive impairment as well as possibly shared mechanisms between RVCL and diabetic retinal vasculopathy.

855/T

A duplication at chromosome 11q12.2 is associated with spinocerebellar ataxia type 20 (SCA20). *M.A. Knight[1], D. Hernandez[2], I. Rafferty[2], S.M. Forrest[3], R.J.M. Gardner[4, 5], E. Storey[5, 6], A. Dutra[7], E. Pak[7], K.H. Fischbeck[1], A.B. Singleton[2].* 1) Neurogenetics Branch, NINDS/NIH, Bethesda, MD, USA; 2) Molecular Genetics Unit, Laboratory of Neurogenetics, NIA/NIH, Bethesda, MD, USA; 3) Australian Genome Research Facility, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia; 4) Genetic Health Services Victoria, Melbourne, VIC, Australia; 5) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, VIC, Australia; 6) Department of Medicine (Neurosciences), Alfred Hospital Campus of Monash University, Melbourne, VIC, Australia; 7) Genetic Diseases Research Branch, Cytogenetic and Microscopy Core, NHGRI/NIH, Bethesda, MD, USA.

SCA20 is an autosomal dominant cerebellar ataxia that is clinically distinct from the other SCAs. It is characterized phenotypically by a slowly progressive ataxia with the additional clinical features of dysphonia and palatal tremor, and the unique observation on neuroradiology of calcification of the dentate nucleus of the cerebellum. SCA20 is linked to chromosome 11. The locus overlaps the SCA5 disease locus, but after discovery of the SCA5 gene, β-III spectrin (*SPTBN2*), it was found that SCA5 and SCA20 were not allelic. Since SCA20 is a separate entity, we studied the structure of the genomic DNA, using Illumina 550 SNP genotyping chips. The results indicated a 2.6Mb duplication within the previously linked region. The duplication was shown to be present in all affected individuals in the single reported SCA20 pedigree. We are currently doing fluorescent *in situ* hybridization analysis on metaphase chromosome spreads to confirm the duplication and determine its orientation. We are also investigating twelve known genes within the duplicated region to determine which, if any, are expressed in the cerebellar Purkinje cells and the dentate nucleus, which we presume to be the neural substrate predominantly affected in SCA20. We are seeking to determine whether the disease is caused by increased dosage of one or more of the genes within the duplicated region, or by a breakpoint in one of the genes resulting in a novel gene product.

856/T

Agenesis of the Corpus Callosum in Three Generations. *J. Li, V. Woo, K. Kronfeld, N. Osbun, R. Jeremy, E. Marco, E.H. Sherr.* Dept Neurology, Univ California, San Francisco, San Francisco, CA.94143.

Agenesis of the corpus callosum (ACC), a failure to develop the large bundle of fibres that connect the cerebral hemispheres, occurs in 1:4000 individuals. Current evidence indicates that a combination of genetic mechanisms, including single-gene mutations, single-gene sporadic mutations and complex genetics might have a role in the aetiology of ACC. Here we report a family in which ACC is present in six individuals of three consecutive generations. The family consists of the proband's parents, his brother and sister and his three children, two girls and a boy. Clinical exams revealed mild learning deficits, but most had IQs in the normal range. Imaging analysis showed either partial or complete ACC within the same family. Although no other cortical abnormalities were detected. The case reported here is consistent with autosomal dominant inheritance with nearly complete penetrance. Linkage analysis was performed using Illumina linkage IV panel SNP markers which include 5861 SNPs; the average genetic distance between mapped SNPs is 0.64 cM. The statistical significance of SNPs data was analysis by Merlin. These preliminary results pointed to several SNP markers on Chr4 and Chr14 which show the maximal possible LOD score, This analysis suggests that genes including Kv channel interacting protein 4, glutamate receptor, RAD51-like 1 isoform 2, regulator of G-protein signaling 6 and checkpoint suppressor 1 as disease candidates. To our knowledge, this is the first report of possible autosomal dominant ACC in three consecutive generations, perhaps suggesting that this is more common than previously appreciated.

857/T

Comparison of spectrum of deletions within the CCM genes between Italian and American populations. *C.L. Liquori[1], S. Penco[2], T.P. Leedom[1], F. Squitieri[2], D.A. Marchuk[1], F. Gianfrancesco[3].* 1) Dept Molecular Gen & Microbiol, Duke Univ, Durham, NC; 2) Neurogenetics Unit IRCCS Neuromed, Pozzilli (IS), Italy; 3) Institute of Food Science, National Research Council, Avellino, Italy.

Cerebral cavernous malformations (CCMs) are vascular abnormalities of the brain that can cause a variety of neurological disabilities, including stroke and seizures. Familial forms of CCM are inherited in an autosomal dominant fashion and three CCM genes have been identified. We recently determined that large genomic deletions in the CCM2 gene represent 15% of mutations in a large CCM cohort from the U.S. A 77.6 kb deletion spanning CCM2 exons 2-10 displays an identical recombination event in 10 CCM families/probands, and haplotype analysis suggests that this common deletion derives from a founder mutation within our cohort. In the current study, we examined an Italian CCM cohort consisting of 24 CCM1, 2, 3 "mutation-negative" proband/families. The common CCM2 deletion spanning exons 2-10 is not present in this population. Further analysis of the Italian cohort by multiplex ligation-dependent probe analysis (MLPA) identified a total of 10 deletions - five in the CCM1 gene, four in the CCM2 gene, and one in the CCM3 gene. A duplication within the CCM2 gene was also identified. We conclude that there appear to be elements within all three of the CCM genes that predispose them to large deletion/duplication events. However, the common deletion spanning *CCM2* exons 2-10 appears to be specific to the U.S. population.

858/T

Variation in novel exons (RACEfrags) and human genetic disorders; the case of Rett syndrome. *P. Makrythanasis[1], P. Kapranov[2], L. Bartoloni[1], A. Raymond[3], S. Deutsch[1], R. Guigo[4], F. Denoeud[4], C. Rossier[1], F. Ariani[5], V. Capra[6], A. Renieri[5], T. Gingeras[2], S.E. Antonarakis[1].* 1) Medical Genetics and Dev., University of Geneva, Switzerland; 2) Affymetrix, Santa Clara, US; 3) University of Lausanne, Switzerland; 4) IMIM, Barcelona, Spain; 5) University of Sienna, Italy; 6) Neurochirurgia, Istituto G.Gaslini, Genova, Italy.

The study of transcription using genomic tiling arrays, strikingly has lead to the identification of numerous additional exons connected to known genes. One example is the MECP2 gene on the X-chromosome; using 5'RACE and RT-PCR in numerous human tissues and cell lines, we have found more than 15 novel exons (RACEfrags) "connecting" to at least one exon of MECP2 gene and map up to 1 Mb telomeric to it. We subsequently asked if variation in the novel exons is causatively associated with Rett syndrome, a monogenic disorder commonly due to pathogenic mutations of MECP2. We sequenced all MECP2-connected exons and flanking sequences in 3 groups: 48 Rett patients without mutations in MECP2 and CDKL5 genes (group 1); 30 Rett patients with mutations in the MECP2 gene (group 2); 100 control individuals from the same geoethnic group (group 3). Approximately 14 kb was sequenced per sample, for a total of 2.6 Mbs of DNA resequencing. We found 75 individuals with rare variants (observed in 1-4 alleles). The individuals with rare variants were 19/48, 11/30, and 45/100 in groups 1 to 3 respectively, i.e. there is not a statistically significant difference among the 3 groups. These results suggest that variants in the newly discovered exons studied do not contribute to Rett syndrome, furthermore if some of these variants are related to a phenotype, this must be different from Rett. Interestingly however, the variants in the novel exons are twice as frequent as those found in flanking sequences (50 vs 24 for approximately 1.3 Mb sequenced for each class of sequences). The significance of this result remains to be elucidated; one hypothesis is that novel exons accumulate variants faster than the rest of the genome (positive selection?) that could underscore the functional importance of these sequences.

859/T

Mutations in the Walker-Warburg Syndrome genes *POMT1*, *POMT2*, *FKRP* and *fukutin* are differentially distributed in populations of Middle Eastern and European descent. *M.C. Manzini[1], D. Gleason[2], B.S. Chang[1], R.S. Hill[1], A. Bodell[1], K. Apse[1], A. Poduri[3], S. Currier[1], W.B. Dobyns[4], M.A. Salih[5], M.Z. Seidahmed[6], P. Galvin-Parton[6], L.R. Shapiro[7], K. Schmidt[8], J.G. Davis[9], C.A. Walsh[1, 2].* 1) Neurology and HHMI, BIDMC, Harvard Medical School, Boston, MA; 2) Genetics, Children's Hospital, Boston, MA; 3) Neurology, Children's Hospital, Boston, MA; 4) Human Genetics, Neurology and Pediatrics, University of Chicago, Chicago, IL; 5) Pediatric Neurology, King Kahlid University Hospital, King Saud University, Riyadh, Saudi Arabia; 6) Pediatrics, Stony Brook University Medical Center, Stony Brook, NY; 7) Pediatrics, Westchester Medical Center, Valhalla, NY; 8) Genetics, Children's Hospital of Pittsburgh, Pittsburgh, PA; 9) Pediatrics, Weill Cornell Medical College, New York, NY.

Walker-Warburg syndrome (WWS [MIM 236670]) is an autosomal recessive disorder characterized by type II (cobblestone) lissencephaly, hydrocephalus, severe cerebellar and ocular malformations, and congenital muscular dystrophy (CMD). WWS is the most severe form of CMD, leading to significant developmental delay and life expectancy of less than 3 years. While WWS presents with a relatively homogeneous phenotype, it is genetically heterogeneous and mutations have been identified in several glycotransferases that modify α-dystroglycan. We analyzed a cohort of patients of diverse ethnic origin with classic WWS (41 patients from 38 families) and sequenced WWS genes (*POMT1*, *POMT2*) and genes that are more rarely mutated in this disease, such as *fukutin* and fukutin related protein (*FKRP*). A striking difference was observed in the geographic distribution of mutations. Middle Eastern families most commonly carried *POMT1* mutations, while a diverse range of mutations in *POMT1*, *POMT2*, *fukutin* and *FKRP* was found in the European and American cases. Moreover, in contrast to previous data suggesting that only 10-20% of WWS cases can be explained by mutations in these genes, we found that 39.5% of patients in our cohort carried *POMT1*, *POMT2*, *FKRP* or *fukutin* mutations.

860/T

Molecular analysis of the *MFN2* gene in familial and sporadic axonal Charcot-Marie-Tooth disease type 2 (CMT2). *M. Milani, D. Pareyson, F. Taroni.* Div Biochem & Genetics, Fondazione IRCCS Ist Neurologico Carlo Besta, Milan, MI, Italy.

Axonal CMT (CMT2) is genetically highly heterogenous, with at least 14 loci and 10 genes identified thus far. Mutations in the gene encoding the mitochondrial protein mitofusin-2 (MFN2) have been shown to be responsible for autosomal dominant CMT2 type A2 (CMT2A2). The *MFN2* gene maps to chromosome 1p36.2 and encodes a 757-amino acid protein which is an essential component of mitochondrial fusion in mammalian cells. The CMT2A2 phenotype is largely indistinguishable from that of CMT2A1 (*KIF1B*), CMT2E (*NEFL*), and CMT2F (*HSPB1*). However, in a subset of CMT2A2 patients, pyramidal involvement and visual impairment have been reported. The disease exhibits reduced penetrance: studies in large families have shown that individuals with *MFN2* mutations may present no signs of disease even at the electrophysiological examination. In order to analyse the mutation spectrum and frequency and to assess the phenotypes associated with the *MFN2* gene, we have screened for *MFN2* mutations a large group (n=196) of index patients with axonal CMT. Familiarity was reported in one third of the cases only. The 17 *MFN2* exons and exon-intron boundaries were screened by DHPLC. Fragments showing an altered profile were directly sequenced. Nine novel and 3 previously reported mutations were found in heterozygous form in 12 unrelated index cases. There were 9 missense mutations, 1 frameshift mutation, and 1 amino acid deletion. The majority of cases (8/12, 66.7%) were sporadic. Age at onset ranged from 4 years to adulthood. Clinical presentations included clinical and electrophysiological sparing of the upper limbs, pyramidal signs, tremor, and optic atrophy in two cases. Two familial cases were characterized by early-onset, severe progression, and proximal involvement. Conclusions: our results indicate that in a raw series of axonal CMT patients the minimum frequency of *MFN2* mutations is approx. 6%, thus indicating that genetic testing for CMT2 should begin from the *MFN2* gene. The results also show that *MFN2* mutation analysis should be performed in both familial and sporadic cases. [Supported by Telethon-UILDM (GUP04009) and Fondazione Mariani (R0544)].

861/T

Role of ubiquitin-proteasome dysfunction in Lafora disease. *S. Mittal, S. Ganesh.* Biological Sciences & Bioengineering, Indian Institute of Technology, Kanpur, UP, India.

Lafora's progressive myoclonus epilepsy (called Lafora disease: LD) results primarily from the formation of Lafora body inclusions and neuronal cell death. LD is invariably a fatal disorder as none of the current treatments halt or retard Lafora body formation and neurodegeneration. The main obstacle in developing therapies for LD is the limited understanding of the key molecular events that provoke Lafora body formation and neurodegeneration. The discovery of NHLRC1 gene, encoding an ubiquitin ligase called malin, has led to the hypothesis that dysfunction of the ubiquitin-proteasome pathway (UPP) is pivotal to LD pathogenesis. In order to understand the role of UPP in LD, we have established a cell-based model to define the substrates for, and their cellular functions of malin, to understand the protective role of malin in neuronal cell-death induced by various stresses, and finally, the question as to how defects in malin lead to the formation of Lafora bodies. We would also like to uncover the synergistic function of laforin, a protein phosphatase coded by a second gene involved in LD, using this cellular model. We show here that malin and laforin co-localize in endoplasmic reticulum and form centrosomal aggresomes when cells were treated with proteasomal inhibitors. Laforin and malin form aggresome when expressed together or otherwise, suggesting that the two proteins are recruited to the centrosome independent of each other. Thus, centrosomal accumulation of malin, possibly with the help of laforin, may enhance the ubiquitination of its substrates and facilitate their efficient degradation by proteasome. It is indeed the case, as the cellular level and toxicity of misfolded substrates of malin diminish when they are co-expressed with malin or laforin. Our results further suggest that, in addition to the UPP, the malin-mediated clearance of misfolded proteins is likely to involve autophagy. Taken together our results suggest that defects in malin or laforin may thus lead to increased levels of misfolded and/or target proteins, which may eventually affect the physiological processes of the neuron leading to the LD phenotype.

862/T

Role of an intermediate SCA2 allele in a patient with spinocerebellar ataxia. *E.M. Ramos[1], S. Martins[1,2], I. Alonso[1], L.B. Jardim[3], P. Coutinho[1,4], J. Sequeiros[1,5], I. Silveira[1].* 1) UnIGENe, IBMC, Univ. Porto, Portugal; 2) IPATIMUP, Porto, Portugal; 3) Hosp. Clínicas de Porto Alegre, Brasil; 4) Hosp. S. Sebastião, St. M. Feira, Portugal; 5) ICBAS, Univ. Porto, Portugal.

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disorder of autosomal dominant inheritance. It is caused by the expansion of an unstable CAG repeat within the exon 1 of the *ATXN2* gene (12q24.1), coding for a polyglutamine (polyQ) tract, in ataxin-2 protein. The interspected repeat is highly polymorphic in length and configuration, with CAA interruptions varying in number and position within the sequence. Normal chromosomes have usually 14 to 31 repeats interrupted by one or more CAAs, whereas expanded alleles display 34 to 59 pure repeats. In this study, ten SCA2 families from different ethnic origins, namely of Portuguese (4), Brazilian (3), Italian (1) and Asian (2) ancestry, were studied. We have analyzed two previously reported single nucleotide polymorphisms (SNPs), proximal to the CAG repeat, along with the CAA interruption pattern. The GT haplotype was described as mainly associated with normal alleles, whereas the CC is shared by all expanded alleles. Expanded haplotypes were reconstructed by cloning a region of 462 bp, encompassing the deleterious repeat and flanking SNPs. All normal chromosomes carrying the GT haplotype shared the $(CAG)_8CAA(CAG)_4CAA(CAG)_8$ repeat pattern, whereas normal alleles with the CC haplotype lack the first CAA interruption. The only expanded chromosome (50 CAGs) assessed showed the CC haplotype. In addition, we found an intermediate allele of 32 repeats in a patient with atypical symptoms of spinocerebellar ataxia. Although sharing the CC haplotype with expanded alleles, the intermediate allele was interrupted at the 3'-end of the repeat: $(CAG)_{23}CAA(CAG)_8$. This pattern may confer increased instability to the repeat tract, predisposing to expansion and disease expression. Since our finding points to the importance of the haplotype and configuration patterns on disease penetrance of intermediate alleles in SCA2, further studies in additional alleles are now proceeding.

863/T

Novel *MFSD8* mutations in variant late-infantile neuronal ceroid lipofuscinosis. *E. Siintola[1,2], M. Kousi[1,2], M. Topcu[3], N. Aula[1,2], H. Lohi[1,4], B.A. Minassian[4], A.D. Paterson[4,5], X.-Q. Liu[4], C. Wilson[6], U. Lahtinen[1,2], A.-K. Anttonen[1,2], S.E. Mole[7], A.-E. Lehesjoki[1,2].* 1) Folkhälsan Institute of Genetics, Finland; 2) Neuroscience Center, University of Helsinki, Finland; 3) Department of Pediatrics, Hacettepe University, Faculty of Medicine, Section of Child Neurology, Turkey; 4) Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada; 5) Department of Public Health Sciences, University of Toronto, Canada; 6) Starship Children's Hospital, Auckland, New Zealand; 7) MRC Laboratory for Molecular Cell Biology, University College London, United Kingdom.

Neuronal ceroid lipofuscinoses (NCLs) are a group of childhood-onset inherited neurodegenerative disorders of which the late-infantile onset group (LINCLs) is genetically the most heterogeneous with mutations identified in five genes. LINCL presents clinically with onset at 2-7 years of age, epileptic seizures, myoclonus, psychomotor deterioration, loss of vision, and premature death. A variant form of LINCL (vLINCL) in Turkish patients was initially considered a distinct clinical and genetic entity (CLN7). Recently, we reported mutations in the *CLN6* and *CLN8* genes in a subset of Turkish patients with vLINCL. In the majority of families, however, the disease is not linked to any of the known NCL loci. After a genome-wide single nucleotide polymorphism scan, homozygosity mapping, and candidate gene sequencing in mainly Turkish families, we identified mutations in the *MFSD8* gene as a novel gene underlying vLINCL. MFSD8 is a novel lysosomal transmembrane protein that belongs to the major facilitator superfamily, members of which have various transporter activities. The substrate specificity and the cellular function of the protein encoded by this novel NCL gene are not known. Subsequently, we have screened the *MFSD8* gene in several additional families with vLINCL and identified new mutations accounting for the disease in patients of various ethnic origins. This implies that MFSD8-associated vLINCL is not limited to the Turkish population.

864/T

Screening of point mutations in genes implicated in spinocerebellar ataxia (SCA) type 13, 14, 27 and SCA linked to chromosome 16q in a large group of SCA families. *I. Silveira[1], E.M. Ramos[1], I.F. Bento[1], I. Alonso[1], P. Magalhães[2], P. Coutinho[3], J. Sequeiros[1,4].* 1) UnIGENe, IBMC - Univ Porto, Portugal; 2) CCGen, IBMC - Univ. Porto, Portugal; 3) Hosp. S. Sebastião, Sta Maria da Feira, Portugal; 4) ICBAS, Univ. Porto, Portugal.

The autosomal dominant spinocerebellar ataxias (SCAs) are clinically and genetically very heterogeneous diseases, mainly characterized by gait and limb ataxia, dysarthria, and a variable degree of oculomotor impairment, usually of adulthood onset. Seven autosomal dominant SCAs (SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA17 and dentatorubro-pallidoluysian degeneration or atrophy, DRPLA) are caused by a (CAG)n expansion within the coding region, producing an extended polyglutamine tract in the mutant protein. In SCA12, the disease results from an expanded (CAG)n, but in the 5'-UTR of the *PPP2R2B* gene. In SCA8 there is an expanded (CTG)n tract in an untranslated region. A pentanucleotide expansion is implicated in SCA10, caused by a tract of hundreds of ATTCT repeats in an intron of a gene with still unknown function. In five dominant forms, including SCA5, 13, 14, 27 and SCA linked to chr. 16, the disease is originated by point mutations or small deletions in the responsible gene. The SCAs are individually rare worldwide, though some may show a high clustering in certain geographic areas. We ascertained 270 SCA families of Portuguese origin and performed mutation analysis for all SCAs caused by a repeat expansion as well as for SCA13, 14, 27 and the single-nucleotide substitution in puratrophin-1 gene implicated in SCA linked to chr. 16q. In our group of families, MJD was the most frequent SCA (52%), followed by DRPLA (4%); SCA1, 2, 6, 7, 8, and 17 were together the cause of only a few cases (less than 8%); a point mutation was found responsible for SCA14 in one family (less than 1%), whereas no mutations where identified in SCA13, 27 and puratrophin-1 genes. In conclusion, point mutations in known autosomal dominant SCA genes seem to be rare in Portuguese families with SCA.

865/T

Disruption of CTNND2 by a de novo t(3;5) is associated with borderline intelligence and avoidant personality disorder. *E.A. Swanson[1], L. Margari[2], P. Ventura[2], A. Presicci[2], M. Gentile[3], F. Margari[4], R. De Blasi[5], M. Buttiglione[2], S.L. Christian[1], W.B. Dobyns[1].* 1) Human Genetics, Univ of Chicago, Chicago, IL; 2) Child Neurological and Psychiatric Service, Department of Neurological and Psychiatric Sciences, Univ of Bari, Italy; 3) Medical Genetic Unit, Hospital Di Venere, ASL BA/04, Bari, Italy; 4) Psychiatric Service, Department of Neurological and Psychiatric Sciences, Univ of Bari, Italy; 5) Neuroradiology Service, Department of Neurological and Psychiatric Sciences, Univ of Bari, Italy.

The genetic basis of subtle developmental and especially behavioral disorders is complex, making gene identification challenging. We ascertained a family segregating the reciprocal translocation t(3;5)(q24;p15.2) that implicate the brain specific gene CTNND2 in brain development and function. A boy with the der (3) had parietal bone foramina, cleft palate and enlarged and lucent right lung associated with pulmonary ectasia, which led to cardiac failure and death. The father and sister were both carriers of the balanced translocation, which was de novo in the father. Both had borderline scores on cognitive testing and an avoidant personality disorder based on history of deficient reciprocal social interactions and communication, as well as anxiety, depression, and poor attention. Brain imaging showed herniation of the mesial parietal lobes through an enlarged tentorial notch (previously associated with parietal foramina) and mild cerebellar vermis hypoplasia (CVH). We used FISH to identify the two breakpoints. The 3q breakpoint was localized to a region ~300 kb telomeric to the ZIC1 and ZIC4 genes that we previously associated with cerebellar hypoplasia and Dandy-Walker malformation (Grinberg et al., 2004). The 5p breakpoint was fine mapped between the first and second exons of the brain-specific gene, CTNND2, using the fosmid G248P87103D2. The gene, located ~11 Mb from the 5p telomere, is deleted in some patients with cri-du-chat syndrome. We hypothesize that altered expression of ZIC1 and ZIC4 are responsible for the mild CVH, while disruption of CTNND2 is responsible for the unusual cognitive and behavioural profile. The cause of the meningeal deficiency is not clear.

866/T

The identification of mutations at the SPG5 locus defines the gene responsible for a pure form of hereditary spastic paraplegia. *M.K. Tsaousidou[1], M.A. Simpson[1], P.A. Wilkinson[1], H. Patel[1], T.T. Warner[3], M.A. Patton[1], T. Siddique[2], A.H. Crosby[1].* 1) Medical Genetics, St George's University of London, London, United Kingdom; 2) Northwestern University Medical School, Chicago, USA; 3) Royal Free & University College Medical School,London, UK.

The hereditary spastic paraplegias (HSPs) comprise a genetically and clinically complex group of inherited diseases of the motor neuron. They are classified according to the mode of inheritance and whether the cardinal clinical feature of leg spasticity occurs alone (pure HSP) or is accompanied by additional neurological or systemic abnormalities (complicated HSP). Despite the mapping to chromosome 8 of the first pure autosomal recessive form (SPG5) of HSP many years ago, the precise nature of the causative gene has remained elusive. In order to refine the chromosomal localisation of SPG5, we undertook linkage studies in a large pure HSP family which maps to this locus. In combination with reports of other families which link to this region, we were able to refine the SPG5 locus to a 21cM (24Mb) interval flanked by markers D8S1115 and D8S1795, a region comprising approximately 100 genes. Systematic sequence analysis of these genes ultimately revealed family specific mutations in all families analysed in a gene located within this region; the identification of this gene defines a novel cause for the motor neuron degeneration characteristic of this disease.

867/T

Screening for melanocortin-4 receptor mutations in a cohort of Dutch obese children. *L. van den Berg[1,2], H. Delemarre-van de Waal[2,3], P. Heutink[1,3].* 1) Section of Medical Genomics and Center for Neurogenomics and Cognitive Research, VU medical center, Amsterdam, Netherlands; 2) department of Paediatrics, VUmc, Amsterdam, Netherlands; 3) Institute for Clinical and Experimental Neurosciences, VUmc, Amsterdam, Netherlands.

The most common monogenic form of obesity is caused by mutations in the gene encoding the melanocortin 4 receptor (MC4R). This receptor integrates orexigenic and anorexigenic signals in the hypothalamus to regulate food intake and energy expenditure. Several aspects of the role of MC4R mutations in obesity remain unclear. For instance, it is unclear which phenotypic characteristics accompany MC4R mutations. We have established a centre for childhood and adolescent obesity. More than 500 obese children have already visited this centre.

We have screened the coding sequence and the minimal promoter region of MC4R of 119 random patients from our cohort. We found 15 variants, two of which were not described previously (-1101C>T and -705A>T). The -705A>T variant may influence gene expression because it is located in a regulatory element (Lubrano-Berthelier et al. 2003 *Diabetes* 52:2996-3000). It was found in a 3-year-old girl with a body mass index standard deviation score (BMI-SDS) of 3.4. We found a Tyr35STOP mutation in a 12-year-old boy with a BMI-SDS of 2.5. This mutation leads to a truncated non-functional receptor. We detected a G231S mutation in a 15-year-old girl with a BMI-SDS of 3.3. This mutation has been shown to reduce the basal activity of the receptor (Govaerts et al. 2005 *Peptides* 26:1909-1919). Three variants remain to be tested functionally (F202L and two variants in the 3'UTR). All other variants that we detected are not expected to be pathogenic because they have failed to show MC4R impairment in *in vitro* studies (Asp37Val, V103I, and I251L) or because they have been found at the same frequency in lean people (-1042C>T, -1005C>T, -896C>T, -178A>C). We will extend our research by screening additional patients, studying co-segregation of mutations and obesity in families, studying phenotypic characteristics of MC4R mutation carriers, and functional studies of mutations.

868/T

Clinical Variability and Mutation Frequency in *REEP1* (SPG31) Hereditary Spastic Paraplegia. *W.A.G. van Zelst-Stams[1,2], S.G.M. Frints[1,2], M. Gerards[1,2], R.G. Janssen[1,2], C.E.M. de Die-Smulders[1,2], C.T.R.M. Schrander-Stumpel[1,2], H.J. Smeets[1,2].* 1) Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, Limburg, Netherlands; 2) Research Institute GROW, Maastricht University, Maastricht, The Netherlands.

Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder characterized by clinical and molecular heterogeneity. In autosomal dominant (AD) spastic paraplegia (SPG), *SPASTIN* (SPG4) and *ATLASTIN* (SPG3A) gene defects account for approximately 40% and 10%, respectively. We performed parametric linkage analysis, using the Affymetrix 10K SNP array, to identify the SPG locus in a nine-generation Dutch pedigree (1050 individuals). A maximum LOD score of 5.03 was obtained at the SPG31 locus (2p11-p12). Mutation analysis of the receptor expression-enhancing protein 1 gene (*REEP1*) was performed in 10 additional AD SPG families from the South-East part of the Netherlands. A truncating four basepair deletion in exon six (c.537_540delCGGC p.Ser179ArgfsX43) was identified which co-segregated with the disorder in the large linked family and in two other small unrelated families, suggesting a founder effect. The clinical features within these families ranged from normal to severe spasticity of legs and the age of onset was from birth till >75 years of age. The search for a founder haplotype is ongoing. Furthermore, we try to identify modifying loci or genes which can contribute to nonpenetrance in HSP. In conclusion, we identified a possible founder *REEP1* mutation in 27% (3/11) of the AD pure SPG families investigated in the South-East part of the Netherlands. Thus *REEP1* gene defects seem to be more common than earlier reported.

869/T

Identification of a novel PD locus on chromosome 6q in Norwegian and Tunisian kindreds. *C. Vilariño-Güell[1], K. Haugarvoll[1], M.M. Hulihan[1], J.M. Kachergus[1], L. Ishihara-Paul[2], S. Oldham[2], R. Amouri[3], S.B. Ahmed[3], M. Kefi[3], R.A. Gibson[2], F. Hentati[3], J. Aasly[4], M.J. Farrer[1].* 1) Dept of Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Research and Development, GlaxoSmithKline, USA and UK; 3) Dept of Neurology, Institut National de Neurologie, Tunis, Tunisia; 4) Dept of Neurology, St Olav's hospital, Trondheim, Norway.

Parkinson's disease (PD) is a prevalent, late-onset movement disorder affecting ~2% of the population by 70 years of age. To date, mutations in 5 genes (SNCA, PARKIN, DJ-1, PINK1, and LRRK2) have clearly been implicated in familial forms of PD. In addition, seven other chromosomal loci have also been mapped for inherited forms of PD. Here we report a new locus for late-onset, levodopa-responsive, autosomal dominant parkinsonism in families from Norwegian and Tunisian populations. Genome-wide genotyping and statistical analysis of 63 multiplex kindreds with familial parkinsonism from Tunisia, North Africa, identified significant linkage to chromosome 6q23.1-q24.2 (MLS=4.5). The same chromosomal 6q23.1-q24.2 locus was concurrently identified by linkage analysis of autosomal dominant parkinsonism in a large Norwegian pedigree (MLS=2.3). Combined, the statistical evidence for a novel gene mutation(s) is unequivocal (MLS=6.), for which the LOD-1 peak spans a genomic interval of 10.8Mb. The geographic origin of linked families in Tunisia and Norway suggests the prevalence of chromosome 6q23.1-q24.2 linked disease may be globally widespread. LRRK2 G2019S provides a good precedent as the mutation appears to have originated in the Tunisian Arab Berber community, but is now found throughout the world and is a major contributor to risk. Preliminary data implicates nine kindreds in total, in which affected families members have been screened for all other known genetic causes of parkinsonism. High resolution molecular genetic mapping in linked pedigrees, including STR, high-density SNP genotyping and copy number assessment have refined the disease-linked genomic interval. Gene sequencing is being pursued in affected family members of the largest Norwegian kindred, for which there is significant linkage to identify the novel causative gene mutation.

870/T

Gene expression profiling of peripheral blood of patients with SCA1 and SCA3 identifies potential disease progression markers. *M. Walter[1], S. Poths[1], T. Schmitz-Hübsch[2], O. Riess[1], M. Bonin[1], The Eurosca Consortium.* 1) Microarray Facility Tübingen, Institute of Human Genetics, Department of Medical Genetics, Eberhard-Karls-University, Tübingen, Germany; 2) Dept. of Neurology, University of Bonn, Germany.

Spinocerebellar ataxias (SCAs) are dominant, late onset hereditary disorders characterized by a progressive ataxia that is variably associated with other neurological symptoms. The clinical hallmarks result from a progressive degenerative process that mostly affects the cerebellum, brainstem and spinal cord. To date at least 28 different loci are associated with SCAs and related diseases. Therefore, we used whole genome expression profiling of peripheral blood to search for easily accessible markers, which should i) differentiate between patients with different SCA types and ii) be able to monitor disease progression. Whole blood of 12 patients with SCA1 and 15 patients with SCA3 were analyzed on Affymetrix U133plus 2.0 Gene Chips. However, using Support Vector Machines (SVM) and Prediction Analysis for Microarrays (PAM) no predictor could be defined that clearly distinguish between the two disease types. This is most likely due to the large heterogeneity of the patients which had disease durations between two and over 20 years. To identify progression specific markers, the patient collective was subdivided in early, intermediate and late stage of disease according to their SARA value (Scale for the Assessment and Scaling of Ataxias). Transcripts, that showed differential expression between early and late stage patients, were identified and subsequently used to define a gene predictor set of 18 genes, which is able to correctly predict the disease stage of all patients of all three disease stages. Neither of these transcripts showed significant changes in age matched healthy control samples. Taken together, grouping of the patient samples according to their stage of disease progression enabled us to identify markers that did not change with age but with severeness of disease and could classify the patients into early, intermediate or late stage.

871/T

Pur Alpha Gene Mutations Are Not a Major Cause of Unexplained Spinocerebellar Ataxia. *T.A. Maher[1], G. Zhao[1], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

The inherited Spinocerebellar ataxias (SCAs) are a group of progressive neurologic disorders that have significant genetic heterogenecity and clinical variability. Clinical molecular testing is available for a growing number of these SCAs, but a significant group of symptomatic patients remain without a known etiology following genetic testing. We evaluated the Pur alpha gene (MIM 600473) as a candidate for causing SCA given its interaction with the FMR1 protein. Our laboratory, retrospectively, bidirectionally sequenced 200 samples for mutations in the Pur alpha gene. The cohort of samples were previously submitted for SCA testing with negative results for SCA1, 2, 3, 6, 7, 8, 10, 12, 17 and DRPLA. This cohort of samples was also screened for the $(CGG)_n$ repeat in the Fragile X mental retardation syndrome gene (FMR1). The FMR1 repeat has been associated with Fragile-X Tremor/Ataxia Syndrome (FXTAS) in patients with repeat sizes in the premutation range of Fragile X. Pur alpha is a ubiquitously expressed sequence-specific DNA- and RNA- binding protein. It has been implicated in the transcripitional control of neuronal genes such as myelin basic protein (MBP), gata2 (mouse), and the nicotinic acetylcholine receptor gene (nAch). Pur alpha also binds triplet $(CAG)_n$ and $(CGG)_n$ repeat domains and interacts with the FMR1 protein. None of our cohort harbor mutations in the Pur alpha gene. Hence, Pur alpha gene mutations appear not to be a major cause of unexplained SCA.

872/T

Molecular survey of human Leber congenital amaurosis disease(LCA) in a consanguineous family collection from Saudi Arabia. *R. Chen[1,2,3], Y. Li[1,2], J. Peng[1,2], H. Wang[1,2], K. Lee[1], R. Gibbs[1,2,5], S.M. Leal[1,2], W. Lewis[2,4,6,7], J. Lupski[2,5,7], M.S. Bray[2], G. Mardon[2,3,4,5].* 1) Human Genome Sequencing Center,; 2) Molecular & Human Genetics,; 3) Program of Developmental Biology,; 4) Department of Ophthalmology,; 5) Program in Cell & Molecular Biology,; 6) Department of Medicine,; 7) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Leber congenital amaurosis (LCA) is the most common hereditary cause of visual impairment in infants and children and affects nearly 1 in 15,000 in the general population. Unfortunately, to date, no medical or surgical intervention has been shown to alter the natural course of LCA, nor has any pharmacologic therapy shown effect on modulating or moderating its progression. However, gene therapy has corrected the blinding phenotype in animal models of selected genetic forms and human clinical trials are in progress.

Analysis of the known LCA genes in patient collections indicates that many additional LCA genes remain to be identified. It has been estimated that mutations in known LCA genes account for about 63% of all cases. To clone additional LCA disease genes, we have collected DNA samples from 38 consanguineous geographically distinct families with recessive LCA. Among them, disease-associated markers in one family were previously mapped to the LCA3 locus on chromosome 14. These families have been screened for mutations in all coding exons of all twelve known LCA genes. As a result, known and novel mutation alleles have been identified in 9 families. Therefore, LCA-associated mutations in the other 28 families remain to be determined. Computer simulations indicated that several families are large enough to independently establish linkage or are sufficiently large to have a high conditional probability of being linked to one locus (LOD score > 3). Progress of analyzing these families will be reported.

873/T

Specific sequence variations within the 4q35 region are associated with FSHD. *R.J.L.F. Lemmers[1], M. Wohlgemuth[2], K. van der Gaag[1], P. van der Vliet[1], P. de Knijff[1], G.W. Padberg[2], R.R Frants[1], S.M. van der Maarel[1].* 1) Department of Human Genetics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands; 2) Department of Neurology, University Medical Center Nijmegen, P.O. box 9101, 6500 HB, Nijmegen, The Netherlands.

Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) is mainly characterized by progressive wasting and weakness of the facial, shoulder and upper-arm muscles. FSHD is caused by contraction of the macrosatellite repeat D4Z4 on chromosome 4q35. The D4Z4 repeat is very polymorphic in length and D4Z4 rearrangements occur almost exclusively via intrachromosomal gene conversions. Several disease mechanisms have been proposed, but none of these models can comprehensively explain FSHD as conditions in addition to repeat contraction need to be met to cause disease. Almost identical D4Z4 repeat arrays have been identified on chromosome 10q26 and on two equally common chromosome 4 variants; 4qA and 4qB. Yet, only repeat contractions of D4Z4 on chromosome 4qA cause FSHD, contractions on the other chromosomes are not pathogenic. We hypothesized that allele-specific sequence differences between 4qA, 4qB and 10q alleles underlie the 4qA specificity of FSHD. Sequence variations between these alleles have been described before, but the extent and significance of these variations proximal, within and distal to D4Z4 have not been studied in detail. We examined additional sequence variations in the FSHD locus including a relatively stable simple sequence length polymorphism (SSLP) proximal to D4Z4, a SNP within D4Z4 and the A/B variation distal to D4Z4. Based on these polymorphisms, we demonstrate that this subtelomeric domain of chromosome 4q can be subdivided into nine distinct haplotypes, of which three carry the distal 4qA variation. Interestingly, we show that repeat contractions in one of these 4qA haplotypes is not associated with FSHD. We also show that each of these haplotypes has its unique sequence signature and propose that specific SNPs in the disease haplotype are essential for the development of FSHD.

874/T

Sequence Analysis of the SCN9A Gene in a Familial Form of Adult-Onset Erythromelalgia. *T.Z. Fischer[1,2,3], S.D. Dib-Hajj[1,2,3], L. Tyrrell[1,2,3], F.M. Hisama[4], S. Novella[1], L. Marshall[1], S.G. Waxman[1,2,3].* 1) Dept Neurology, Yale Univ, New Haven, CT; 2) Center for Neuroscience & Regeneration Research, Yale Univ, New Haven, CT; 3) Rehabilitation Research Center, VA CT Healthcare System, West Haven, CT; 4) Dept Genetics, Children's Hospital Boston, Boston, MA.

Voltage-gated sodium channels play a major role in the pathogenesis of chronic pain in peripheral neuropathies. Alterations in the expression and targeting of specific sodium channels within the DRG neurons appear to predispose the neurons to abnormal firing in acquired channelopathies, and mutations in one channel, Nav1.7, have been linked to inherited painful neuropathies. Early-onset primary erythromelalgia is an autosomal dominant disorder that is characterized by episodic burning pain associated with redness and warmth of the affected extremities, often relieved by cooling. The etiology of this disease was unknown until recently when mutations were identified in the SCN9A gene, encoding the Nav1.7 voltage-gated sodium channel, indicating that erythromelalgia is a neuronal channelopathy. Mutations in Nav1.7 from erythromelalgia patients lower the threshold for single action potentials and induce higher firing frequency of nociceptive DRG neurons. The molecular basis of familial adult-onset erythromelalgia is less well understood, however. We enrolled a large family who met the clinical criteria for this disease, and screened for mutations within SCN9A by direct sequencing of PCR amplicons of exons and the immediately flanking intron sequences. A polymorphic substitution, R1150W, was identified in the proband. This allele was previously identified in sporadic cases of early- and adult-onset erythromelalgia, but importantly, it is present in 14% of a control sample. Thus, the disease-causing potential of this substitution is not clear. The chromosomal localization of a potential disease locus for adult-onset erythromelalgia is being investigated by genome-wide SNP analysis. Increasing the database of mutations in SCN9A will enable us to establish a genotype-phenotype relationship, and the identification of a new locus will hopefully permit the design of mechanism-based treatment for these painful neuropathies.

875/T

Type-2 NF1 deletions are highly unusual by virtue of the absence of non-allelic homologous recombination hotspots and an apparent preference for female mitotic recombination. *H. Kehrer-Sawatzki[1], K. Steinmann[1], D.N. Cooper[2], L. Kluwe[3], N.A. Chuzhanova[4], C. Senger[1], E. Serra[5], C. Lazaro[6], M. Gilaberte[7], K. Wimmer[8], V.F. Mautner[3].* 1) Institute of Human Genetics, University of Ulm, Germany; 2) Institute of Medical Genetics, Cardiff University, Heath Park, CF14 4XN, Cardiff, UK; 3) Department of Maxillofacial Surgery, University Hospital Eppendorf, Hamburg, Germany; 4) Department of Biological Sciences, University of Central Lancashire, Preston, UK; 5) Centre de Genètica Mèdica i Molecular-Institut de Recerca Oncològica (IRO)-Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Hospital Duran i Reynals, Barcelona, Spain; 6) Translational Research Laboratory-IDIBELL, Institut Català d'Oncologia, Hospital Duran i Reynals, Barcelona, Spain; 7) Department of Dermatology, Hospital del Mar-IMAS, Barcelona, Spain; 8) Department of Medical Genetics, Medical University of Vienna, Vienna, Austria.

Five percent of patients with NF1 exhibit gross deletions that encompass the NF1 gene and its flanking regions. The breakpoints of the common 1.4 Mb (type-1) deletions are located within low-copy repeats (NF1-REPs) and cluster within a 3.4 kb hotspot of non-allelic homologous recombination (NAHR). Here we present the first comprehensive breakpoint analysis of type-2 deletions, a second type of recurring NF1 gene deletions. Type-2 deletions span 1.2 Mb and the breakpoints are located within the SUZ12 gene and its pseudogene. Breakpoint analysis of 13 independent type-2 deletions did not reveal any hotspots of NAHR. Intriguingly, 12 of the 13 type-2 deletions are characterized by somatic mosaicism indicating a positional preference for NAHR within the NF1 gene region. All mosaic type-2 deletions were found in females what contrasts with the equal gender distribution noted for type-1 NF1 deletions. Whereas type-1 deletions are caused by interchromosomal meiotic NAHR, type-2 deletions are generated by intrachromosomal NAHR between SUZ12 and its pseudogene during mitosis. Such a clear distinction between the preferred sites of mitotic versus meiotic NAHR is unprecedented in any other genomic disorder.

876/T

Coinheritance of a novel deletion of the entire SPINK1 gene with a CFTR missense mutation (L997F) in a family with chronic pancreatitis. *E. Masson[1, 2], C. Le Maréchal[1, 2, 3, 4], P. Lévy[5], N. Chuzhanova[6], P. Ruszniewski[5], D. Cooper[7], J. Chen[1, 3], C. Férec[1, 2, 3, 4].* 1) INSERM U613, BREST, France; 2) Faculté de Médecine de Brest et des Sciences de la Santé, Université de Bretagne Occidentale,Brest, France; 3) Etablissement Français du Sang - Bretagne,Brest, France; 4) Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Universitaire de Brest, Hôpital Morvan, Brest, France; 5) Pôle des Maladies de l'Appareil Digestif, Service de Gastroentérologie-Pancréatologie, AP-HP, Hôpital Beaujon, Clichy, France; 6) Department of Biological Sciences, University of Central Lancashire, Preston PR1 2HE, United Kingdom; 7) Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom.

Quantitative fluorescent multiplex PCR (QFM-PCR) was established in order to make possible the rapid and efficient analysis of the pancreatic secretory trypsin inhibitor (SPINK1) gene. Using QFM-PCR, a novel deletion encompassing the entire SPINK1 gene was identified in one of nine newly recruited French Caucasian families with chronic pancreatitis. The breakpoints were fully characterized and the ~30 kb deletion was termed c.1-15969_c.240+7702del30588bp. Whilst sequences with the potential to form non-B DNA structures were found to span both the 5' and 3' deletion breakpoints, the generation of this gross deletion is potentially explicable in terms of non-homologous end-joining facilitated by the presence of a 1-bp microhomology at the two ends. The SPINK1 gene deletion identified in the index patient was also detected in her affected father and paternal uncle but not in 50 healthy French Caucasians. Remarkably, in all three affected individuals, the SPINK1 deletion was found to occur in trans with a p.L997F missense mutation in the unlinked CFTR gene, a lesion which has been previously reported to be associated with a variety of cystic fibrosis-related diseases including idiopathic pancreatitis. Given that the SPINK1 deletion constitutes a clear-cut disease-causing factor, it may be that the CFTR missense mutation acts as a disease modifier in the context of this particular family.

877/T

Comprehensive analysis of breakpoints of PARK2 rearrangements in patients with autosomal recessive juvenile parkinsonism (AR-JP) employing a high-density tiling array-based comparative genomic hybridization (array-CGH) system. *J. Mitsui[1], Y. Takahashi[1], H. Tomiyama[2], H. Yoshino[2], J. Goto[1], Y. Mizuno[2], N. Hattori[2], S. Tsuji[1].* 1) Department of Neurology, the University of Tokyo, Tokyo, Japan; 2) Departmen of Neurology, Juntendo University, Tokyo, Japan.

[INTRODUCTION] Autosomal recessive juvenile Parkinsonism (AR-JP) is one of the most common hereditary Parkinson diseases, which is caused by mutations in PARK2. Among PARK2 mutations, 50-90% of mutants have been reported to be gross deletions involving exons. Although PCR-based gene dosage analysis has been commonly employed to identify rearrangements, there are limitations in the accuracy of the quantification of the gene dosage, in the sensitivity for identification of compound heterozygous deletions and in the determination of the exact breakpoints. To overcome these limitations, we have developed a high-density tiling array-based comparative genomic hybridization (array-CGH) system focusing on PARK2. [SUBJECTS AND METHODS] 124 AR-JP patients with exon rearrangements determined by the TaqMan assay were enrolled in this study. For the array-CGH analysis, we originally designed 35,668 probes consisting of 45-60 mer oligonucleotides to cover the entire PARK2 gene with its flanking regions of 300 kb employing the Agilent's platform. The average interval between the neighboring probes was 112 bp. [RESULTS] We determined exact breakpoints of 199 mutated alleles of the 124 AR-JP patients consisting of 100 deletion and 6 duplication breakpoints. 92 breakpoints were observed individually, suggesting that founder effects account for relatively limited proportions and many deletions occur independently. The 5' and 3' ends of breakpoints were clustered within intron 2 and intron 4, respectively. [DISCUSSION] With the array CGH analysis system, we were able to precisely determine the rearrangements including those in compound heterozygous states. The clustering of breakpoints clearly indicated existence of the hotspots. Interestingly, the hotspots are located in the center of the common fragile site, FRA6E, raising the possibility that the hotspots of rearrangements in PARK2 share properties as the common fragile site.

878/T

Evolutionary conservation of a coding function for D4Z4, the tandem DNA repeat mutated in facioscapulohumeral muscular dystrophy. *J.E. Hewitt[1], J. Clapp[1], L.M. Mitchell[1], D.J. Bolland[2], J. Fantes[3], A.E. Corcoran[2], P.J. Scotting[1], J.A.L. Armour[1].* 1) Institute of Genetics, Queens Medical Centre, Univ Nottingham, Nottingham, UK; 2) Laboratory of Chromatin & Gene Expression, Babraham Institute, Cambridge, CB2 4AT, UK; 3) MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, UK.

The autosomal dominant neuromuscular disorder facioscapulohumeral muscular dystrophy (FSHD) is caused by an unusual type of mutation; deletions within the polymorphic DNA tandem array D4Z4. Each 3.3kb D4Z4 repeat unit has an open reading frame (ORF), termed *DUX4*, containing two homeobox sequences. However, because the repeat appeared to be poorly conserved and there has been no evidence for a transcript from the array, D4Z4 is generally believed to have only a non-coding function. Accordingly, D4Z4 deletions are thought to cause FSHD by a chromatin position effect on the expression of genes in *cis* on chromosome 4q. We used data from whole genome sequencing projects to identify D4Z4 homologues in the genomes of rodents and Afrotheria (elephants and related species). We found conservation of both the *DUX4* ORF and the tandem array organization in these homologues. This is the first identification of D4Z4 sequences in any species other than apes. Phylogenetic analysis indicates that primate and Afrotherian D4Z4 arrays are orthologous and originated from a retrotransposed copy of an intron-containing DUX gene. RT-PCR, RNA fluorescence and tissue *in situ* hybridization data indicate transcription of the mouse Dux array. Our data strongly supports a coding function for human D4Z4 and necessitates re-examination of current models of the FSHD disease mechanism.

879/T

First Evidence of a Pathogenic Insertion in the NOTCH3 Gene Causing CADASIL. *C. Ungaro[1], F.L. Conforti[1], D. Guidetti[2], M. Muglia[1], G. Cenacchi[3], P.L. Lanza[1], A. Patitucci[1], T. Sprovieri[1], A. Magariello[1], A.L. Gabriele[1], L. Citrigno[1], R. Mazzei[1].* 1) Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone, Cosenza; 2) UOC di Neurologia, Ospedale Guglielmo da Saliceto di Piacenza; 3) Department of Radiological and Istocytopathological Sciences, Section of Pathology, University of Bologna.

CADASIL is an autosomal dominant disorder leading to cognitive decline and dementia. Mutations in the NOTCH3 gene are responsible. These highly stereotyped mutations are located within the 22 exons, encoding for the 34(EGF)-like repeats of the extracellular domain of the Notch3 receptor, all mutations resulting either in a gain or loss of a cysteine residue. Therefore it has been suggested that the unpaired cysteine residues, generated by these mutations may cause aberrant interaction of the Notch3 receptor with its ligands. In the present study we examined the NOTCH3 gene exons in a subject with clinical and radiological findings consistent with CADASIL having distinctive GOM deposits in her skin biopsy. The proband underwent MRI investigation of the brain that revealed a severe diffuse leukoencephalopathy and multiple lacunar lesions. The proband was analyzed for mutations in the NOTCH3 gene using the DHPLC analysis and direct sequence. The examination was extended to the proband's family: an affected mother and an unaffected sister. DHPLC analysis revealed a variant profile in exon 3 of both proband and her mother. Sequencing of the exon 3 showed a 3bp insertion (nt 357ins TGC) in the second EGF-like repeat, resulting in an insertion of a cysteine residue. This mutation was not observed in 560 control chromosomes. Using both DHPLC analysis and direct sequence, in the subjects carrying the mutation in exon 3 no mutation was found in the other exons containing EGF-like repeats (exons 2-23). The insertion together with the neurological and clinical phenotype suggests it is the pathogenic mutation in our patient. This novel pathogenic mutation represented by the first insertion found in a CADASIL patient, suggests that the change towards an unpaired reactive cysteine residue is a very critical molecular event in CADASIL.

880/T

Frequency analysis of autosomal dominant Spinocerebellar Ataxia (AD-SCA) in the patients from southern Italy. *E. Mannarino, P. Tarantino, D. Civitelli, I.C. Cirò Candiano, S. Carrideo, F.E. Rocca, F. Annesi, E.V. De Marco, G. Provenzano, V. Greco, G. Annesi.* Inst Neurological Sci, National Research Council, Cosenza, Italy.

Autosomal dominant spinocerebellar ataxias (AD-SCA) constitute a clinically, genetically and pathologically heterogeneous group of neurodegenerative disorders characterized by degeneration of spinocerebellar pathways with variable involvement of other neural systems. These disorders are caused by variable trinucleotide repeat expansions within SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17 and DRPLA genes. The aim of our study was to estimate the frequency of AD-SCA in patients from Southern Italy. We analyzed 945 subjects affected by progressive ataxia as a cardinal clinical feature, by pyramidal and extrapyramidal signs and by peripheral neuropathy. The known trinucleotide repeat expansions were assessed in the SCA1, SCA2, SCA3 SCA6, SCA7, SCA8, SCA12, SCA17 and DRPLA genes. Genomic DNA was amplified with fluorescent primers spanning the SCA expansions and PCR products were separated onto a capillary sequencer. Genetic analysis showed the presence of pathological repeat expansions in SCA1, SCA2 and SCA17 genes in a portion of the examined patients, and allowed us to identify 33 individuals affected with SCA1 belonging to 18 families; 42 individuals affected with SCA2 belonging to 28 families and 3 individuals affected with SCA17 belonging to 3 families. No cases of SCA3, SCA6, SCA7, SCA8, SCA12 and DPRLA were identified. Compared with the overall Italian distribution, the mutation distribution within the AD-SCA genes in Southern Italy appears to be peculiar. Indeed, the frequency of SCA1 expansion is higher than in Middle and in Northern Italy and, on the contrary, SCA3, SCA6, SCA7, SCA8, SCA12 and DRPLA expansions are absent in our sample. SCA2 expansion is the most frequent, while only a few patients carrying SCA17 expansion were detected. Moreover, our results suggest an involvement of additional loci associated with AD-SCA in the patients in whom genetic analysis excluded the presence of pathological repeat expansions in the herein explored genes.

881/T

Investigating CAG repeat instability in Huntington's disease. *M. Swami, E. Dragileva, A. Teed, T. Gillis, E. Lopez, M.E. MacDonald, V.C. Wheeler.* CHGR, MGH, Boston, MA.

Huntington's disease (HD) is a progressive neurodegenerative disease caused by the expansion of a polymorphic CAG repeat in exon 1 of the *HD* gene over 35 repeats. Mutant expanded CAG repeats are unstable both in the germline and somatic tissues of HD patients. The highest levels of somatic instability are observed in the striatum and cortex, which are the tissues most susceptible to HD pathogenesis. We are investigating the mechanisms underlying *HD* CAG repeat instability and the role of somatic instability in HD pathogenesis. Genetic modifiers of instability are being investigated in Hdh^{Q111} mice, an accurate genetic knock-in mouse model of the disease, previously shown to recapitulate both the germline and tissue-specific somatic repeat instability observed in HD patients. Hdh^{Q111} mice have been crossed to various lines of mice deficient in DNA repair proteins and analysed for germline and somatic repeat instability and for the nuclear localization of mutant huntingtin, an early striatal-specific phenotype. Crosses with mice deficient in mismatch repair protein Msh2 and its binding partners Msh3 and Msh6 reveal that Msh2 and Msh3, but not Msh6, are needed for expansions in the male germline and in the striatum. This suggests that germline and somatic instability are mediated via Msh2-Msh3 complexes rather than Msh2-Msh6 complexes. Loss of Msh2 or Msh3 slows the nuclear accumulation of mutant huntingtin. Crosses with mice deficient in Xpc, a component of the nucleotide excision repair pathway, indicate that this pathway is not a major player in *HD* CAG repeat instability. The delayed nuclear huntingtin accumulation in the absence of Msh2 or Msh3 suggests that somatic expansion contributes to the disease process. We are investigating this hypothesis in HD patient brain samples. Using a sensitive small-pool PCR analysis we are quantifying the degree of somatic repeat instability in the cortex of HD patients that exhibit "extreme early" or "extreme late" age of onset as predicted by their mutant CAG repeat length in order to test whether patients with higher levels of somatic expansion have earlier ages of disease onset.

882/T

Inhibition of caspase-7 proteolytic cleavage of ataxin-7 markedly ameliorates polyglutamine neurotoxicity in SCA7 transgenic mice. *S.J. Guyenet[1], A. Lin[2], B.L. Sopher[1], S.K. Custer[1], J.E. Young[1], L.M. Ellerby[2], A.R. La Spada[1].* 1) Dept Laboratory Medicine, Univ Washington, Seattle, WA; 2) Buck Institute for Ageing Research, Novato, CA.

Spinocerebellar ataxia type 7 (SCA7) is a dominantly inherited neurodegenerative disease caused by a CAG repeat expansion located in the 5' coding region of the ataxin-7 gene. The CAG repeat expansion encodes an abnormally long glutamine tract that is expressed in the ataxin-7 protein, rendering it toxic. Neuronal loss and gliosis occur in the retina, cerebellum, and associated brainstem structures. This leads to progressive blindness and a debilitating lack of coordination. Neuronal intranuclear inclusions, composed of truncated ataxin-7, accumulate in many brain regions. Among the polyglutamine diseases, Huntington's disease, SCA3, SBMA, DRPLA and SCA7 all exhibit proteolytic cleavage that exacerbates aggregation and toxicity. Ataxin-7 contains a predicted caspase cleavage site at an aspartic acid residue at position 266, and we have shown that caspase-7 can cleave the ataxin-7 protein at this site. Furthermore, cleavage-resistant polyglutamine-expanded ataxin-7 is less toxic than wild-type ataxin-7 in vitro. To test the hypothesis that caspase-7 cleavage of ataxin-7 contributes to SCA7 disease pathogenesis, we generated SCA7 transgenic mice with or without a point mutation (D266N) targeted to this site. After confirming that we had derived independent SCA7 transgenic lines with comparable levels of transgene expression, we evaluated the biochemical and phenotypic features of the SCA7-92Q-D266N mice. In contrast to SCA7-92Q-wt mice, ataxin-7 protein expressed by SCA7-92Q-D266N mice is resistant to proteolytic cleavage by caspase-7. Based upon behavioral phenotyping, survival, and histopathology analysis, SCA7-92Q-D266N mice display a markedly attenuated neurodegenerative phenotype in comparison to SCA7-92Q-wt mice. Our findings indicate that caspase-7-mediated proteolytic cleavage of ataxin-7 constitutes a key step in the SCA7 pathogenic cascade, and provides a rational target for directed therapy development for this currently untreatable disorder.

883/T

MNGIE disease: Four novel mutations in five Mexican families. *N. Monroy, L. Macías, J. Arteaga, O. Mutchinick.* Genetics, National Institute of Medical Sciences and Nutrition,Salvador Zubirán. Mexico City, Mexico.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare autosomal recessive disease caused by mutations in the nuclear gene encoding thymidine phosphorylase (TP) that generate diverse alterations of mitochondrial DNA (mtDNA). MNGIE is clinically defined by gastrointestinal dismotility, cachexia, ptosis, opthalmoparesis and peripheral neuropathy. At this point of time, 87 cases have been described as the result of 52 different TP mutations. We describe the clinical and molecular characteristics of 8 affected individuals in five Mexican families. A total of 28 relatives were also included; 18 of which were heterozygous carriers and 10 were wild type homozygous. Clinical data, neuro-physiological, and manometric tests were performed. Genomic DNA (and total RNA, when necessary) was isolated and the complete gene sequenced. mt DNA was also studied. Computational analyzes of the DNA and protein sequences were done. Consanguinity was confirmed in 3 families and suspected in the other two. Families were grouped in two categories: 1) with diarrhea and 2) with vomiting and intestinal pseudo-obstruction. Individuals of group 1 are still alive, while 5/6 patients of group 2 died. Additionally other clinical variations regarding progression of the disease and main symptoms were observed. Sequencing of the TP gene revealed 4 new mutations: L133P, G152R, P300S, IVS5+110_111insAG and one previously described (S471L), all of them in homozygous state. In the fourth family (AG insertion) it is supposed that the mutation generated a new splicing acceptor site, but not mutant transcript was observed. In this case, we propose a mechanism of nonsense-mediated mRNA decay. The mtDNA analysis showed multiple deletions and a 65-96% depletion in all patients. An interesting finding is the interfamilial variability for age onset, progression and gastrointestinal symptoms and the high intrafamilial concordance for the same variables. The last could be explained because the patients share the same mutations.

884/T

Detection of deletion and duplication of dystrophin gene with MLPA in a subset of Chinese DMD/BMD patients. *X-Z. Wang[1,2], Z. Wang4[4], M. Yan[1,2], S-J. Song[1,2], Y-Z. Zhang[1,2], J-H. Zou[1,2], S-Z. Huang[4], T.J. Chen[5], N. Zhong[1,2,3].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Peking University Health Science Center; 3) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 4) Peking Union Medical College, Beijing, China; 5) University of South Alabama, Mobile, AL.

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are common X-linked recessive neuromuscular degeneration diseases. DNA rearrangement including deletion and duplication was determined as the major mutation underlying the DMD/BMD. To further investigate the details of deletion and duplication, we applied multiplex ligation-dependent probe amplification (MLPA) analyzed 143 unrelated Chinese DMD/BMD patients and 19 obligated female carriers. The overall detection rate was 72%, which includes 67.1% deletion and 4.9% of duplication. We determined that exon 51 was the most common exon deleted in single-exon deletion, and exons 48-50 and exons 45-50 are the most common in multi-exons deletion. We observed that about 90% of clinically diagnosed DMD/BMD cases carry small size deletion that involves 10 exons or less, including 25% cases carry a single-exon deletion. The most common region of the DMD gene deletions was further determined between exon 48 and exon 51. There is no 5' hotspot region can be characterized in this study, however, the 3' hotspot region was between exon 45 and exon 55. Most of small deletion, as well as the larger duplication, resulted in out-of-frame mutation. The rate of deletion and duplication in dystrophin gene is similar to that of western countries in Chinese population, however we did not find the 5' hotspot region in this study. We conclude that MLPA is a sensitive and effective method to detect duplication and deletion mutation, which should be applied as a first line screening for DMD/BMD in clinical practice.

885/T

Startle disorder, Hyperekplexia, is primarily a recessive disorder. *S. Chung[1], A. Robertson[1], C. Hammond[1], R.J. Harvey[2], M.I. Rees[1].* 1) Neuroscience, University of Wales Swansea, Swansea, United Kingdom; 2) 2Department of Pharmacology, The 2Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London, UK.

Glycinergic neurotransmission is a major inhibitory system in the central nervous system (CNS) and defects in glycinergic genes are associated with a startle disorder, hyperekplexia. This rare, but potentially fatal, neurological disorder is primarily a hereditary disorder, typically associated with dominant mutations in the glycine receptor α1 subunit, GLRA1. As part of an ongoing 10-year screening program, we have analysed the entire coding regions of GLRA1 within 56 sporadic patients. Of the 21 hyperekplexia mutations identified in this study, 16 were inherited in a recessive mode or part of compound heterozygosity. Consistent with previous studies, all deletion and nonsense mutations were associated with recessive onset of phenotype, where as missense mutations could exert an effect either as dominant or recessive traits depending on the position of the mutation in the polypeptide. This study indicates, that on a population basis, recessive hyperekplexia is more common than expected and that the previous label and reference towards a 'dominant disorder' was an ascertainment bias on familial presentation and founder linkage analysis cohorts. The discovery of mutations in the glycine transporter-2 gene in hyperekplexia in 2006 also displays predominantly recessive inheritance and compound heterozygosity. In contrast to other diseases caused by dysfunction of ion channels or murine hyperekplexia models, patients with recessive mutations/null hyperekplexia mutations in GLRA1 were not particularly associated with severe cases of hyperekplexia. The explanation for tolerance of null GLRA1 gene function in humans is likely due to a compensatory mechanism by other neuro-inhibitory mechanisms. The discovery of hyperekplexia associated mutations and biophysical studies of the mutations will provide invaluable opportunities to study the pathophysiology of this neuromotor disorder.

886/T

SPASTIN GENE MUTATIONS IN ITALIAN PATIENTS WITH PURE AND COMPLICATED FORMS OF SPASTIC PARAPLEGIA. *A. Magariello[1], A. Patitucci[1], RL. Mazzei[1], F.L. Conforti[1], A.L. Gabriele[1], T. Sprovieri[1], C. Ungaro[1], L. Citrigno[1], A. Gambardella[2], F. Bono[2], T. Piccoli[3], F. Patti[4], M. Zappia[4], M. Muglia[1].* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, University 'Magna Graecia', Catanzaro, Italy,; 3) Department of Clinical Neurosciences, University of Palermo, Palermo, Italy; 4) Department of Neurosciences, University of Catania, Catania, Italy.

Mutations in the spastin gene are the commonest cause of spastic paraplegia accounting for up to 40% of autosomal dominant (ADHSP) and 12% of sporadic cases. The phenotype associated with disease due to mutations in the spastin gene (SPG4) tends to be pure. However, there is increasing evidence of patients with complicated forms of spastic paraplegia in which spastin mutations have been identified. To characterize in more detail the genetic and phenotypic characteristic of SPG4, we examined twenty unrelated Italian patients. The mutational screening of the spastin gene was performed by Denaturing High Performance Liquid Chromatography (DHPLC) and sequence analysis. Sixteen patients showed a pure form of spastic paraplegia (11-ADHSP and 5-sporadic) and four patients had a complicated phenotype of whom 2-ADHSP resulted with ataxia and 2-sporadic with cerebellar signs. We detected 8 different mutations, 4 of which were novel (Glu143fsX, c.1687-3C>G, Asp548Asn, Gln568X). One possible pathogenic variant (2*G>T) was also identified in the 3'UTR of the gene after two nucleotides from the stop codon. The overall rate of mutation in the spastin gene in our sample was 40% (8/20). Two out of 8 mutations were detected in sporadic patients affected by a complicated phenotype with cerebellar signs, whereas six mutations were found in patients with a pure ADHSP. The obtained results confirm that the spastin screening has be performed in complicated cases of HSP. The identification of mutations in the spastin gene in patient with complicated forms is important in order to characterize in more detail the genetic and phenotypic characteristic of SPG4.

887/T

Charcot-Marie-Tooth X-linked: five novel mutations in Italian patients. *A. Patitucci[1], A. Magariello[1], A.L. Gabriele[1], R. Mazzei[1], F.L. Conforti[1], T. Sprovieri[1], C. Ungaro[1], L. Citrigno[1], P. Valentino[2], C. Rodolico[3], A. Mazzeo[3], A. Toscano[3], M. Muglia[1].* 1) ISN-CNR, Mangone Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Department of Neurology, University of Messina, Italy.

Charchot Marie Tooth disease represents a clinically and genetically heterogeneous group of disorders affecting the peripheral nervous system. The most common form, CMT1A, is usually due to a 1.4 Mb duplication in the chromosome 17p11.12 containing the PMP22 gene. CMT X-linked is the second most common form of CMT disease caused by mutations in the gap junction protein beta 1 (GJB1) gene encoding for the connexin 32 (Cx32) located in the X-q13 region. We studied nine subjects from unrelated Italian families with a possible X-linked peripheral neuropathy, and one sporadic subject with clinical and electrophysiological features similar to those observed in X linked neuropathy. The coding region of the GJB1 gene was amplified using primers reported by Bergoffen et al. Three reactions were performed to obtain three overlapping fragments and analyzed by Denaturing High Liquid Chromatography WAVE system (DHPLC-Transgenomic). Direct sequencing of the exons with variant profiles was performed with a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) on the ABI3130 sequencer. The screening of the GJB1 gene revealed in ten unrelated Italian index cases five novel mutations (Ser49Phe, Ala88Val, Ser128Leu, Arg164Leu, and Phe153Leu) and five known mutations (Tyr7Cys, Arg164Gln, Arg183Hys, Arg183Cys, and Met194Val). The sporadic patient carried the Ser49Phe mutation that is a de novo mutation because it was not found in his mother. The Ser49Phe occurs in the first EC domain of the Connexin 32 protein which is thought to play a role in interconnecting Cx32 hemichannels. Phe153Leu and Arg164Leu occur in the EC domain too, instead Ser128Leu and Ala88Val occur in the intracellular domain and transmembrane domain respectively; however all five aminoacids are also highly conserved in the GJB1 proteins among all mammalian species suggesting a functional role for the above mentioned aminoacids at these positions.

888/T

Mutational spectrum of spatacsin-associated spastic paraplegia (SPG11). *P. Bauer[1], U. Hehr[2], B. Winner[3], R. Schuele[4], W. Koehler[5], G. Uyanik[3], A. Engel[1], A. Hehr[2], S. Ploetz[3], J. Gamez[6], A. Rolfs[7], A. Oelmez[8], M. Bonin[1], H. Topaloglu[8], U. Bogdahn[3], B.H.F. Weber[2], L. Schoels[4], O. Riess[1], J. Winkler[3].* 1) Dept Medical Genetics, Univ Tubingen, Tubingen, Germany; 2) Dept of Human Genetics, Univ Regensburg, Regensburg, Germany; 3) Dept of Neurology, Univ Regensburg, Regensburg, Germany; 4) Research Division for Clinical Neurogenetics, Centre of Neurology and Hertie-Institute for Clinical Brain Research, Univ Tuebingen, Tuebingen, Germany; 5) Dept of Neurology, Hospital Hubertusburg, Wermsdorf, Germany; 6) Dept of Neurology, Hospital General Vall d'Hebron, Barcelona, Spain; 7) Dept of Neurology, Univ Rostock, Rostock, Germany; 8) Dept of Pediatric Neurology, Hacettepe University, Ankara, Turkey.

Hereditary spastic paraplegia (HSP) comprise a heterogeneous group of neurodegenerative disorders resulting in progressive spasticity of the lower limbs. One form of autosomal recessive HSP (ARHSP) with thin corpus callosum (TCC) was linked to chromosomal region 15q13-15 (SPG11) and recently associated with mutations in the Spatacsin gene. By means of direct sequencing, a total of 11 causal mutations were identified in the KIAA1840 coding sequence (now designated Spatacsin) including 3 in consanguineous pedigrees from Turkey, one from Saudi Arabia and Germany as well as in 4 sporadic patients of German or Spanish origin. All mutations identified represent truncating mutations and include one previously reported and 6 novel frame-shift mutations as well as two novel nonsense mutations. Furthermore, we report two different splice mutations associated with the SPG11 phenotype. These mutations have been validated by mRNA analyses in peripheral blood cell transcripts. Mutations are distributed throughout the first 30 exons of the Spatacsin gene without obvious clustering in mutational hotspots. In conclusion, we could (i) demonstrate Spatacsin-mutations in our linked SPG11 families, (ii) extend the mutational spectrum for Spatacsin-mutations being scattered throughout the coding sequence, (iii) demonstrate that SPG11 is a recognizable clinical condition even in unlinked sporadic paraplegic patients with thin corpus callosum (TCC).

889/T

A NOVEL MISSENSE MUTATION OF THE NF2 GENE IN A SEVERELY AFFECTED BOY AND HIS HEALTHY FATHER. *A.L. Gabriele[1], M. Ruggieri[2,3], C. Nucifora[3], A. Patitucci[1], T. Sprovieri[1], A. Magariello[1], R. Mazzei[1], F.L. Conforti[1], C. Ungaro[1], M. Muglia[1], A. Quattrone[1,4].* 1) Institute of Neurological Sciences (ISN)-CNR, Mangone , Cosenza, Italy; 2) ISN, CNR, Section of Catania, Italy; 3) Department of Paediatrics, University of Catania, Italy; 4) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy.

Neurofibromatosis type 2 (NF2) is an autosomal dominant disease characterised by the development of multiple nervous system tumours and skin and ocular abnormalities. Inactivating mutations in the NF2 tumour-suppressor gene, located on 22q12, causes the disease. We studied the NF2 gene in a severely affected boy and his family. An 8-year-old boy was referred because of multiple cutaneous café-au-lait spots and scoliosis. General examination revealed besides the café-au-lait spots, a moderate scoliosis and NF2-plaques. Magnetic resonance imaging (MRI) of the brain and spine, revealed a massive high signal lesion extending over almost the entire spine (ependymoma). At age 11 years, MRI revealed bilateral vestibular schwannomas and spinal schwannomas in the lumbar spine. There were no other NF2 stigmata after full clinical and imaging NF2 screening in the boy and his 12-year-old sister and in both parents. Screening of the entire coding region sequence of the NF2 gene by DHPLC analysis showed a modified pattern for exon 12. Direct sequencing revealed a heterozygous missense mutation at the nucleotide 1127 resulting in an aminoacid substitution arginine-376 by glutamine (R376Q). The mutation was also detected in the father's patient and was not found in 100 normal chromosomes. Impairment of the functional domain of the missense mutation here reported could abolish the NF2 tumour- suppressor activity determining the NF2 clinical phenotype we recorded. Notably, however, missense mutations are usually mild, often causing the mildest form of NF2. Remarkably, in this family we observed an early onset and severe phenotype in the boy and lack of clinical/imaging signs in his father who harboured the same NF2 gene mutation.

890/T

A comprehensive mutational analysis system using resequencing microarray delineates molecular epidemiology of hereditary spastic paraplegias in the Japanese population. *H. Ishiura, Y. Takahashi, J. Goto, S. Tsuji.* Dept. of Neurology, Univ. of Tokyo, Tokyo, Japan.

[Objective] To establish a high-throughput comprehensive mutational analysis system for hereditary spastic paraplegia (HSP) and to describe molecular epidemiology of HSP in the Japanese population. [Background] HSP is a neurodegenerative disorder characterized by progressive lower limb spasticity. Up to present, SPGs 1-36 have been identified as the genetic loci and the number of causative genes has been increasing. Since HSP is genetically heterogeneous and little is known about genotype-phenotype correlations, comprehensive analysis system is necessary. [Methods] We established a high-throughput DNA microarray resequencing system based on GeneChip™ (Affymetrix), capable of analyzing complete nucleotide sequences of all the coding exons and splicing junctions of 9 causative genes (SPG1: *L1CAM*, SPG2: *PLP1*, SPG3A: *atlastin*, SPG4: *spastin*, SPG6: *NIPA1*, SPG7: *paraplegin*, SPG10: *KIF5A*, SPG20: *spartin*, and SPG31: *REEP1*). Given the high frequency of insertion/deletion mutations in *spastin*, conventional direct nucleotide sequence analysis was also employed. The study enrolled 80 Japanese HSP patients: 29 patients with autosomal dominant inheritance (ADHSP), 8 patients born to consanguineous parents, 5 patients with possible family histories and 38 apparently sporadic patients. [Results] We identified 15 *spastin* mutations among the ADHSP patients (15/29: 52%), and 1 *atlastin* mutation (1/29: 3.4%). Another *spastin* mutation was found in a patient with family history. Two mutations in *spastin* and 1 novel amino acid substitution in *atlastin* were found among apparently sporadic patients (2/38: 5.3% and 1/38: 2.6%, respectively). Of the 18 patients with *spastin* mutation, 10 were null mutations and 2 were splice site mutations, suggesting that haploinsufficiency is a major mechanism responsible for SPG4. [Conclusions] SPG4 is the most common ADHSP. The frequency of SPG4 among ADHSP is higher than previously reported. SPG3A in young onset ADHSP also exists in the Japanese population. The system is highly effective for comprehensive mutational analysis of such heterogeneous disorders as HSP.

891/T

A Novel missense mutation in the SCN1A gene associated with hepatic coma and brain damage in a child. *D. Lev[1], M. Abu- Rashed[2], L. Blumkin[2], S. Zuberi[3], T. Lerman-Sagie[2].* 1) Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel; 2) Pediatric Neurology Unit,Wolfson Medical Center, Holon, Israel; 3) Fraser of Allander Neurosciences Unit, Royal Hospital for Sick Children, Yorkhill, Glasgow UK.

The spectrum of the infantile encephalopathies related to mutations in alpha-subunit type A of voltage-gated sodium channel (SCN1A) is rapidly expanding and now includes: severe myoclonic epilepsy of infancy (SMEI), SMEI-borderland, vaccine related encephalopathy, severe infantile multifocal epilepsy and even familial hemiplegic migraine. We report a 4 year-old boy with an atypical presentation of a SCN1A mutation. He presented at 7 months with recurrent episodes seizures usually associated with fever.. Myoclonic epilepsy of infancy was suspected and he was put on VPA therapy. At the age of 11 months he developed febrile status epilepticus. His seizure was stopped with phenobarbital. He developed liver and kidney failure with hyperammonemia. The diagnosis of Alpers-Huttenlocher disease was considered because of the myoclonic epilepsy combined with liver failure on valproic acid therapy. A muscle biopsy showed decreased complex II and complexes II +III of the respiratory chain. The skeletal muscle biopsy disclosed minor changes consistent with mitochondrial dysfunction. Sequencing of POLG1 gene did not detect any mutations. MtDNA depletion was ruled out. A liver biopsy at 12 months was normal. Following the acute deterioration, his clinical picture was consistent of a static encephalopathy. He continued to suffer from febrile induced episodes of status epilepticus that were not controlled by anticonvulsants. The unequivocal association between the seizures and fever lead us to suspect severe myoclonic epilepsy of infancy as the primary epileptic syndrome. SCN1A sequence analysis was performed at 3.4 years old. A novel amino acid change p.Val 1637 Glu was detected. This missense mutation changes a neutral amino acid to an acidic amino acid in segment 4 of domain IV of the SCN1A protein. Liver failure in an epileptic child may be the presentation of a SCN1A mutation.

892/T

Phosphatidylinositol pathway defects in arthrogryposis: autosomal recessive lethal congenital contractural syndrome caused by mutations in *PIP5K1C* and in *ERBB3*. *G. Narkis[1], O. Ofir[1], E. Manor[2], D. Landau[2], M. Volokita[1], K. Elbedour[2], O.S. Birk[1,2].* 1) Dept Development Genetics, Ben-Gurion University, Beer-Sheva, Israel; 2) The Genetics Institute, Soroka Medical Center, Beer-Sheva, Israel.

Lethal congenital contractural syndrome type 2 (LCCS2) is a neonatally lethal form of Arthrogryposis prevalent in Israeli Bedouins. The phenotype is characterized by multiple joint contractures, anterior horn atrophy in the spinal cord, and a markedly distended urinary bladder, suggesting a spinal cord neuropathic etiology. We previously mapped this syndrome to 4.6 Mb (harboring 150 genes) on chromosome 12q13. We now describe a third LCCS phenotype (LCCS3) - similar to LCCS2 yet without neurogenic bladder. Using 10K SNP arrays followed by fine mapping with microsatallite markers, we localized the LCCS3 gene to 3.4 Mb (harboring 120 genes) on chromosome 19p13. Of these genes, 30 candidates were sequenced, identifying a single homozygous mutation in *PIP5K1C*. *PIP5K1C* encodes phosphatidylinositol-4-phosphate 5-kinase, type I, gamma (PIPKIγ), an enzyme that phophorylates phosphatidylinositol 4-phosphate (PI4P) to generate phosphatidylinositol-4,5-bisphosphate (PIP_2). The mutation causes substitution of aspartic acid to asparagine at amino acid 253 (D253N), abrogating the kinase activity of PIPKIγ. Based on this finding, we sequenced genes in the LCCS2 locus that encode proteins in pathways interacting with the phosphatidylinositol pathway. We demonstrate that LCCS2 is caused by aberrant splicing of *ERBB3* (Her3), leading to a predicted truncated protein. ERBB3 is known to modulate phosphatidylinositol-3-kinase (PI3K), an enzyme that phosphorylates PIP_2 to produce phosphatidylinositol-3,4,5-triphosphate (PIP_3). ERBB3, an activator of the PI3K/Akt pathway - regulating cell survival and vesicle trafficking - is essential for the generation of precursors of Schwann cells that normally accompany peripheral axons of motor neurons. We suggest that defects in the phosphatidylinositol pathway affecting PIP_2, a molecule active in endocytosis of synaptic vesicle proteins, culminate in lethal congenital arthrogryposis.

893/T

Autosomal dominant distal myopathy associated with a recurrent missense mutation in the gene encoding the nuclear matrix component, matrin 3. *A. Roos[1], J. Senderek[1], S.M. Garvey[2], I. Tournev[3], C. Stendel[1], A. Urtizberea[4], V. Guergueltcheva[3], V. Mihailova[3], H. Feit[5], J.J. Tramonte[6], P. Hedera[7], J. Weis[8], J.S. Beckmann[9], E. Seboun[10], M.A. Hauser[2], C.E. Jackson[6].* 1) Institute of Human Genetics, Aachen University of Technology, Aachen, Germany; 2) Duke Center for Human Genetics, Duke University, Durham, USA; 3) Department of Neurology, Sofia Medical University, Sofia, Bulgaria; 4) Hôpital Marin, Hendaye, France; 5) Department of Neurology, Henry Ford Hospital, Detroit, USA; 6) Scott & White Memorial Hospital, Temple, USA; 7) Department of Neurology, Vanderbilt University Medical Center, Nashville, USA; 8) Institute of Neuropathogy, Aachen University of Technology, Aachen, Germany; 9) Service of Medical Genetics, CHUV, Lausanne, Switzerland; 10) Division de Génétique et de Microbiologie, Université Pierre et Marie Curie, Paris, France.

Distal myopathies represent a heterogeneous group of inherited skeletal muscle disorders. One type of adult-onset, progressive autosomal dominant distal myopathy, frequently associated with dysphonia and dysphagia, has been mapped to chromosome 5q31 in a North American pedigree (vocal cord and pharyngeal weakness with distal myopathy; VCPDM). Here we report identification of a second VCPDM family of Bulgarian descent and fine mapping of the critical interval. The refined candidate region includes the MAT3 gene that encodes a protein of the nuclear matrix, a filamentous protein network in vertebrate nuclei. MAT3 is a candidate for VCPDM as mutations in the genes for the nuclear envelope proteins lamin A/C and emerin cause muscular dystrophies. Screening of MAT3 for mutations led to the identification of a non-conservative missense mutation affecting a highly conserved serine residue (S85C) in both pedigrees. Different disease related haplotype signatures were observed in the two families, providing evidence that two independent mutational events at the same position in MAT3 cause VCPDM. Our data provide evidence that the nuclear matrix is crucial for normal skeletal muscle structure and function and put VCPDM on the growing list of monogenic disorders associated with the nuclear proteome.

894/T

A novel Angiogenin gene mutation in a sporadic patient with Amyotrophic Lateral Sclerosis from Southern Italy. *T. Sprovieri, R. Mazzei, A. Patitucci, C. Ungaro, A. Magariello, L. Citrigno, F. Condino, A.L. Gabriele, M. Muglia, F.L. Conforti.* Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy.

Amyotrophic Lateral Sclerosis (ALS) is a devastating untreatable neurodegenerative disorder. It is characterized by progressive wasting and weakness of limb, bulbar and respiratory muscles due to degeneration of motoneurons in the spinal cord, brain stem and motor cortex. Disease onset is usually in the fifth or sixth decade of life and, in most affected individuals, progresses to death due to respiratory failure 3-5 years after onset. Genetic analysis of ALS patients has identified mutations in the Cu/Zn superoxide dismutase (SOD1) gene in approximately 33% of familial cases (FALS) and 2.5% of apparently sporadic cases (SALS). Mutations in the SOD1 gene account for such a small proportion of familial cases of ALS that a concerted effort has been made to identify other disease-causing genes. Mutations in the Angiogenin gene (ANG), linked to 14q11.2 have been recently discovered to be associated with Amyotrophic Lateral Sclerosis (ALS) in Irish and Scottish populations. In our study we investigated the role of ANG gene in ALS patients from Southern Italy. We found a novel mutation (M1I) of the ANG gene in a sporadic patient with ALS (SALS). The molecular analysis of the ANG gene also demonstrated an allelic association with the rs11701 single nucleotide polymorphism (SNP) in familial ALS (FALS) but not in SALS patients. Our finding supports the evidence that the ANG gene is involved in ALS.

895/T

A Murine Model for Mucolipidosis Type IV. *C. Curcio-Morelli[1], B. Venugopal[1], M.F. Browning[1], A. Varro[2], N. Michaud[3], N. Nanthakumar[1], S.U. Walkley[4], J. Pickel[5], S.A. Slaugenhaupt[1].* 1) Massachusetts General Hospital and Harvard Medical School, Boston, MA; 2) University of Liverpool, UK; 3) Massachusetts Eye and Ear Infirmary, Boston, MA; 4) Albert Einstein College of Medicine, Bronx, NY; 5) NIH, Bethesda, MD.

Mucolipidosis Type IV (MLIV) is an autosomal recessive lysosomal storage disorder caused by a disruption in cellular membrane trafficking. The MLIV gene, MCOLN1, maps to chromosome 19p13.2-13.3 and encodes a 580 amino acid protein named mucolipin-1. We have created the first murine model for this disease and our model accurately replicates the human disease phenotype, which includes progressive neurodegeneration, ophthalmologic abnormalities, constitutive achlohydria, and elevated plasma gastrin levels. The *Mcoln1*$^{-/-}$ mice were generated by replacing exons 3, 4 and 72 bp of exon 5 of Mcoln1 with a PGK-neomycin cassette. *Mcoln1*$^{-/-}$ are born at Mendelian ratios and both male and female *Mcoln1*$^{-/-}$ mice are fertile. Electron microscopy of the brain reveals the presence of numerous dense inclusion bodies in all cell types, particularly in neurons. Plasma gastrin, measured by radioimmunoassay, was dramatically increased in *Mcoln1*$^{-/-}$ mice (*Mcoln1*$^{-/-}$ =158.6 pM and *Mcoln1*$^{+/+}$=76.25 pM). Histology of the stomach mucosa showed the presence of vacuolization in parietal cells. Retinas from *Mcoln1*$^{-/-}$ mice were analyzed by electron microscopy and revealed a severe degeneration, presenting a dystrophic outer nuclear layer and a significantly reduced outer plexiform and inner nuclear layer. Analysis of clasping and gait confirmed the presence of a neurological defect in *Mcoln1*$^{-/-}$ mice. The creation of the first murine model for MLIV provides an excellent system for elucidating disease pathogenesis. Moreover, this model will provide an invaluable resource for testing potential therapeutic agents. Recently, two studies have shown resolution of lysosomal storage in MLIV patient fibroblasts following treatment with chloroquine and nigericin. Evaluation of these compounds in *Mcoln1*$^{-/-}$ animals and primary cell cultures is underway and will yield important insights into treatment development for this devastating disorder.

896/T

Neuronal ceroidlipofuscinoses (NCLs) in Czech and Slovak patients: Two novel mutations in CLN2 gene and high amount of unexplained NCL6-like cases. *H. Vlaskova, L. Dvorakova, M. Hrebicek, L. Stolnaja, H. Myskova, M. Elleder.* Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine and University Hospital, Charles University, Prague.

Ninety one cases of NCLs from 77 families have been diagnosed in our Institute that serves as a diagnostic center for the Czech Republic and Slovakia (15 mil. inhabitants). The diagnosis was carried out using histology, electron microscopy, histochemistry, biochemistry and recently mutation analysis. NCL1 and NCL4 proved to be extremely rare (2 cases of NCL1, one case of NCL4 - the dominant Parry type). NCL2 was diagnosed in 35 patients. The cases corresponded to the classical clinical phenotype, neuropathology, and electron microscopy. In postmortem samples there was a uniform generalized SCMAS (subunit c of mitochondrial ATP synthase) storage. The diagnosis of NCL2 was proved by enzyme assay in 8 patients. Mutation analysis, performed in 11 patients, showed a high prevalence of p.R208X (16/22). Other identified mutations were IVS5-1G>C (3/22), p.S475L (1/22) and two novel substitutions, c.1439T>G (p.V480G) and c.1642T>C (p.W548R). DNA of one patient was not available, analysis of the parental samples revealed a probable patient's genotype (p.R208X/p.V480G). This patient presented with an atypical onset (at 6 years) and course of the disease (prolonged span of life until 15 years), which suggests p.V480G to be a mild mutation. NCL3 was diagnosed in 2 patients, both homozygous for the prevalent deletion of 996bp. The rest of the series represented by 34 families with high incidence in the Gipsy population (about 50% of cases) is classified as NCL6 - like. The current DNA analysis carried out in 8 unrelated patients did not reveal any mutations neither in CLN6 nor in CLN5 and CLN8 genes. The diagnosis of NCL6 is thus made per exclusionem and open for further investigation. Our laboratory is a member of The Rare NCL Gene Consortium (www.ucl.ac.uk/ncl/RNGC.shtml). Support: IGA MZ CR NR/8351-3, VZ MSM CR 0021620806, VZ MZ CR 64165.

897/T

Identification of Sequence Variants in Glutamate Receptor and Interacting Protein Genes in Patients with Mental Retardation Using High-throughput CE-SSCP. *A. Adamczyk[1], S. Bhat[1], A.K. Srivastava[2], C.E. Schwartz[2], D. Valle[1], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Greenwood Genetic Center, Greenwood, SC.

Mental retardation (MR) is a common cause of severe handicap in children and young adults, affecting 2-3% of the general population. Genetic defects account for >50% of the identifiable causes of MR. L-glutamate is the predominant excitatory neurotransmitter in CNS via the activation of ionotropic and metabotropic receptors. Glutamate signaling is essential for the induction and maintenance of long-term potentiation (LTP) and long-term depression (LTD), two cellular models of learning and memory. To determine the nature and spectrum of genetic defects in glutamate-signaling that cause MR, we initiated a study on a large cohort (n=1,200) of patients with MR of unknown cause to identify pathological sequence variants (SV) in coding and key regulatory regions of genes that encode glutamate receptors (n=25) and direct interacting proteins using a high-throughput capillary electrophoresis single strand conformation polymorphism (CE-SSCP) screen. We conducted a proof of principle study using DNA samples from 29 patients, each with a different and sequence confirmed mutation, in the ornithine-?-aminotransferense (*OAT*) gene and identified aberrant SSCP tracings in 25 of the 29 (86%;) samples. In a pilot screen of *GRIA1, GRIA2* and *GRIP1* in 768 samples, we found 6 nonsynonymous coding variants (CV) that involve highly conserved functional domains and are absent in SNP database in 8 patients and 6 synonymous CV in 9 patients. in vitro functional studies on these CV are ongoing. We conclude that CE-SSCP is a powerful method to achieve a high-throughput, low cost, and reliable screen to identify potentially pathological SVs in genes that are essential in glutamate signaling in patients with MR.

898/T

New approaches to understand the genetic differences between classic Rett syndrome and Preserved Speech Variant. *F. Ariani[1], E. Scala[1], R. Caselli[1], F. Papa[1], R. Artuso[1], MA. Mencarelli[1], I. Meloni[1], F. Mari[1], M. Zappella[2], G. Hayek[2], DH. Yasui[3], JM. LaSalle[3], A. Renieri[1].* 1) Medical Genetics, Dept Molecular Biol, Univ Siena, Siena, Italy; 2) Child Neuropsychiatry, Univ Siena, Siena, Italy; 3) Medical Microbiology and Immunology, Rowe Program in Human Genetics, School of Medicine, University of California, Davis, CA.

In classic Rett (RTT), the III stage is characterized by mild improvement of eye contact. At the same stage, the Preserved Speech Variants (PSV) recover the ability to speak and to use hands. Both phenotypes are due to similar or identical de novo mutations in MECP2 (http://www.biobank.unisi.it and Sampieri, Hum Mut 2007). In order to understand the genetic differences between the two phenotypes, we have: i) searched for statistically significant differences in allele frequencies of polymorphisms in genes associated to a similar phenotype (CDKL5) or target genes (BDNF); ii) analyzed differences in genomic variations by array-CGH in two sisters with discordant phenotype, balanced XCI, and the same MECP2 partial deletion absent in parents. We have established that the p.Q791P CDKL5 polymorphism is not involved in the modulation of epilepsy in RTT (p=0.373). Array-CGH analysis on the above described RTT sisters showed a duplication of 390 Kb on chromosome 16 in the classic RTT girl inherited from the healthy father and absent in the PSV sister. The duplication includes 10 genes. Two are disease-genes: one related to a known myopathy and the other to a CNS disease. Chromatin immunoprecipitation promoter array (ChIP-chip) analysis identified three potential MeCP2 target genes within the duplicated region. Real Time RT-PCR experiments are ongoing to analyze the combined effect of MECP2 defect and 16p duplication on the expression of these three genes. Understanding the genetic differences between classic RTT and PSV will help in designing therapeutic strategies.

899/T

Genetic and functional characterization of sequence variants in GRIPAP-1, a neuronal rasGEF protein and a candidate gene for X-linked mental retardation. *Y.W. Jiang[1], S. Bhat[1], F. Abidi[2], Y. Wu[1], L.L. Zhang[1], E. Marcocci[4], I. Meloni[4], A. Renieri[4], C.E. Schwartz[2], R. Huganir[3], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Greenwood Genetic Center, Greenwood, SC; 3) Department of Neuroscience, Johns Hopkins University, Baltimore, MD; 4) Department of Medical Genetics, University of Siena, Italy.

X-linked mental retardation (XLMR) occurs in 1 in 600 males and is genetically highly heterogeneous. Among the estimated 150-200 XLMR genes, <60 were cloned. Using a human X chromosome cDNA microarray screen, we identified GRIP-associated protein-1 (GRIPAP1) mapped to Xp11.2 as a candidate gene for XLMR. GRIPAP-1 has a predicted GEF-activation domain and a PDZ-like protein interaction domain. GRIPAP-1 expresses abundantly in neurons and is involved in AMPA receptor trafficking (Neuron 26:603, 2000). By sequencing DNA samples from 288 males with XLMR, we found four nonsynonymous (S73N, P179L E578A, R822Q) and three synonymous (E161E, L504L, H554H) coding variants in GRIPAP1. S73N was found in two unrelated XLMR males and is absent in 500 control males. Both P179L and E578A were also found in control males and are likely nonpathogenic variants. R822 in the PDZ-like domain is a highly conserved residue from zebrafish to human. R822Q co-segregates with MR phenotype in the proband family and is absent in 500 normal males. R822Q results in a stable protein and is predicted to have a significant impact on phosphorylation status of nearby serine [NetPhos2.0]. Functional studies of GRIPAP-1-knockout mice suggest that GRIPAP-1 may be involved in the regulation of ERK1/2 pathway. Further genetic and functional characterization of molecular defects in GRIPAP1 in XLMR patients will contribute to our understanding of the physiological function of GRIPAP1 and potential roles of these defects in the pathogenesis of MR in humans.

900/T

Mutation in a C2 domain-containing gene cause autosomal recessive non-syndromic mental retardation. *A. Noor[1], C. Windpassinger[1,2], M. Azam[3], M. Ayub[4], J.B. Vincent[1].* 1) Neurogenetics, Centre For Addiction & Mental Health, Toronto, Ontario, Canada; 2) Institute of Medical Biology and Human Genetics, University of Graz, Graz, Austria; 3) Pakistan Institute of Medical Sciences, Islamabad, Pakistan; 4) St. Luke's Hospital,Middlesbrough, United Kingdom.

Mental retardation (MR) is defined by an intelligence quotient (IQ) of less than 70 associated with functional deficits in adaptive behaviour and it has a prevalence of 1-3%. Although, autosomal recessive forms of MR (ARMR) are believed to be more common, yet only three genes, the PRSS12, CC2D1A and CRBN have been reported so far to cause this. In this study, we ascertained a consanguineous family from Pakistan affected with non-syndromic autosomal recessive mental retardation. The phenotype was present in 5 individuals from three branches of the family. We used the Affymetrix ~260K NspI chip to perform homozygosity mapping and identified a homozygous and haploidentical region of 11.2-Mb on chromosome 4p15.33-p15.2, but only in four out of five affected individuals. Further analysis of the SNP microarray data for copy number variants (CNVs) revealed the duplication of entire chromosome X in the fifth affected individual who did not share the 4p15 homozygous region. Subsequent cytogenetic analysis has indicated the karyotype 48,XXXX in this individual. We also performed genotyping using 11 microsatellite markers across the 4p region; Analysis confirmed a common haplotype spanning 11.2 Mb in four affected individuals. Linkage analysis was also performed and a maximum two-point logarithm of odds (LOD) score of 3.59 at theta= 0.0 was obtained at markers D4S419. The 11.2 Mb critical region, containing approximately 39 known genes was fine mapped by genotyping and sequencing of flanking SNPs rs6814906 and rs7664104. Furthermore, we sequenced genes in the critical region and identified a splice site mutation segregating with the phenotype in a C2 domain-containing gene. This splice site mutation is predicted to result in truncated protein lacking the C2 domain. This sequence variant was not found in 230 healthy controls. Further studies are required to understand the role of this gene in development.

901/T

Investigation the effect of Iron accumulation on different parts mtDNA of Iranian patients comparing with normal mtDNA. *S. EtemadAhari[1], S. Kasraie[1], M. Moin[2], M. Houshmand[1], M. Shafa Shariat Panahi[1].* 1) Dept Molecular Medical Gen, NIGEB, Tehran, Iran; 2) Immunology,Asthma & Allergy Research Institute, Tehran,Ian.

Friedreich's ataxia (FRDA)1 is the most common inherited ataxia. Clinically, Friedreich's ataxia is characterized by multiple symptoms including progressive gait and limb ataxia, dysarthria, diabetes mellitus, and hypertrophic cardiomyopathy. There is much evidence to suggest that FRDA results from mitochondrial iron accumulation leading to cellular damage and death by the production of toxic free radicals by Fenton chemistry.Presence of free radicals in mitochondria of patients with FRDA motive us to investigate different parts of mtDNA in 20Iranian FRDA patients, by PCR and automated DNA sequencing and compare it with normal mtDNA to find any probable point mutation that can be adjunctin the pathogenesis of FRDA.

902/T

Investigation of tRNA $^{Leu/lys}$ and ATPase 6,8 genes mutation in Huntington's Disease. *S. Kasraie[1], S. EtemadAhari[1], M. Houshmand[1], M. Moin[2], M.A. Bahar[3], M. Shafa Shariat Panahi[1].* 1) Department of Medical genetics, National Research Center of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 2) Immunology, Asthma & Allergy Research Institute, Tehran, Iran; 3) Shahid Motahari Burn & Reconstruction Research Center, Iran University of Medical Sciences & Health Services, Tehran, Iran.

Huntington disease (HD) is a genetically dominant condition caused by expanded CAG repeats coding for glutamine in the HD gene product huntingtin. Huntingtin is expressed in almost all tissues, so abnormalities outside the brain might be expected. Mitochondria dysfunction is reported in HD brains. Mitochondria are organelles that among other functions regulate apoptotic cell death. Involvement of nuclei and mitochondria in HD pathophysiology has been suggested. The tRNA gene mutations are one of hot spots that cause mitochondrial disorders. We performed mutation screenings of tRNA $^{leu/lys}$genes and also ATPase 6,8 genes in 20 patients with HD and 100 aged-matched controls. Mitochondrial tRNA $^{leu/lys}$ genes and ATPase 6,8 genes were studied by PCR method and automated DNA sequencing to evaluate any possible mtDNA damage. We found novel mutations in HD patients including G8950A, T8395C, A8656G, A8460G, and C8300T. We propose that A8656G could be considered as a modifier factor in HD severity. Understanding the role of mitochondria in the pathogenesis of neurodegenerative diseases could potentially be important for the development of therapeutic strategies in HD.

903/T

Sporadic POLG1 mutations in two cases; one with acute liver failure and the other with encephalopathy. *E. Schmitt[1], R.E. Lutz[2], Q. Zhang[1], C. Reyes[2], E. Truemper[2], R. McComb[2], A. Hernandez[3], A. Basinger[4], L.-J. Wong[1].* 1) Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Department of Genetics, Endocrinology, and Metabolism, Munroe-Meyer Institute, University of Nebraska Medical Center, Omaha, NEB; 3) Department of Neurology, Cook Children's Medical Center, Ft. Worth, TX; 4) Division of Metabolic Genetics, Cook Children's Physician Network, Ft. Worth, TX.

PURPOSE: To identify the molecular etiology of suspected of mtDNA depletion syndrome in two patients; one with an acute liver failure triggered by infection and the other with repetitive seizures. METHODS: The POLG1 gene, responsible for mitochondrial DNA (mtDNA) biogenesis, was analyzed by direct DNA sequencing. Real time quantitative PCR was used to measure the mtDNA copy number. Respiratory chain enzyme complex activities, histochemistry, and ultrastructural studies were performed on a liver specimen from one of the patients. RESULTS: Three mutations, p.T251I, p.P587L, and p.K1191R in the POLG1 gene were identified in a baby girl with acute liver failure. Liver biopsy showed swollen hepatocytes with microvesicular steatosis and a drastic reduction of mtDNA content. Analysis of parental DNA revealed that T251I and P587L were in cis on the maternal chromosome, while the novel K1191R mutation was not detected in either parent. Two heterozygous mutations, p.A467T and c.2157+5_6 GC> AG were found in a baby boy with seizures. Subsequent analysis of parental blood samples revealed that the mother is heterozygous for the p.A467T allele; both parents were negative for the c.2157+5_6 GC> AG allele. CONCLUSIONS: Neither of our patients have typical clinical presentation of autosomal recessive Alpers syndrome, which is characterized by infantile liver failure and intractable seizures. Infection may be a precipitating factor in acute liver failure for patients harboring POLG1 mutations without CNS problems. Patients with Alpers syndrome may alos present with CNS symptoms only, without liver involvement. We report the first two sporadic cases with novel POLG1 mutations. Both de novo mutations are likely to be on the paternal copy of the POLG1 gene.

904/T

Ribosomal fameshifting on expanded *ATXN3* transcripts: a *Drosophila* model. *C. Gaspar[1], S. Stochmanski[1], J. Laganiereè[1], D. Rochefort[1], M. Therrien[1], P. Dion[1], F. Blondeau[1], D. Van Meyel[2], G.A. Rouleau[1].* 1) Center for the Study of Brain Diseases, CHUM Research Center, Montreal, QC, Canada; 2) Center for Research in Neuroscience, MGH Research Institute, Montreal, QC, Canada.

Rationale: Spinocerebellar ataxia type 3 (SCA3) is caused by the expansion of a coding CAG repeat in the *ATXN3* gene. We have previously shown that the expanded CAG repeat in SCA3 is prone to -1 ribosomal frameshifting, leading to the production and aggregation of proteins containing polyalanine stretches. These frameshifted molecules confer increased toxicity to cells when compared to constructs containing expanded CAA repeats, which code for polyglutamine in the main frame but lack the ability to frameshift into an alanine frame. Anisomycin (a ribosome interacting antibiotic that reduces -1 frameshifting) decreases frameshifting in expanded CAG tracts and ameliorates the cellular toxic phenotype. **Aims:** To model expCAG repeat -1 frameshifting *in vivo*; to assess the contribution of -1 frameshifting to expCAG toxicity in *Drosophila.* **Methods:** Full-length *ATXN3 Drosophila* transgenic lines carrying either wtCAG, expCAG or expCAA constructs containing epitope tags in the three possible reading frames were generated and comparatively analysed. **Results:** We show that: (1) transgenic expression of expCAG *ATXN3* constructs is deleterious in the fly; (2) transgenic expression of expCAA *ATXN3* constructs, despite adequate levels of protein expression, is not toxic; (3) -1 frameshifting occurs in *Drosophila* and is restricted to the expanded CAG transgenic lines. **Conclusions:** We propose that -1 ribosomal frameshifting is a major contributor to the toxicity observed in expanded CAG repeat diseases. This novel pathological mechanism may open new therapeutic opportunities for these diseases.

905/T

In depth investigation of -1 frameshifting in expanded CAG repeat tracts using time-lapse live-cell imaging. *S. Stochmanski, C. Gaspar, D. Rochefort, P. Hince, J. Laganiere, G.A. Rouleau.* Centre for the Study of Brain Diseases, CHUM Research Centre, Montreal, QC, Canada.

Rationale: Spinocerebellar ataxia type 3 (SCA3) results from an expansion of a polyglutamine-encoding CAG tract in the *ATXN3* gene. We have previously demonstrated that this expanded CAG tract is subject to -1 ribosomal frameshifting into the alanine frame, which seems to confer an increased toxicity, and that the antibiotic anisomycin reduces both -1 frameshifting and cell toxicity. **Aims:** The objectives of this work are (1) to perform a characterization of the mechanism of -1 frameshifting within large CAG repeats and (2) to compare the inherent properties of constructs containing expanded CAG *versus* CAA repeats, using a real-time live-cell assay. **Methods:** Time-lapse live-cell two-wave fluorescent microscopy was performed using several doubly tagged constructs: pDsRED was ligated N-terminally to *ATXN3* constructs, to be expressed in the main (glutamine) frame, whereas EGFP was fused at the C-terminus in the -1 (alanine) reading frame. Reporter constructs had either 14 CAG repeats, 89 CAG repeats or 92 CAA repeats. Constructs were transfected into COS-1 cells and live cells were monitored for the production of red or green fluorescent signals on a Leica live-stage microscope, for periods of 48 hours. **Results:** We confirmed the occurrence of -1 frameshifitng for the CAG_{89} construct, at a rate of approximately 20 percent (measured by the ratio of green to red fluorescent cells). Constructs bearing wild-type CAG or expanded CAA repeats did not show significant frameshifitng. We also determined that pDsRED expression (glutamine frame) first appears approximately 8 hours post transfection, whereas EGFP is expressed approximately 16 hours post transfection. **Conclusions:** These results suggest that there is a marked time delay between the onset of glutamine-containing protein expression and the production of frameshifted species. This finding argues in favor of local glutamine codon starvation, followed by a shift in the reading frame to resume translation of the protein in the alanine frame.

906/T

A CLINICAL AND MOLECULAR STUDY IN TWO MEXICANS FAMILIES WITH X-LINKED SPINAL AND BULBAR MUSCULAR ATROPHY. *M.A. LOPEZ, A. MONARRES, J.J. MORALES, B. CACHO, G. RAMOS, O.M. MUTCHINICK.* GENETIC AND NEUROLOGY DEPARTMENTS, INCMNSZ.MEXICO,D.F.

X-linked Spinal and Bulbar Muscular Atrophy (SBMA; Kennedy disease) is a hereditary neurodegenerative disease characterized by slow progressive muscle weakness and atrophy of bulbar, facial and limb muscles, accompanied by signs of androgen insensitivity such as gynecomastia and reduced infertility. The cause of SBMA is a expansion of trinucleotide CAG repeat, wich encode the polyglutamine tract, in the first exon of the androgen receptor (AR) gene. SBMA mainly occurs in adult males, whereas neurological symptoms are rarely detected in females having mutant AR gene. The aim of this work is to report the detailed phenotypic study and the molecular analysis in a serie of 34 individuals evaluated in two mexicans kindreds. Males with suspected of SBMA phenotypic and females carriers probably were examined. DNA was isolated from peripheral blood leukocytes and used for further PCR amplification of the segment of AR gene containing CAG repeats. The number of these repeats was determinated by electrophoresis on a 3.5 percent of agarose gel and confirmed by sequencing. The molecular genetic diagnosis showed an abnormal number of CAG repeats in nine males SBMA patients and seven females carriers, the first one family with 52 repeats and the last one with 51 repeats. The healthy individulas of both families showed of normal size (18-26 repeats). The two kindreds showed a wide spectrum of different clinical characteristics. All males with SBMA had gynecomastia and neurological manifestations. This is the first report in mexicans families with SBMA confirmed. Although an early diagnosis may not be crucial for the treatment, given the lack of effective therapy, the molecular testing can be of great relevance for carrier detection, disease prognosis and genetic counseling.

907/T

Novel PANK2 gene mutations in three Pakistani patients with pantothenate kinase-associated neuro-degeneration (PKAN). *P.M. Frossard[1], Z. Aly[2], T. Ali[2], M.H. Arshad[2], B. Khealani[3], D. Saleheen[1,4].* 1) Dept. Biological & Biomedical Sciences; 2) Medical College; 3) Neurology Section, Dept. Medicine; Aga Khan University Medical College, Karachi, Pakistan; 4) Dept. Public Health and Community Medicine, Cambridge University, UK.

Pantothenate kinase-associated neuro-degeneration (PKAN) is an autosomal recessive disorder associated with iron accumulation in basal ganglia. The disease can present either as a rapidly progressive disorder with early childhood onset, or as a slowly progressive disorder with late onset and decreased severity. Signs include dystonia, rigidity and gait impairment leading to restriction of activities and loss of ambulation. T2-weighted MRI's show a characteristic 'eye of the tiger sign' in the patients. The disease is caused by defective iron metabolism due to pantothenate kinase defects, which is encoded by PANK2 gene. We report here a mutation screen conducted on three patients who had clinical symptoms suggestive of PKAN.

The three patients belonged to two unrelated, Pakistani families. MRI findings revealed a marked ring of hypodensity circumscribing a hyperdense area in the basal ganglia ('eye of the tiger' sign), particularly the globus pallidus and the substantia nigra, in all three patients.

DNA was extracted by a phenol-chloroform reference protocol and *PANK2* was amplified by multiplex PCR's on the patients and all available family members. Mutation screen was carried out by direct DNA sequencing using an MJ Research PTC-225 Peltier Thermal Cycler. Two novel mutations (H173Y and Y176C in *PANK2* exon 2) were identified in the patients; neither of the two mutations was found in 200 control alleles. The two *PANK2* mutations found in these Pakistani patients form the basis for the development of a pre-symptomatic genetic test for PKAN. The molecular diagnosis also confirms the specificity of the clinical finding of the 'eye of the tiger' sign on T2-weighted MRI in the diagnosis of PKAN.

908/T

Mutation analysis of SIX3, ZIC2, SHH and TGIF in a series of holoprosencephaly patients. *J. Herbergs, A. Paulussen, S. Spierts, D. Tserpelis, H. Smeets.* Dept Clinical Genetics, Academic Hosp Maastricht, Maastricht, Netherlands.

Holoprosencephaly (HPE) is a common severe malformation of the brain that involves abnormal formation and septation of the developing central nervous system. The prevalence is 1:250 during early embryogenesis, but the live born prevalence is only 1:16000. The etiology of HPE is extremely heterogeneous and can include both a teratogenic and/or genetic basis. We studied four genes known to be involved in HPE, namely SHH, ZIC2, SIX3 and TGIF by sequence and MLPA analysis. A series of in total 66 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 14 pathogenic mutations (21%), 4 out of 58 sporadic cases and 7 out of 9 familial cases. One of the familial cases was caused by a mutation in parental germ cells. Seven mutations were detected in the SIX3 gene, four mutations in the ZIC2 gene and three mutations in the SHH gene. The familial mutations displayed great phenotypic heterogeneity of the disease, which makes it difficult to establish genotype-phenotype correlations. This phenotypic variability may be due both to environmental factors and to potential modifier genes. HPE development is probably a multihit process , which implicates more genes; illustrating the importance of further identification of new genes.

909/T

Translational profiles of neuronal ceroid lipofuscinoses (NCLs). *N. Zhong[1,2], P-R. Wang[2].* 1) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 2) Peking University Center of Medical Genetics, Beijing, China.

Neuronal ceroid lipofuscinoses (NCLs) are a group of clinically and genetically heterogeneous neurodegenerative disorders. NCLs comprise ten variant forms. Seven genes have being identified. The precise function of all the NCL proteins is currently unclear. One hypothesis is that the proteins defective in NCLs may, at least partially, be involved in a common metabolic pathway. To test our hypothesis, we have investigated protein expression profiles among NCL1, NCL2, NCL3, and NCL8 disease-derived, compared to wild type, fibroblasts. PF2D two-dimensional chromatography was applied. In the first dimension, proteins were separated by chromatofocusing, based on isoelectric point (pI). In the second dimension, proteins with same pI were resolved by reversed-phase chromatography detected by UV. Translational profiles were displayed as an UV/pI map using a ProteoVue* software. Differentially displayed protein pattern was further applied to LC-MS to identify protein sequence. One protein band was found to be at a translational level of 0.166 in wild type fibroblasts but at -0.017 or -0.008 in two NCL1 fibroblast cell lines, at 0.104 in one NCL2 line, 0.066 or 0.047 in two NCL3 lines, and 0.062 in one NCL8 line. Protein sequencing analyses showed this protein has a function of binding calcium and phospholipids to promote membrane fusion. Our results suggested that identification of this protein may open a new avenue to understand the molecular pathogenic mechanism underlying the NCLs.

910/T

Somatic instability in Friedreich ataxia progresses throughout life, and includes large, age-dependent expansions in dorsal root ganglia. *I. De Biase[1], R. Clark[1], A. Rasmussen[1,2], S. Al-Mahdawi[3], A. Monticelli[4], S. Cocozza[4], M. Pook[3], S.I. Bidichandani[1].* 1) Dept Biochemistry, Univ Oklahoma HSC, Oklahoma City, OK; 2) Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico; 3) Brunel University, Uxbridge, UK; 4) Univ Federico II, Naples, Italy.

Friedreich ataxia (FRDA) patients are homozygous for large expansions of a GAA triplet-repeat (GAA-TR) sequence in the FXN gene. The neurodegeneration involving primarily the dorsal root ganglia (DRG) results in the progressive ataxia. The high sensitivity of DRG to frataxin deficiency is the likely cause of this selective degeneration, but the progressive nature remains unexplained. The expanded GAA-TR is highly unstable in somatic and germ cells. To test whether somatic instability contributes to the tissue-specific and progressive nature of FRDA, we analyzed GAA-TR instability in multiple tissues from six autopsies of FRDA patients. Small-pool PCR analysis showed that DRG had a significantly higher frequency of large expansions compared with all other tissues (P<0.001). There was a significant age-dependent increase in the DRG large expansions frequency, which ranged from 0.5% at 17y to 13.9% at 47y (R=0.78; P=0.028). A transgenic mouse carrying the entire human FXN locus with an expanded tract showed the same age-dependent, DRG-specific increase in large expansions indicating that the DRG-specific somatic instability is not secondary to the disease process. Progressive pathology involving the DRG is therefore likely due to age-dependent accumulation of GAA-TR large expansions. Compared with adult-derived tissues, SP-PCR analysis of multiple tissues of an 18-week fetus homozygous for large expansions revealed a remarkably low level of instability (4.2% vs 30.6%, P<0.0001). The overall mutation load in vivo, measured in blood samples, increased with patient age, ranging from 7.5% at 18-weeks gestation to 78.7% at 49 years of age (R=0.91; P=0.0001). FRDA somatic instability occurs mostly after early embryonic development, progresses throughout life, and possibly contributes to disease pathogenesis and progression. Postnatal repeat expansion in specific tissues is a common theme in triplet-repeat diseases pathogenesis.

911/T

The fragile X protein controls GFP transgene expression in mouse neurons. *C. Dobkin[1], D. Ziemnicka[1], G. LaFauci[1], X. Ding[1], W.T. Brown[1], A. El Idrissi[2].* 1) Department of Human Genetics, NYS Institute for Basic Research, Staten Island, NY; 2) Department of Biology and Center for Developmental Neuroscience and Developmental Disabilities. College of Staten Island, CUNY, Staten Island, NY.

We analyzed Fmr1 protein (Fmrp) and enhanced green fluorescent protein (EGFP) expression in adult female mice that carried the Fmr1 knock out mutation on one X chromosome and the EGFP transgene on the other X. As expected, confocal fluorescent microscopy showed that, due to X inactivation, approximately 50% of brain cells were negative for both Fmrp and EGFP. Surprisingly we found that Fmrp immunoreactivity was discordant with EGFP fluorescence, even though both the functional Fmr1 gene and the EGFP transgene were located on the same X chromosome. Cells that were strongly immunopositive for Fmrp showed little or no EGFP fluorescence. In vitro analysis of cerebellar granule cells from male mice carrying this X-linked EGFP transgene also showed substantial discordance between EGFP fluorescence and Fmrp immunoreactivity. Activation of glutamate receptors increased EGFP fluorescence, as has been observed in other systems (Job & Eberwine, 2001) and also appeared to increase the coincidence of EGFP fluorescence and Fmrp immunoreactivity. These observations suggest that Fmrp represses translation of the EGFP message and that the repression may be relieved by glutamate receptor activation. Translation repression by Fmrp is thought to be relieved through a signal cascade initiated by metabotropic glutamate receptor activation (Baer, Huber & Warren, 2004). It is possible that the EGFP transgene can serve as an indicator of Fmrp activity and possibly of the mGluR signaling cascade. To illustrate the potential linkage between mGluR activation, Fmrp and EGFP expression, we will show the effects of translation inhibitors, mGluR receptor agonists and antagonists on EGFP fluorescence in cerebellar granule cells in vitro.

912/T

Investigating Protein-Protein Interactions Relevant to SCA6 and SCA7 Pathogenesis. *J.J. Kahle[1, 2], J. Lim[1], H.Y. Zoghbi[1, 2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute.

Several dominantly inherited Spinocerebellar ataxias (SCAs) are caused by expansion of a translated CAG repeat encoding a polyglutamine (polyQ) tract in the respective proteins. The normal function of the ataxia proteins is proving relevant to pathogenesis, suggesting that disease proteins and their interacting partners play a role in neuronal health and survival. We generated an interactome using ataxia-causing proteins, however, we did not identify interactors for some full-length bait proteins, including CACNA1A (calcium channel alpha-1A subunit) and ataxin-7, the proteins mutated in SCA6 and SCA7, respectively. Our data suggest that rescreening with multiple fragments of these proteins may yield more interactions. CACNA1A is a large protein that has different splice forms, including one that expresses a polyQ tract in the cytoplasmic tail of the channel, and another, that has a stop prior to the polyQ tract. Using different wild-type splice isoforms of CACNA1A, as well as constructs containing different polyQ lengths we generated seventeen fragments of the cytoplasmic domain of the protein. We also generated eleven protein fragments of ataxin-7 with different length polyQ repeats. These baits were used in a high-stringency yeast 2-hybrid screen against an adult human brain cDNA library. We identified 254 total different interacting proteins; surprisingly, a subset of these are common to both ataxin-7 and CACNA1A. Ataxin-7 fragments interacted with many proteins reported to be expressed in the retina and a few that are suspected to have a role in eye disease, which is interesting given that patients with SCA7 exhibit macular degeneration in addition to ataxia. We identified 161 proteins interacting with CACNA1A, of these, 23 were in common to both splice forms. Currently, we are validating the interactions by co-affinity purification. Adding the new data to the existing ataxia interactome will enable us to look for additional common points of convergence for the various ataxias and will provide further insight about the normal function of the disease proteins and perhaps pathways involved in neurodegeneration.

913/T

Multiple sclerosis-like disorder in OPA1-related autosomal dominant optic atrophy. *P. Amati-Bonneau[1], C. Verny[2], D. Loiseau[1], C. Scherer[2], P. Lejeune[2], A. Chevrollier[1], N. Gueguen[1], V. Guillet[1], M. Ferré[1], P. Reynier[1], D. Bonneau[1].* 1) Département de Biochimie et Génétique, CHU Angers, INSERM U694, Angers, France; 2) Département de Neurologie, CHU Angers, France.

Autosomal dominant optic atrophy is a progressive ophthalmologic disorder caused by mutations in the Optic Atrophy 1 (OPA1) gene, a nuclear gene encoding a mitochondrial protein. We report a patient affected by bilateral optic atrophy associated with multiple sclerosis-like (MSL) features due to a novel OPA1 mutation. A 44 yrs-old man was referred to our neurology unit for a sudden onset of pain in the lower left limb. He presented progressive loss of visual acuity associated with trigeminal neuralgia that appeared two years earlier. Neurological examination revealed proprioceptive dysfunction, brisk tendon reflexes, and ankle clonus in the left lower limb. Ophthalmologic examination indicated bilateral visual acuity of 4/10, cæcocentral scotoma, blue-yellow dyschromatopsia, and moderate bilateral optic atrophy. MRI of the cerebrum and the spinal cord showed T2-weighted high intensity lesions, and fluid-attenuated immersion recovery (FLAIR) revealed white matter hyperintensities predominantly in the calloso-septal interface and in the periventricular region. None of the three main mutations or the seven rare mutations in mitochondrial DNA responsible for Leber's hereditary optic neuropathy was found. The sequencing of the OPA1 gene revealed a novel heterozygous S646L mutation absent in 200 controls. Biochemical studies performed on fibroblasts from the patient showed a significant mitochondrial coupling defect associated with reduced ATP production and respiratory function in comparison to 7 controls. Most OPA1 mutations lead to isolated optic atrophy. MSL features have not been reported so far in association with optic atrophy in patients harbouring OPA1 mutations. A more severe energetic defect was found in fibroblasts from this patient suggesting a relationship between the level of mitochondrial dysfunction and central demyelination. These findings reinforce the hypothesis of the implication of mitochondrial energy metabolism in neurodegenerative disorders, particularly in MS.

914/T

Mutations in the brand-new lebercillin gene account for 7.9 % of Leber congenital amaurosis (LCA) type II. *S. Gerber[1], S. Hanein[1], I. Perrault[1], N. Delphin[1], J.-L. Dufier[2], C. Leowski[3], A. Munnich[1], J. Kaplan[1], J.-L. Rozet[1].* 1) Genetics Dpt & Research Unit INSERM U781, Hopital Necker, Paris, France; 2) Ophthalmology Dpt, Hopital Necker, Paris, France; 3) Institut d'Education Sensorielle, Paris, France.

Purpose: Leber congenital amaurosis (LCA) is the earliest and most severe form of inherited retinal dystrophy responsible for blindness or severe visual impairment at birth or within the first months of life. Up to date, ten LCA genes have been identified. Three of them account for ca. 43% of families and are responsible for a congenital severe stationary cone-rod dystrophy (Type I, 60 % of LCA in our series) while the seven remaining genes account for 32% of patients and are responsible for a progressive yet severe rod-cone dystrophy (Type II, 40 % of LCA in our series). The purpose of this study was to evaluate the involvement of the brand-new LCA gene Lebercillin and to look for genotype-phenotype correlations. Patients & Methods: 95 LCA families including one large multiplex and consanguineous families (5/7 affected sibs)linked to LCA5 were considered. LCA genes were excluded in all 95 families prior to the present study. Mutations in the lebercillin gene were searched by direct sequencing. Results and discussion: Two lebercillin mutations were identified in 3/95 families. The p.Q204X mutation was homozygous in the multiplex consanguineous family linked to LCA5 and heterozygous in a French family while the p.E396X mutation was homozygous in a multiplex consanguineous Maroccan family (2/7 affected sibs). The natural history of the disease in the 3 families allowed drawing preliminary genotype-phenotype correlations as all three were affected with a severe LCA type II: early-onset yet progressive disease, night blindness followed by photophobia, no hyperopia, fundus appearance of RP with macular rearrangement, visual acuity measurable at early stages and reduced to light perception or counting fingers by the end of the second decade onwards. Conclusion: The 11th LCA gene, lebercillin, accounts for about 3% of all LCA cases. Interestingly, lebercillin is the 8th gene responsible for LCA type II (7.9%).

915/T

Molecular characterization of Leber congenital amaurosis in Korea. *M.W. Seong[1,2], S.Y. Kim[1], Y.S. Yu[3], J.M. Hwang[4], H.S. Ko[1], J.Y. Kim[1], S.S. Park[1].* 1) Department of Laboratory Medicine, Seoul National University College of Medicine & Seoul National University Hospital Clinical Research Institute, Seoul, Korea; 2) Department of Laboratory Medicine, National Cancer Center, Goyang, Korea; 3) Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea; 4) Department of Ophthalmology, Seoul National University Bundang Hospital, Seongnam, Korea.

Leber congential amaurosis (LCA) is the most severe form of all the inherited retinal dystrophies leading to congenital blindness. LCA is genetically heterogeneous disorder as well as clinically. Although more than 8 genes have been identified with an association of LCA so far, these genes are estimated to account for about half of LCA. We performed the comprehensive mutation analysis of known LCA genes in 20 unrelated Korean patients. All exons and flanking regions were analyzed by direct sequencing for nine genes: the *AIPL1, CRB1, CRX, GUCY2D, RDH12, RPE65, RPGRIP1, LRAT and TULP1.* We identified 9 different mutations in six patients (31.6% of all cases): one frameshift, one nonsense, one splicing and six missense mutations. Seven except one nonsense and one splicing mutation were novel mutations. None of them was recurrent. Mutations were most frequent in the *RPGRIP1* (13.2%) gene, and followed by *RPE65* (5.3%) and *CRB1* (5.3%). Three patients were compound heterozygte harboring two different mutations. The other three patients were found to be single heterozygous for a missense mutation. All novel missense mutations were predicted to be harmful to protein structure or function by analysis of amino acid conservation, characteristics of substituted amino acid and protein structural information. Similar predictions were obtained by in-silico analysis softwares such as Polyphen, SIFT and PMut. These results showed severe genetic heterogeneity in Korean LCA patients and different mutation spectrum from previous reports, suggesting that different strategy might be applied to molecular diagnosis of LCA in Korea.

916/T

The molecular pathophysiology of Borjeson Forssman Lehman Syndrome. *M.A. Corbett[1], L. Vandeleur[1], J. Crawford[1], C. Wilkinson[2], C. Shoubridge[1], E. Parkinson-Lawrence[3], D. Brooks[3], L.S. Nguyen[1], W. Just[4], J. Gecz[1,5].* 1) Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, Australia; 2) School of Mathematical Sciences, The University of Adelaide, Adelaide, Australia; 3) Division of Health Sciences, School of Pharmacy and Medical Sciences, City East Campus, The University of South Australia,Adelaide, Australia; 4) Department of Human Genetics, University Clinic Ulm, Ulm, Germany; 5) Department of Paediatrics, University of Adelaide, Adelaide, Australia.

Mutations in PHF6 cause Borjeson-Forssman-Lehmann Syndrome (BFLS), a syndromic form of X-linked mental retardation. The main clinical features of BFLS are intellectual disability, truncal obesity with gynecomastia, hypogonadism and large ears. To date there are 12 different PHF6 mutations known in 19 unrelated BFLS families and isolated cases. PHF6 is a ubiquitously expressed nucleolar protein, which has four nuclear localisation sequences and two PHD-like zinc finger motifs. To elucidate the role of PHF6 in the cell and in BFLS we performed microarray gene expression profiling on lymphoblastic cell lines (LCL) from six BFLS patients. We observed significant changes in RNA processing, DNA replication and the cell cycle genes, consistent with cellular processes of the nucleolus. We also noted that two unrelated patients with a recurrent c.1024 C>T, p.R342X mutation had significantly reduced levels of PHF6 transcript, due to nonsense mediated decay of the predominant PHF6 transcript (PHF6a) but not of an alternate transcript (PHF6b). PHF6a and PHF6b differ by the alternative exclusion or inclusion of a 330bp sequence in the 3'UTR. Examination of PHF6 expression in different tissues detected increased amounts of PHF6b in the brain compared to PHF6a levels. We showed by luciferase reporter assay that the 330bp 3'UTR sequence in PHF6b increases expression. Thus PHF6 expression can be regulated post-trancriptionally. We detected variable levels of PHF6 protein in LCL of patients with BFLS, including low levels of the truncated p.R342X mutant. All cases of BFLS are similar, thus we predict that PHF6 has a single functional domain that is disrupted by any mutation in the protein.

917/T

Significant Reductions of SMN and Gemin3 in X-linked SMA: Implications for Common Disease Pathways. *K.O. Yariz, L. Baumbach.* Miller School of Medicine, University of Miami, Miami, FL.

Our group has described an X-linked form of lethal infantile spinal muscular atrophy (MIM 301830), which is very similar clinically to Type I SMA, but with additional features of early onset/congenital contractures and/or fractures. Due to this phenotypic overlap, whether the SMN-Gemin complex (altered in autosomal recessive) is perturbed in XL-SMA is an important fundamental biological question. We have quantitatively measured SMN, Gemin-2 and Gemin-3 levels in lymphoblastoid cell lines from XL-SMA patients, SMA Type I patients, and controls. Preliminary results based on two unrelated XL-SMA patients suggest that XL-SMA patients have a significant reduction in steady-state levels of SMN and Gemin3 proteins as compared to a healthy control (40% less for SMN; 50% less for Gemin3), but they still have more of these proteins than a SMA (Type I) patient. The two unrelated XL-SMA patients display almost identical SMN and Gemin3 protein levels. Gemin2 protein levels were not altered in the XL-SMA cell lines. RNA expression studies were performed for SMN1 and Gemin3 in the same cell lines used above. These results indicate no significant difference in gene expression of SMN and Gemin3 in XL-SMA and SMA cell lines compared to the healthy control. This implies that these genes are expressed normally in XL-SMA, but protein levels are altered. Unexplained post-transcriptional, translational, or a post-translational events may likely account for this observation. It is intriguing that autosomal recessive SMA and XL-SMA share many clinical similarities; therefore, one could hypothesize common disease mechanisms. Our results suggest that SMN protein and at least one other component of the SMN complex is substantially reduced in XL-SMA patients, thus accounting in part, for the phenotypic overlap of these genetically distinct disorders. These observations also suggest new insights into therapeutic targets for XL-SMA.

918/F

An On-line Database System for Knock-down Analysis of KAO-NASHI Genes. *A. Shimizu[1], S. Asakawa[1], T. Sasaki[1], N. Shimizu[2].* 1) Dept Molecular Biol, Keio Univ Sch Medicine, Tokyo, Japan; 2) GSP Center, The Leading Institute of Keio University, Tsukuba, Japan.

The human genome project has provided a computer-estimation of 23,000 protein-coding genes in the human genome. However, many of these protein-coding genes are not fully proven for their existence by experimental evidence. In general, proteins with known motifs are readily classified, but substantial numbers of protein have no obvious motifs in their sequences. We designated these genes/proteins without obvious motifs as KAO-NASHI (Faceless) and initiated a project to unveil their face (kao) by comparative genomics and knock-down analysis.

We extracted 1,000 KAO-NASHI genes from human genome sequence by step-wise data filtration with InterPro motif analysis, BLAST homology search and PubMed document search. A small fish medaka (Oryzias latipes) was chosen as an experimental system to knockdown medaka orthologs of human KAO-NASHI genes with morpholino-antisense oligos. As an initial study, we designed antisense oligos to target translation initiation sites of 100 medaka kao-nashi genes. When these antisense oligos were micro-injected into medaka fertilized eggs, their morphogenesis at early developmental stages was occasionally disturbed and morphological changes were observed. Thus, we obtained initial information how these medaka kao-nashi genes are involved in the developmental process of patterning and organ formation. About 60 genes were found to cause morphological defects and these were further classified in terms of developmental sub-stages and expression profiles. Especially, suppression of the three kao-nashi genes resulted in brain vesicle expansion. All these knockdown data were combined with the pictures of knockdown medaka embryo and stored in our original database.

Thus, our approach using medaka will eventually provide functional information on the human KAO-NASHI genes/proteins.

919/F

DIMORPHIC EFFECTS OF NOTCH SIGNALING IN BONE HOMEOSTASIS AND DYSREGULATION IN OSTEOSARCOMA VS. AGE RELATED OSTEOPOROSIS. *F. Engin[1], T. Yang[1], G. Zhou[1], T. Bertin[1], M.M. Jiang[1,2], Y. Chen[1,2], L. Wang[3], H. Zheng[1], Z. Yao[4], B. Boyce[4], B. Lee[1,2].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston, TX; 3) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 4) Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY.

Notch signaling plays an important role in various developmental processes including cell fate determination, differentiation, proliferation, and apoptosis. Dysregulation of Notch pathway has been implicated in many different diseases including spondylocostal dysostosis and cancer. However, its in vivo function in bone homeostasis remains largely unknown. Here, we show that osteoblast-specific gain of function of Notch1 results in severe osteosclerosis. Transgenic mice over-expressing Notch1 intra cellular domain (N1ICD) from the *Col1a1* promoter have increased proliferation of immature osteoblasts that produce immature woven bone. Under these pathological conditions, Notch stimulates early osteoblastic proliferation by up-regulating *Cyclin D*, *Cyclin E* and *Osterix*. Notch also regulates osteoblastic terminal differentiation by binding *Runx2*, an essential transcription factor for osteoblastogenesis, and repressing its transactivation function. Consistent with this proliferative effect, human osteosarcomas show evidence of increased Notch signaling and its inhibition by a γ-secretase inhibitor in vitro decreases the proliferation of human osteosarcoma cells. In contrast, loss of all physiologic Notch signaling in osteoblasts, generated by deletion of *Presenilin 1* and *2* in osteoblasts, is associated with late onset, age-related osteoporosis. Double knock-out mice show decreased expression of *Osteoprotegrin* (*Opg*) expression indicating an increased osteoblast-dependent osteoclastic activity. Moreover, co-culture and flow cytometric analyses reveal increased differentiation of osteoclast precursors explaining the low bone mass phenotype in these mice. Together, these findings highlight the potential dimorphic effects of Notch signaling in bone homeostasis, and importantly, they may provide direction for novel therapeutic applications.

920/F

Loss of *Smad1* and *Smad5* in proliferating chondrocytes leads to a shortened growth plate and craniofacial phenotype. *B. Keller[1], P. Hermanns[2], B. Zabel[2], B. Lee[1].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, Tx; 2) Centre for Pediatrics and Adolescent Medicine, University Hospital of Freiburg, Germany.

Bone morphogenetic proteins (BMPs), members of the TGFβ superfamily, play a pivotal role in the development of the vertebrate skeleton. The BMP-Receptor BMPR-1B (ALK-6) is expressed in all types of cartilage and expression of a dominant-negative form of BMPR-1B is able to block chondrogenesis and osteogenic differentiation. Smad1, Smad5 and Smad8 are the mediators of the BMP signaling pathway. Binding of BMP to the receptor leads to the phosphorylation of the Smads. They undergo a conformational change and are able to bind the Co-receptor (Co-Smad) Smad4. This complex translocates into the nucleus and activates or represses the transcription of target genes. *Smad1* and *Smad5* are expressed in proliferating and maturating chondrocytes, whereas Smad1 and Smad8 have been shown to be expressed in mesenchymal cells. We examined the role of *Smad1* and *Smad5* in chondrogenesis by knocking out *Smad1* in proliferating chondrocytes using the Cre-loxP system on a *Smad5* heterozygous background. P1 mutant mice skeletons stained with alcian blue and alizarin red appeared to be normal and no obvious patterning defect could be observed. On cellular level we could detect a slight shortening of both, the prehypertrophic and hypertrophic zones due to a decrease in the proliferation rate. *Ihh* expression in mutant animals is reduced as well. Additionally, adult mutant mice exhibit a subtle craniofacial phenotype with a shortening of the head and missing nasal septum.

921/F

E-Selectin Ligand 1 Negatively Regulates TGFβ in the Golgi during Skeletogenesis. *T. Yang[1], R. Mendoza[1], H. Lu[1,5], K. Li[1], B. Keller[1], M.M. Jiang[1,2], Y. Chen[1,2], T.K. Bertin[1], B. Dabovic[4], D.B. Rifkin[4], J. Hicks[3], A.L. Beaudet[1], B. Lee[1,2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Department of Pathology, Baylor College of Medicine and Texas Children's Hospital; 4) Department of Cell Biology, New York University Medical Center; 5) Department of General Internal Medicine, UT MD Anderson Cancer Center.

E-Selectin Ligand-1 (ESL-1), the cysteine rich protein originally isolated as a ligand for E-Selectin, was also found to interact with FGFs and to be co-purified with TGFb1 in a large protein complex. To elucidate its in vivo function, we generated Esl-1-/- KO mice. The newborn Esl-1-/- mice are notably smaller with narrow chests and generalized shortening and thinning of all bony elements. The severe growth retardation was observed from E15.5 to maturity. Histologically, P1 Esl-1-/- mice showed shortening of the growth plates in both the proliferating zone and hypertrophic zone. Moreover, the notable less bone density was also detected in the adult mutant mice. Further molecular assays show that ESL-1 acts as a negative regulator of TGFβ production by binding TGFβ precursors via the laternt activation domain (LAP) in the Golgi in a cell autonomous fashion. In vivo, loss of ESL1 function causes increased TGFβ signaling resulting in decreased cell proliferation and delayed terminal differentiation in the growth plate, independent of effects on BMP and FGF signaling. Moreover, in vivo genetic models of gain vs. loss of TGFβ signaling in the growth plate confirm this effect. Transforming growth factor β (TGFβ) signaling plays critical roles on regulating the growth and differentiation during development and diseases. Its context dependent action is specified by numerous control mechanisms at the extracellular level and downstream of ligand-receptor interactions, but little is known about the regulation of its post-translational trafficking. Our data not only identified ESL-1 as a critical regulator for skeletogenesis, cartilage and bone homeostasis, but also revealed a novel mechanism for regulating TGFβ intracellular pool in these processes.

922/F

Identification of Genes Involved in Neural Tube Defects and Neurogenesis. *A. katz[1], T. Hare[1], S. Khateeb[1], R. Ofir[1], V. Caspi[2], O.S. Birk[1].* 1) The Morris Kahn Laboratory of Human Genetics, the National Institute for Biotechnology in the Negev, Ben-Gurion University, Beer-Sheva, Israel; 2) the National Institute for Biotechnology in the Negev, Ben-Gurion University, Beer-Sheva, Israel.

Neural tube defects (NTDs) have an incidence of approximately 1 in 1,000 births worldwide. Numerous models of NTDs exist in the mouse. Studies of these models have led to the elucidation of specific molecular pathways critical to neural tube closure. The neural tube, formed from neuroephitheilum, closes at approximately the fourth week post-conception in the human foetus, and at E8.5 -E10 in the mouse. In an attempt to enhance and expand our understanding of the molecular mechanisms and pathways underlying neural tube closure, we undertook a high-throughput gene expression analysis (Affymetrix Mouse Genome 430 2.0 expression arrays) of open and closed sections of the mouse neural tube as it forms in the mouse embryo.

An array of known genes as well as novel genes (possibly associated with tube closure), were found to be over-expressed in the open sections of the neural tube as compared to the closed section. Other gene clusters were shown to be expressed more in the closed sections of the neural tube - suggesting a possible role in neurogenesis.

(TH, SK and AK contributed equally to this study).

923/F

Spatiotemporal expression in mouse brain of *Kiaa2022*, a gene disrupted in two patients with severe mental retardation. *A.M. Lossi[1], V. Cantagrel[1], R. Haddad[1], P. Ciofi[2], D. Andrieu[3], L. van Maldergem[4], J.C. Roux[1], L. Villard[1].* 1) Faculté de Médecine, INSERM U491, Marseille, France; 2) INSERM U378, Bordeaux, France; 3) CNRS UMR6156, IBDML, Campus de Luminy, Marseille, France; 4) Centre de Génétique Humaine, Université de Liège, Liège, Belgium.

We previously reported two male patients suffering from severe mental retardation in whom the *KIAA2022* gene was disrupted by an intrachromosomal rearrangement and no longer expressed. Virtually nothing is known about the function of *KIAA2022*, encoding a predicted protein of 1516 aminoacids with no homology to other known proteins. Therefore, to better understand the function of *KIAA2022* in brain function, we have cloned its murine ortholog, *Kiaa2022*. We have determined its genomic structure and we have studied its expression during mouse development. Using quantitative RT-PCR and in situ hybridization, we show that *Kiaa2022* is preferentially expressed in the central nervous system although its transcript can also be detected in other tissues. The expression of *Kiaa2022* is temporally and spatially regulated. It is initially detected at E11 in postmitotic neurons of the central nervous system. The expression increases rapidly during the development to reach a maximum at P3 where *Kiaa2022* is expressed in the hippocampus, the entorhinal cortex and very strongly in the ventral premammillary nucleus. After P3, the expression of *Kiaa2022* decreases rapidly and is maintained at low levels during adulthood. These results suggest that *Kiaa2022* plays a role in postmitotic neurons during brain development.

924/F

Neuron-specific enhanced expression of TAF1 and its isoform. *S. Makino, G. Tamiya.* Division of Human Molecular Genetics, Department of Neurology, Tokushima University Graduate School of Medicine, Japan.

We previously found a neuron-specific isoform of the *TAF1* (TATA-binding protein-associated factor 1) gene, which is the disease causative gene of X-linked recessive dystonia-parkinsonism showing severe neurodegeneration in striatum (XDP/DYT3; MIM314250). The *TAF1* gene encodes the largest component of the TFIID complex involved in RNA polymerase II-mediated expression of many genes related cell division. The neuron-specific isoform of the *TAF1* gene, named *N-TAF1*, may have an essential role in neuronal survival in the striatum through transcriptional regulation of many neuron-specific genes. To investigate the detailed function of the neuron-specific isoform of the *TAF1* gene, we performed knockdown of the neuron-specific isoform using a specific siRNA in the human dopaminergic neuroblastoma cell line SH-SY5Y. The siRNA significantly reduced mRNA of the neuron-specific isoform to approximately 1/6 of that in the negative control siRNA with no induction of the interferon response. We subsequently performed microarray analysis for the knockdown cell line using an Affymetrix Human Genome Focus Array representing 8,793 annotated genes. In addition, we carried out over-expression of *N-TAF1* in the mouse N2a cell line and subsequent microarray analysis. Through these *in vitro* experiments, we demonstrated that the neuron specific enhanced expression of *TAF1*/*N-TAF1* regulate the neuron-specific gene transcriptions.

925/F

Loss of necdin in the mouse impairs the migration of neurons in the developing nervous system. *A.A. Tennese, J.R. Bush, R. Wevrick.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder characterized by failure to thrive, neonatal hypotonia, hyperphagia, childhood-onset obesity, and global developmental delay. PWS is caused by the inactivation of a subset of genes on chromosome 15. Necdin is one of the genes inactivated in individuals with PWS and is located in a syntenic region on chromosome 7C in the mouse. The expression of necdin in the mouse is highest in tissues relevant to PWS, including the central and peripheral nervous systems, and muscle. We previously determined that loss of necdin in mice causes axonal extension, bundling, and branching defects in cultured sympathetic chain ganglia neurons. Therefore, we examined the sympathetic nervous system during prenatal development in necdin-null mouse embryos using immunohistochemistry. We identified a defect in the size and location of the superior cervical ganglia (SCG), the most rostral ganglia in the sympathetic chain, in necdin-null embryos. The SCG appears normal at midgestation, but does not migrate towards the head at later stages in development as is normally observed in control embryos. In later stage necdin-null embryos, a decrease in innervation of SCG target tissues and an increase in cell death are also observed. As the survival of neurons requires nerve growth factors produced by target tissues, the reduction in axonal outgrowth likely causes the increased apoptosis in the maturing SCG neurons. To address the molecular mechanisms by which necdin might promote proper migration, we cultured fibroblasts from necdin-null embryos and control littermates. Necdin-null fibroblasts show reduced migration in cell culture wound-healing assays, suggesting that loss of necdin may affect the ability of the cytoskeleton to reorganize itself in response to environmental cues. We propose a novel role for necdin in the migration of neurons and other cell types in which it is expressed. Understanding the function of necdin in cytoskeletal reorganization and cellular migration may identify a cause for many characteristics of the PWS phenotype.

926/F

Identification of a ciliary protein as a novel contributor to sporadic heterotaxia. *E.E. Davis[1], J.W. Belmont[2], H. Omran[3], N. Katsanis[1].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore MD 21205, USA; 2) Department of Molecular and Human Genetics and Division of Cardiology, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA; 3) Department of Pediatrics and Adolescent Medicine, University Hospital Freiburg, Freiburg, Germany.

Heterotaxia is defined as the spectrum of birth defects where asymmetric left-right (LR) patterning is perturbed; typical manifestations include cardiovascular, gastrointestinal, pulmonary, and genitourinary anomalies. Despite its frequent occurrence in the population (as high as 1:500 live births), the genetic basis for heterotaxias remains largely unknown. It has been shown that defects in cilia in the embryonic node can cause a constellation of phenotypes including LR determination defects, as in the case of Kartagener syndrome, or, less frequently, in Bardet-Biedl syndrome, we hypothesized that mutations in ciliary protein-encoding genes could contribute to sporadic heterotaxia. To explore this possibility, we took advantage of a recently defined integrated ciliary proteome to screen a series of known and novel ciliary genes in a panel of heterotaxia patients. For one transcript, CP007, we have found both nonsense and missense mutations in highly conserved amino acids in ~2% of patients. To confirm the pathogenic potential of the missense variants, we pursued a functional analysis in zebrafish, where we have measured the ability of mutant mRNA to rescue LR-determination defects caused by cp007 suppression; our preliminary data suggest that some of the cp007 mutations compromise but do not completely abrogate the function of the CP007 protein and support the potential role of this transcript in sporadic heterotaxias. Overall, our data suggest that partial loss of ciliary function predisposes to LR determination defects in humans and that evaluation of additional ciliary genes will expand our appreciation of the total mutational load in this phenotype. Finally, the implementation of an in vivo model to evaluate all variants found represents an indispensable tool that will likely be important in the emerging medical resequencing era.

927/F

Analysis of Fibroblast growth factor 15 cis-elements reveals two conserved enhancers which are closely related to cardiac outflow tract development. *H. Saitsu[1, 2], K. Shiota[2, 3], M. Ishibashi[2].* 1) Department of Human Genetics, Graduate School of Medicine, Yokohama City University; 2) Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University; 3) Congenital Anomaly Research Center, Graduate School of Medicine, Kyoto University.

Fibroblast growth factor 15 (Fgf15) is expressed in the developing mouse central nervous system and pharyngeal arches. Fgf15 mutant mice showed defects of the cardiac outflow tract probably because of aberrant behavior of the cardiac neural crest cells. In this study, we examined cis-elements of the Fgf15 gene by transient transgenic analysis using lacZ as a reporter. We identified two enhancers: one directed lacZ expression in the hindbrain/spinal cord and the other in the posterior midbrain (pmb), rhombomere1 (r1) and pharyngeal epithelia. Interestingly, human genomic regions which are highly homologous to these two mouse enhancers showed almost the same enhancer activities as those of mice in transgenic mouse embryos, indicating that the two enhancers are conserved between humans and mice. We also showed that the mouse and human pmb/r1 enhancer can regulate lacZ expression in chick embryos in almost the same way as in mouse embryos. We found that the lacZ expression domain with this enhancer was expanded by ectopic Fgf8b expression, suggesting that this enhancer is regulated by Fgf8 signaling. Moreover, over-expression of Fgf15 resulted in up-regulation of Fgf8 expression in the isthmus/r1. These findings suggest that a reciprocal positive regulation exists between Fgf15 and Fgf8 in the isthmus/r1. Together with cardiac outflow tract defects in Fgf15 mutants, the conservation of enhancers in the hindbrain/spinal cord and pharyngeal epithelia suggests that human FGF19 (ortholog of Fgf15) is involved in early development and the distribution of cardiac neural crest cells and is one of the candidate genes for congenital heart defects.

928/F

Adrenergic system in adult cardiac myocytes. *X. Bao[1], J. Lopez[2], B. Myagmar[2], C.M. Lu[3], P.C. Simpson[2], M.G. Ziegler[1].* 1) Dept Medicine, Univ California, San Diego, Novato, CA; 2) Cardiology Division, VA Medical Center, University of California San Francisco; 3) Department of Laboratory Medicine, VA Medical Center and University of California San Francisco.

Endogenous norepinephrine (NE) and epinephrine (E) play an important role in augmenting cardiac function. Although an intrinsic cardiac adrenergic cell independent of sympathetic neuron cell has been identified in rodent and human heart, it is not clear whether adult cardiac myocyte itself synthesizes catecholamine (CA). To address this question, we inserted the Cre-recombinase gene into the locus encoding phenylethanolamine N-methyltransferase (PNMT), a last enzyme in CA biosynthsis pathway, and crossed these PNMT-Cre mice with ROSA 26 and Z/EG reporter mice to activate lacz and green fluorescent protein (GFP) expression in cells that were selectively derived from the adrenergic lineage. We found that both LacZ and GFP expression were activated in about 10 % adult cardiac myocytes isolated from left ventricle. RT-PCR analyses demonstrated that the cardiac myocytes contained mRNA of catecholamine biosynthetic enzymes aromatic-L-amino-acid decarboxylase, dopamine b-hydroxylase, and PNMT except for tyrosine hydroxylase. Further radioenzymatic assays revealed that there were PNMT activity (6.2 ± 0.4 pmol/g/h) and CA synthesis (66± 6 pg/mg for dopamine, 2605 ± 142 pg/mg for NE, and 46 ± 8 pg/mg for E, respectively) in the cardiac myocytes. These findings suggest that a subset of adult cardiac myocytes constitute an adrenergic signaling system capable of synthesizing CA.

929/F

The genetic architecture of congenital heart disease. *J.B. Winston, J.M. Erlich, P.Y. Jay.* Pediatrics, Washington University, St. Louis, MO.

Many genetic mutations have been discovered in the past decade that cause congenital heart defects in man and mouse models, but little is known regarding the genetic pathways that lead to specific defects. Heterozygous mutations of the cardiac transcription factor NKX2-5 cause a broad spectrum of heart defects with incomplete penetrance. Modifier genes may contribute to variable NKX2-5 phenotypes. An unbiased genetic approach to their discovery can offer insight into pathways and phenotypic variability. A large-scale linkage analysis project was conducted to identify modifier genes that have a main or epistatic effect on the Nkx2-5 mutant phenotype by crossing Nkx2-5$^{+/-}$ mice in an isogenic C57Bl/6 background to FVB/N or A/J inbred strains. Nkx2-5$^{+/-}$ animals in the C57Bl/6 background have a 15-20% incidence of ventricular or atrial septal defects (VSD, ASD). Nkx2-5$^{+/-}$ F1 progeny of C57Bl/6 mice crossed to inbred strains FVB/N or A/J have a nil or rare incidence of defects. Defects are recovered in F2 progeny of inter- and parental backcross backgrounds, suggesting an effect of homozygosity of unknown modifier loci. To map loci that modify the Nkx2-5 mutant phenotype, neonatal hearts of 1,426 F2 Nkx2-5$^{+/-}$ mice were serially sectioned to identify congenital heart defects. Thus far, 256 defects have been found with the vast majority being VSD. Genome wide linkage analysis for VSD susceptibility loci was carried out on F2 progeny to localize inbred strain polymorphisms that influence Nkx2-5$^{+/-}$ hearts toward or away from defects. Analysis revealed a suggestive main effect locus on chromosome 10, and 16 significant epistatic QTL involved in 11 epistatic interactions. Four loci representing 2 epistatic interactions were also found to be significant in the C57Bl/6-A/J intercross. Seven epistatic loci form an interaction network suggesting the existence of a complex regulatory pathway associated with Nkx2-5. The results offer a conceptual framework to place genes into genetic pathways. Finally, an appreciation of the role of modifier genes and genetic interactions in disease pathogenesis should suggest novel, unprecedented strategies to prevent or ameliorate serious congenital heart disease, a leading cause of death in children.

930/F

Copy number analysis of patients with gonadal dysfunction using high-density microarrays and MLPA. *S.J. White[1], S.E. Gustin[1], C.A. Smith[1], S. Forrest[2], M. Bahlo[3], H. Bengtsson[3], K. Bell[1], T.P. Speed[3], A.H. Sinclair[1].* 1) Murdoch Children's Research Institute, Melbourne, Australia; 2) Australian Genome Research Facility, Melbourne, Australia; 3) Walter and Eliza Hall Institute, Melbourne, Australia.

Intersex disorders, ranging in severity from genital abnormalities to complete sex reversal, are surprisingly common and as such represent a major paediatric concern. The cause of these problems is most often the failure of the complex network of genes that regulate development of testes or ovaries. However, we understand relatively little of this regulatory network. Mutations in the critical testis-determining genes SRY and SOX9 account for approximately 20% of XY females with gonadal dysgenesis. We have little idea about what other genes may be involved to account for the remaining 80% of patients. In contrast, 90% of XX males with gonadal dysgenesis are due to Y translocations that include SRY. DNA from 15 sex-reversed patients with gonadal dysgenesis (XX males lacking SRY and XY females without mutations in SRY) has been collected. We have used the Affymetrix 500K SNP arrays to screen the genome of sex-reversed patients for copy number changes. In these 15 patients 26 deletions and duplications were detected that covered at least one gene. These and other candidate regions are currently being confirmed with MLPA, and de novo status is being checked in parental DNA. This powerful approach will identify new genes involved in sex determination.

931/F

Searching for an embryonic lethal mutation associated with DNA methylation. *K.M. Baumgarner[1], M.J. Cramer[1], M.J. Justice[2], A.C. Lossie[1].* 1) Animal Science, Purdue University, West Lafayette, IN; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Virtually all human diseases result from genetic mutations and/or errors in gene expression that can be studied using mouse models, which are important tools for identifying disease-causing and developmentally regulated genes. The peri-implantation window is crucial for DNA methylation and subsequent determination of an individual's epigenome; disruptions in this process often cause embryonic lethality in mammals. This project utilized the *l11Jus1* line of embryonic lethal, homozygous mutant mice, which were derived from an ENU mutagenesis screen targeted to a 35 Mb region of MMU11. Homozygotes die shortly after implantation and demonstrate defective DNA methylation at the *Commd1/U2af1-rs1* locus. The phenotype of the mutants is much more severe than what would be predicted from disruption of these reciprocally imprinted genes. Therefore, we hypothesize that the mutated gene will either disrupt global DNA methylation imprinting or be essential for embryonic development. The goal of this project is to positionally clone *l11Jus1* in order to better understand the epigenetic factors that are involved in epigenome establishment during early mammalian development. The first objective was to narrow the region by meiotic mapping of recombinant animals derived from an F_2 intercross. The second objective was to sequence the best candidate genes in the critical interval (CI), including: *Ap2b1*, *Suz12*, *Evi2a*, *Cct6b*, *Riffl*, and the *Slfn* family of genes. After mapping 527 animals, we narrowed the CI to a 4.3 Mb region flanked by *Wsb1* and *D11MIT120*. During sequence analysis, we identified many single nucleotide polymorphisms (SNPs) between the two background strains (129S6/SvEvTac and C57BL/6J). We have yet to locate the mutation, but have found 25 expressed SNPs, 35 intronic SNPs, and 9 small deletions to date. These SNPs and deletions will be added to the SNP databases, expanding the coverage of the 129S6/SvEvTac genome. We are further narrowing the CI by generating more recombinant animals, and sequencing additional candidate genes.

932/F

PITX2 gain-of-function induced defects in mouse forelimb development. *J. Holmberg[1], G. Gustavsson[2], C. Johansson[2], P. Leander[2], T.A. Hjalt[1].* 1) Experimental Medical Science, Lund University, LUND, Sweden; 2) Radiation Physics, Lund University, MALMOE, Sweden.

Limb development and patterning originates from a complex interplay between the skeletal elements and muscles of the limb. One of the genes involved in patterning of limb muscles is the transcription factor Pitx2 but its role in forelimb development is uncharacterized. Pitx2 is expressed in the majority of premature presumptive forelimb musculature at embryonic day 12.5 and then maintained throughout embryogenesis to adult skeletal muscle. In vitro studies have shown that over-expression of Pitx2 in myoblasts arrests differentiation. To further study the role of Pitx2 in forelimb development we have generated transgenic mice that exhibit a pulse of Pitx2 over-expression at embryonic day 13.5 in the developing forelimb. These mice exhibit a distal misplacement of the biceps brachii insertion during embryogenesis, which twists the forelimb musculature resulting in severe skeletal malformations. These skeletal malformations have some similarities to the pathogenesis of Leri-Weill dyschondrosteosis, which is characterized by disproportionate short stature and a characteristic curving of the radius, known as the Madelung deformity. Taken together, the tendon, muscle, and bone anomalies further support a role of Pitx2 in forelimb development and may also shed light on the interaction between the skeletal elements and muscles of the limb during embryogenesis.

933/F

***Chd7* loss of function phenotypes in mice resemble those in human CHARGE syndrome and include variable and highly penetrant inner ear defects and postnatal growth delays.** *D. Martin[1,2], E. Hurd[1], M. Adams[3], K. Cheng[1], W. Layman[2], D. Swiderski[3], L. Beyer[3], Y. Raphael[3].* 1) Pediatrics, The University of Michigan, Ann Arbor, MI; 2) Human Genetics, The University of Michigan, Ann Arbor, MI; 3) Otolaryngology, The University of Michigan, Ann Arbor, MI.

CHARGE syndrome is a multiple anomaly condition characterized by ocular Coloboma, Heart defects, Atresia of the choanae, Retarded growth and development, Genital hypoplasia, and Ear defects including deafness and semicircular canal dysgenesis. Heterozygous loss of function *CHD7* mutations are present in 60-80% of CHARGE individuals, yet little is known about the pathogenesis of tissue specific defects associated with *CHD7* deficiency. To explore developmental roles of CHD7, we have generated a *Chd7* gene trapped *lacZ* reporter allele, $Chd7^{Gt}$. Homozygous $Chd7^{Gt/Gt}$ mice are embryonic lethal, whereas heterozygous $Chd7^{Gt/+}$ mice exhibit circling behaviors and postnatal growth delays reminiscent of human CHARGE syndrome. Here we show that $Chd7^{Gt/+}$ mice have variable and highly penetrant defects of the lateral and posterior semicircular canals, with severe defects in innervation of vestibular sensory epithelia despite the presence of intact sensory hair cells. Using a newly available anti-CHD7 antibody, we observed absence of CHD7 protein in homozygous $Chd7^{Gt/Gt}$ embryos, confirming that $Chd7^{Gt}$ is a null allele. Tissue specific expression of anti-CHD7 and β-galactosidase in $Chd7^{Gt/+}$ mice show the utility of $Chd7^{Gt}$ for tracking the fates of CHD7-expressing cells. CHD7 is expressed in dividing cells of the embryonic brain and ear, and in mouse embryonic fibroblasts (MEFs), validating the use of MEFs for *in vitro* studies of CHD7 function. These results improve our understanding of developmental CHARGE phenotypes. Inner ear malformations are one of the most highly penetrant clinical features in CHARGE and in $Chd7^{Gt/+}$ mice, suggesting the mammalian inner ear has a unique requirement for *Chd7* function. Ongoing studies in our laboratory are aimed at exploring the downstream target genes and molecular mechanisms of *Chd7* loss of function in the developing inner ear and craniofacial structures.

934/F

***SUMO1* and primary palatogenesis in humans and mice.** *H. Mishima[1], M.A. Mansilla[1], M.K. Johnson[1], S.A. Bullard[1], T. Busch[1], L.M. Moreno[1], M. Arcos-Burgos[2], C. Valencia[3], A. Hing[4], E.J. Lammer[5], M. Jones[6], M.L. Marazita[7], J.C. Murray[1], A.C. Lidral[1].* 1) U. Iowa, IA; 2) NIH, MD; 3) U. Antioquia, Colombia; 4) Children's Hosp., Seattle, WA; 5) Children's Hosp., Oakland, CA; 6) Children's Hosp., San Diego, CA; 7) U. Pittsburgh, PA.

Purpose: *SUMO1* (2q33.1) is a gene involved in posttranslational modification of proteins. A recent report of a patient with cleft lip and palate with a balanced translocation breaking *SUMO1* has suggested that *SUMO1* has a causal role in CL/P. Inactivation of *Sumo1* in mice also supported a role in secondary palatogenesis. However its role in primary palatogenesis is still unclear. Human studies of *SUMO1* have not been reported. This characterized *Sumo1* expression during primary palatogenesis using mouse embryos. Human studies were performed to assess association between *SUMO1* and CL/P; and search for mutations in coding sequence of *SUMO1*. **Methods:** C57BL/6 mouse embryos were evaluated via in situ hybridization for *SUMO1* expression between embryonic days E9.5 and E12.5. Colombian (546), Filipino (372), and US (301) familial triads having probands affected with CL/P were genotyped for 3 SNPs nearby *SUMO1*. Statistical testing for association was performed using FBAT. *SUMO1* was also sequenced in 184 cases and 183 controls. **Results:** At E9.5, no specific signal was detected. At E10.5, the nasal processes had weak expression. At E11.5, *Sumo1* was expressed in the maxillary, medial nasal and lateral nasal processes as they are fusing. At E12.5, expression was not observed in the fused primary palate, whereas the nasal pits and primary choanae had moderate expression. Human studies revealed significant association only in the Colombian families with a SNP (rs13383137, p=0.0016). Sequencing of *SUMO1* revealed three novel noncording SNPs. **Conclusions:** *SUMO1* is expressed during the later stages of primary palatogenesis. Human studies reveal that *SUMO1* variants may contribute to CL/P. However, the discrepancy between study populations suggests genetic heterogeneity of CL/P at this locus. These data provide evidence that *SUMO1* is important for primary palatogenesis. **NIH grants:** R01-DE014667, K02-DE015291, R37-DE08559, P50-DE016215.

935/F

Zebrafish dax1 Has Novel Functions in Enamel-Forming Ameloblasts and Primary Tooth Development. *J. Powers[1], Y. Zhao[2,3], E.R.B. McCabe[1,2,4].* 1) Pediatrics, UCLA, Los Angeles, CA, USA; 2) Human Genetics, UCLA, Los Angeles, CA, USA; 3) Cell and Developmental Bio, UCLA, Los Angeles, CA, USA; 4) Bioengineering, Henry Samueli School of Eng and App Sci, UCLA, Los Angeles, CA, USA.

Dax1 is a nuclear receptor encoded by NROB1 in the human Xp21 chromosome region. Mutations in DAX1 cause X-linked Adrenal Hypoplasia Congenita (AHC) and Hypogonadotropic Hypogonadism (HH). Zebrafish dax1 is the mammalian Dax1 ortholog and the gene structures are absolutely conserved across species. In situ hybridization (ISH) of dax1 in zebrafish revealed an unrecognized dax1-expressing structure. Double ISH studies with appropriate markers showed that this structure is not the ears, pronephric tubule, or fin buds, but does localize to the fifth branchial arch, where pharyngeal tooth development is known to occur. Tooth markers, dlx2a, dlx2b, and pitx2a appearing around 48 hpf do not co-localize with dax1, and dax1 MO had no effect on the expression of these markers. Double ISH with dax1 and eve1, a gene known to be involved in differentiation of the enamel-forming ameloblasts and initiation of the first pharyngeal tooth, showed co-localization of their signals. Mismatch control and MO studies revealed that zebrafish dax1 reduced expression of eve1. We conclude that zebrafish dax1 has novel functions outside the Hypothalamic-Pituitary-Adrenal-Gonadal axis, specifically in influencing amelogenesis and primary pharyngeal tooth development, and therefore is the earliest tooth marker for zebrafish tooth development. DAX1 has been reported to be upregulated in sarcomas and to have a role in osteoblast development. We speculate that dax1 may involve similar NR partners to those involved in its roles in bone, and that DAX1 may be involved in abnormalities in primary tooth development and enamelogenesis.

936/F

ENU-induced, targeted and sporadic mutations reveal a critical role for *Prdm16* during mouse and human craniofacial development. *B.C. Bjork[1], A.R. Vieira[2], S.W. Davis[3], S.A. Camper[3], J.C. Murray[2], D.R. Beier[1].* 1) Div Genetics, Brigham & Women's Hospital, Boston, MA; 2) Dept. Pediatrics, Univ. of Iowa, Iowa City, IA; 3) Depts. Human Genetics and Internal Medicine, Univ. of Michigan, Ann Arbor, MI.

Our ongoing recessive ENU mutagenesis screen generates phenotypes that model human birth defects, such as clefting. The *cleft palate only 1 (cpo1)* mutation is an excellent model of nonsyndromic clefting.

The *cpo1* mutant is a hypomorphic allele caused by a mutation that reduces splicing efficiency to the 7th exon of the zinc finger transcription factor *Prdm16*. Mutant palate shelves fail to elevate. Skeletal preparations, histology and palate culture show that this failure is due to physical obstruction by the tongue. *Prdm16* expression in palate epithelium and mesenchyme, craniofacial and tongue musculature and developing craniofacial cartilage is consistent with it playing a critical role during palatogenesis. We confirmed the etiology of *Prdm16* in *cpo1* mutants with the observation that homozygous mutants carrying a gene trap allele of *Prdm16* have a cleft palate and consistent pattern of *LacZ* expression.

Previously, we identified 3 potential etiologic missense mutations in *PRDM16* in a screen of 200 NSCL/P cases from Iowa and the Philippines. Since isoforms of the *PRDM16* paralog *MDS/EVI-1* are known to inhibit TGFβ signaling, we utilized a TGFβ-responsive luciferase reporter to show that *Prdm16* isoforms similarly inhibit TGFβ signaling. One human missense mutation significantly impairs this function.

Expression analysis of TGFβ pathway genes in *cpo1* embryos to identify interacting proteins and downstream targets is ongoing. We have generated a targeted conditional gene trap allele of *Prdm16* that will allow us to further our study of *Prdm16* during mouse development. Finally, we are developing a general *Sleeping Beauty*-mediated RNAi transgenesis strategy to rapidly validate positionally cloned ENU mutations.

937/F

Cooperative role of tfap2a and tfap2g in zebrafish neural crest induction. *T. Hoffman, T. Schilling.* Cell and Developmental Biology, University of California, Irvine, Irvine, CA.

Transcription factor AP2 (Tfap2) genes play essential roles in development of the epidermis and migratory cells of the neural crest in vertebrate embryos. These transcriptional activators are among the earliest genes expressed in the ectoderm and specify fates within the epidermis/crest through both direct and indirect mechanisms. The Tfap2 family arose from a single ancestral gene in a chordate ancestor that underwent gene duplication to give up to five family members in living vertebrates. This coincided with the acquisition of important roles in neural crest development by Tfap2 genes suggesting that this gene family was important in ectodermal evolution and possibly in the origin of neural crest. Here, we show that a zebrafish tfap2g is expressed in the non-neural ectoderm during early development and functions redundantly with tfap2a in neural crest specification. In zebrafish embryos depleted of both tfap2a and tfap2g, neural crest cells are virtually eliminated. Cell transplantation experiments indicate that tfap2g functions cell-autonomously in NC specification. Cells of the enveloping layer, which forms a temporary skin layer surrounding the ectoderm, also fail to differentiate or to express appropriate keratins in tfap2g deficient-embryos. The role of Tfap2 genes in epidermal and neural crest development is considered here in the broader context of ectodermal evolution. Distinct, tissue-specific functions for Tfap2 genes in different vertebrates may reflect subfunctionalisation of an ancestral gene that consequently led to the gain of novel roles for different subfamilies in patterning the epidermis and neural crest.

938/F

The Impact of Glucose Metabolism Genes on the Location of the Rostral Edge of Spinal Lesion in Patients with Spina Bifida Meningmylelocele. *T.M. King[1], C.M. Sullivan[1], C.M. Davidson[1], J.M. Fletcher[2], D.J. Francis[2], A.M. Walker[2], K.K. Stuebing[2], G.J. Cote[3], C.N. Singletary[1], K.S. Au[1], H. Northrup[1,4].* 1) Pediatrics, UT Houston Medical School, Houston, TX; 2) Psychology, University of Houston, Houston, TX; 3) Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, Houston, TX; 4) Shriners Hospital for Children, Houston, TX.

Derangements in maternal glucose metabolism may be causative in neural tube defect formation, including spina bifida meningomyelocele (SBMM). Evidence suggests that when a fetus is exposed to hyperglycemia, the ensuing malformations are not due to increased glucose, but rather a cascade of events starting with fetal hypoglycemia. This cascade leads to premature apoptosis and results in the malformation of various emerging organ structures. Our study involved 235 individuals with SBMM with detailed lesion descriptions who were genotyped for 12 glucose homeostasis genes: solute carrier family 2, member 1 (*GLUT1*) and member 4 (*GLUT4*), insulin (*INS*), insulin receptor (*INSR*), leptin (*LEP*), leptin receptor (*LEPR*), hexokinase 1 (*HK1*) and 2 (*HK2*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), tumor protein p53 (*TP53*), catalase (*CAT*), and superoxide dismutase 2 (*SOD2*). Our case population consisted 132 Caucasian (52.2%), 90 Hispanic (35.6%) and 31 other ethnicities (12.3%). Exact vertebral location of the rostral edge was available for all patients. Rostral edges were located in the T4-T12 region in 38 (15.0%) patients and in the L1-L5 in 215 (85.0%) patients. Our findings indicate that *LEPR* is significantly associated with the location of the rostral edge of the spinal lesion. The *LEPR* P1019 SNP altered rostral edge location in both Caucasians and Hispanics, while the N656K, R223Q and the S343 SNPs were significant in Hispanics. Two *LEPR* haplotypes (N656K-P1019 and R223Q-S343) were each significant in Hispanics. However, the full *LEPR* haplotype (N656K-P1019-R223Q-S343) was not significant. We found suggestive evidence of a gene-gene interaction between *GLUT1* and *SOD2*. In conclusion, our study shows that *LEPR* may play a role in modulating the location of the spinal lesion in SBMM.

939/F

Identifying the Molecular Basis of Morphological Variation in Beaks. *K. Powder[1], S. Brugmann[2], J. Helms[2], M. Lovett[1].* 1) Washington University, St Louis, MO; 2) Stanford University, Stanford, CA.

Both humans and birds exhibit remarkable morphological variation in craniofacial structures. In both species, cranial neural crest (CNC) cells give rise to the facial skeleton. In avians, the neural crest cells contain molecular information that regulate species-specific facial variation. However, the identity of these regulators is unknown. We measured changes in gene expression between microdissected CNC cells from the frontonasal prominences of three bird species (chickens, quails, and ducks) during embryonic development. These changes were measured on microarrays that interrogated transcription factor genes plus a wide variety of signaling pathways. Samples were isolated at two developmental stages, before (HH20) and after (HH25) morphological distinctions between the species are evident. Our data indicate that by HH20, CNC cells have established a species-specific expression profile that is maintained, largely unchanged, until morphological differences are evident. Of the 387 genes found to be differentially expressed (>2-fold with p-values < 0.05) between the three bird species, one third were found to be up-regulated in the duck compared to either the chicken or quail, at either developmental stage. These included developmental regulators, such as *Fgfs*, as well as genes previously implicated in mammalian craniofacial development, such as *Hoxb2, Jagged2, Osr2,* and *Satb2*. The duck also exhibited a dramatic up-regulation of WNT signaling components including *Dkk2, Fzd1, Apc, Lrp5, Wnt1,* and *Wnt10b*. By contrast, members of the TGF-Beta signaling pathway, including *Bmp10, Tgfb2, Tgfb3,* and *Bmpr1a*,were down-regulated in the duck. We also observed a down-regulation of the Calmodulin 2 gene in the duck. This pathway has previously been identified as playing a role in facial variation in Darwin's finches. We confirmed differential expression patterns of numerous genes by RNA *in situs,* which also revealed spatial patterns of expression. These data provide a valuable list of candidate genes to investigate human craniofacial abnormalities and variation, and the molecular mechanisms that lead to species-specific craniofacial form.

940/F

Transcriptome Analysis of Genes Involved in Neural Tube Closure during Human Embryonic Development Using Long-SAGE. *P.-T. Xu[1], S. Thomas[2], A. Dellinger[1], H.C. Etchevers[2], M. Vekemans[2], J.R. Gilbert[1], M.C. Speer[1].* 1) Center for Human Genetics, Dept Medicine, Duke Univ Medical Ctr, Durham, NC; 2) INSERM U781, Hôpital Necker - Enfants Malades, Paris, France.

Neural tube defects (NTD) are one of the most common birth defects in humans with an incidence of ~1/1000. Little is known about the human genes that regulate these disease processes. To identify potential new genes and pathways that confer susceptibility to neural tube defects, we have generated and analyzed four Long-SAGE libraries using total RNA isolated from the rostral portion (future brain) and the caudal portion (future spinal cord) of neural tubes from normal human embryos at Carnegie (C) stages 12 (C12, at the time of neural tube closure) and 13 (C13, neural tube closure completed). A total of 269,043 SAGE tags, with an average of 67,260 tags for each library, were extracted from these four libraries. These tags represent 137,486 unique transcripts. Preliminary stage specific comparisons of C13 with C12 tag databases demonstrated that a total of 1,433 tags in the caudal region and 1,131 tags in the rostral region were found to have significant Long-SAGE tag count differences ($p < 0.05$). Of this total, 794 tags were up-regulated over time and 639 tags down-regulated in the comparison of caudal libraries, and 523 tags were up-regulated and 608 tags down-regulated over time in the comparison of rostral libraries. Gene Ontology of molecular functional categories analyzed by the EASE program demonstrates that transcripts of nucleic acid/DNA/RNA binding, structure molecules and ribosomal structure proteins, and transcription regulators are among the most up-regulated gene categories in the C13 libraries compared to C12 libraries in both caudal and rostral regions. The most down-regulated gene categories include transporters, hydrolases, signal transducers, protein and metal ion binding proteins. Many differentially transcribed genes fall within NTD linkage subregions or NTD-associated areas, providing a detailed and quantitative picture of known, novel and/or new genes conferring susceptibility to human neural tube defects and/or associated with normal neural tube closure in humans.

941/F

Mouse Mutants as Models for Human Developmental Malformations: The Extra-Toes Spotting (*Xs^{J}*) Mouse. *D.E. Gildea[1,2], S.K. Loftus[1], Y. Yang[1], W.J. Pavan[1], L.G. Biesecker[1].* 1) GDRB, NHGRI, NIH, Bethesda, MD; 2) Genetics, The George Washington University, Washington, DC.

Greig cephalopolysyndactyly syndrome (GCPS) is a malformation syndrome that includes limb anomalies, specifically polydactyly and syndactyly. GCPS is caused by mutations in the Glioma-associated oncogene-3 (GLI3), which plays a role in Sonic hedgehog (SHH) signaling. The GLI3/SHH pathway regulates many developmental processes, including limb patterning. Dysregulation of this pathway due to mutations in GLI3 can result in limb malformation. The Extra-toes (*Gli3$^{Xt.J}$*) mouse is an excellent animal model for GCPS. Like the human phenotype, *Gli3$^{Xt.J}$* mice exhibit preaxial polydactyly. Another mouse model for polydactyly, Extra-toes spotting (*Xs^{J}*), shares a similar phenotype with the *Gli3$^{Xt.J}$* mouse. *Xs^{J}* mice exhibit preaxial polydactyly and/or belly spotting. Both the *Gli3$^{Xt.J}$* and the *Xs^{J}* phenotypes are inherited semidominantly. Previous linkage mapping has excluded mouse *Gli3* as the *Xs^{J}* gene, and the gene and *Xs^{J}* mutation remain unknown. To identify the gene, we are performing recombination mapping in *Xs^{J}* mice. To map the locus, we needed to outbreed our *Xs^{J}* animals to castaneus mice to introduce a distinct chromosomal background, as we encountered substantial homozygosity in the candidate interval. Offspring from this outcross do not exhibit the *Xs^{J}* phenotype. When breeding carriers from the outcross to B6C3FeF1/J mice, we experienced a penetrance of 36%. These data show variable penetrance of the *Xs^{J}* phenotype that is greatly dependent upon mouse genetic background. We have mapped the *Xs^{J}* locus to a 322 kb region on mouse chromosome 7 and are currently evaluating candidate genes. Since *Gli3$^{Xt.J}$* and *Xs^{J}* mice overlap phenotypically, we hypothesize that the gene mutated in the *Xs^{J}* mice is a gene in the Gli3/Shh pathway. To test this hypothesis, we are evaluating by *in situ* hybridization the expression of *Shh*, *Gli3*, and other members of the Gli3/Shh pathway in *Xs^{J}* embryos. Preliminary data show no change in *Shh* expression in *Xs^{J}* embryos. Here we present our genetic analysis strategy, our phenotypic characterization, the mapping data, and our plan for developmental analysis of the animals.

942/F

The *C. elegans Hya-1* mutant: insights for human hyaluronidase and MPS IX. *J.A. Hobert[1], A. Chatel[2], R. Hemming[2], B. Triggs-Raine[2], M.R. Natowicz[1], D.C. Merz[2].* 1) Genomic Medicine Institute, The Cleveland Clinic, Cleveland, OH; 2) Department of Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, MB, Canada.

Deficiency of human hyaluronidase-1 (EC# 3.2.1.35) is the underlying cause of mucopolysaccharidosis IX (OMIM #601492). Human hyaluronidase-1 is a lysosomal endoglycosidase that functions primarily in the degradation of hyaluronan, but also exhibits activity with the related glycosaminoglycan, chondroitin. Invertebrates, such as *C. elegans*, are unable to synthesize hyaluronan yet contain an orthologue of human hyaluronidase, T22C8.2, which we have termed *hya-1*. We have created a deletion mutant of *C. elegans hya-1* and have assessed both the phenotype and relative enzymatic activity against the substrates hyaluronan and chondroitin. Initial observations indicate that animals deficient in *hya-1* are viable and grossly normal but have phenotypic abnormalities in both egg-laying and vulval morphogenesis. In agreement with observations in hyaluronidase-deficient human tissues, microscopic analysis of *hya-1* deficient *C. elegans* revealed some cell types contain enlarged lysosome-related granules. Consistent with the hypothesized function of *hya-1*, mutant animals have increased Alcian Blue staining, suggesting an accumulation of glycosaminoglycan. Enzymatic analyses of extracts from wild-type and *hya-1* deficient *C. elegans* extracts indicate that hya-1 protein is enzymatically active against chondroitin but not hyaluronan and has both acidic (pH 3.8 and 4.4) and near neutral (pH 6.6) optima. Our data suggest that chondroitinases are evolutionary precursors of the vertebrate hyaluronidases. This *C. elegans hya-1* mutant model will: (1) facilitate studies of the trafficking and regulation of this important lysosomal enzyme that, in humans, traffics to lysosomes by an as yet unknown pathway; (2) further our understanding of structure-function issues of the catalytic regions of hyaluronidases and chondroitinases; and (3) serve as a tool to dissect the roles of glycosaminoglycans in the developmental biology of *C. elegans*.

943/F

Mitochondrial respiratory chain assembly and function during human fetal development. *L. Minai[1], D. Chretien[1], F. Razavi[2], A. Munnich[1], A. Rotig[1].* 1) INSERM U781, Hopital Necker, Paris, France; 2) Service Histologie Embryologie Cytogenetique, Hopital Necker, Paris, France.

Although a number of metabolic diseases undergo a symptom-free period, respiratory chain deficiency may have an early antenatal expression, presumably related to the time course of the disease gene expression in the embryofoetal period. We present the investigation of the assembly and function of the mitochondrial respiratory chain during human foetal development. The study was carried out on human foetuses aged from 4 to17 weeks of gestation and aborted for genetic diseases unrelated to mitochondrial function. For each foetus, several different tissues (brain, heart, liver, kidney and muscle) were examined. The assembly of the various respiratory chain complexes studied by blue native gel electrophoresis showed a similar size and amount of these complexes whatever the stage of development in the various tissues tested. Moreover, this pattern was the same as observed for post-natal tissues. SDS-PAGE followed by Western blot revealed that all complexes were fully assembled. However, a slightly faster migrating α-ATPase subunit of CV was detected in fetal brain compared to other pre- and post-natal tissues. Finally, enzyme measurement of respiratory chain complexes showed a similar repartition of the five complexes in pre- and postnatal tissues. Nevertheless, prenatal values were constantly lower than pos-natal values. Our results showed that the subunits of the respiratory chain are fully assembled and functional in the very early steps of fetal development. Therefore, the time dependent expression of respiratory chain deficiency should be related to different spatiotemporal variations of genes involved in regulation and maturation of these multienzymatic complexes.

944/F

The essential role of *Ikbkap* in embryogenesis: Developing a model for Familial Dysautonomia. *Y.T. Chen[1,2], R.S. Shetty[1,2], M.M. Hims[1,2], M. Leyne[1], L. Liu[1], J. Mull[1], J. Pickel[3], S.A. Slaugenhaupt[1,2].* 1) Center for Human Genetic Research, MGH, Boston, MA; 2) Harvard Medical School, Boston MA; 3) NIMH Transgenic Core, National Institutes of Health, Bethesda, MD.

Familial dysautonomia (FD) is one of the best known recessive sensory and autonomic neuropathies. Among the disease-causing mutations identified, an intronic non-coding point mutation (IVS20+6T>C) is present in all FD patients. Studies from our lab have revealed that this mutation causes variable skipping of exon 20 in the *IKBKAP* transcript. This point mutation does not completely abolish all wild-type *IKBKAP* mRNA synthesis, but rather reduces the level of normal message and protein. In fact, the existence of both the wild-type and mutant *IKBKAP* mRNA were observed in various tissues from FD patients with the lowest relative amount of wild-type isoform in the neuronal tissues. To better understand the role of the *IKBKAP* gene in vivo, we have established an *Ikbkap* knock-out mouse model. Analyses of homozygous *Ikbkap* knock-out (*Ikbkap*-/-) embryos shows that deletion of mouse *Ikbkap* results in failure during embryogenesis, which leads to the reabsorption of these defective embryos at later embryonic stages (E12.5). Gross morphological analyses of *Ikbkap*-/- embryos at earlier stages (E8.5-E11.5) revealed several abnormal configurations, including a failure of germ layer inversion, a disruption of cephalic neural tube closure and a smaller body size when compared with wild-type littermates. Further, the expression of several genes required in early embryogenesis is reduced in the *Ikbkap*-/- embryos, suggesting a crucial role of IKAP during development. We have also created transgenic mouse lines using the human FD *IKBKAP* gene, which exhibit tissue-specific mis-splicing of human *IKBKAP* in a pattern similar to that observed in FD patients. Currently, we are in the process of generating an accurate phenotypic model of FD by crossing the knock-out and transgenic lines. These mice should accurately model the human disease and will not only allow us to examine the role of IKAP during development, but will also serve as a platform for testing potential therapeutic agents.

945/F

***Irf6* allelic series in mouse shows differential defects in oral adhesions without cleft palate.** *A. Kinoshita[1, 4, 5], M. Dunnwald[1], K.J. Trout[1], R. Richardson[2], M. Dixon[2], B. Yang[3], B.C. Schutte[1].* 1) Pediatrics, University of Iowa, Iowa City, IA; 2) Life Sciences and School of Dentistry, University of Manchester, Manchester, United Kingdom; 3) Obstetrics and Gynecology, University of Iowa. Iowa City, IA; 4) Human Genetics, Nagasaki University, Nagasaki, Japan; 5) Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology, Kawaguchi, Japan.

IRF6 (interferon regulatory factor 6) is a member of the IRF family of transcription factors. We reported that mutations in *IRF6* cause Van der Woude (VWS) and popliteal pterygium syndromes (PPS) (Kondo *et al.*, 2002). These autosomal dominant syndromes are characterized by cleft lip/palate and lip pits, and PPS also has pterygium (skin webbing). To establish a murine model for these orofacial clefting syndromes, we generated *Irf6* mutant mice with a gene trap allele (*Irf6^{gt}*). This allele does not produce detectable levels of *Irf6* in skin and palate. Heterozygous mice do not exhibit a cleft palate, but have intraoral adhesions in 10% of embryos. Homozygous mice exhibit craniofacial, skin and limb abnormalities, including cleft palate (Ingraham *et al.*, 2006). To generate a murine model that better represents VWS, we made an *Irf6* allele with *Pgk-neo* cassette (*Irf6^{neo}*) inserted in intron 4. All embryos (6/6) that are compound heterozygotes for the gene trap and *Pgk-neo* alleles (*Irf6$^{gt/neo}$*) showed intraoral adhesions, along with hind paw syndactyly and kinked tail. Immunoblotting confirmed a gradient of Irf6 expression *Irf6$^{+/+}$* > *Irf6$^{+/gt}$* > *Irf6$^{gt/neo}$* > *Irf6$^{gt/gt}$*, demonstrating that *Irf6^{neo}* is a hypomorphic allele. The phenotype of mice heterozygous for the most common PPS-associated allele R84C (Richardson *et al.*, 2006) appears to be between *Irf6$^{+/gt}$* and *Irf6$^{gt/neo}$* and is consistent with a dominant-negative effect for this mutation. Finally, we generated a floxed *Irf6* allele without *Pgk-neo* (*Irf6^{fl}*) and a null allele that deleted exons 3 and 4 (*Irf6^{-}*) by cre-loxP recombination. To establish better models for VWS and PPS, we are crossing the *Irf6* conditional allele with transgenic mice that express Cre in craniofacial tissues.

946/F

The gene-trapped allele of mouse Fkbp8 gene represents another null mutation that has human spina bifida phenotype and is not embryo-lethal. *L. Wong[1], B. Wlodarczyk[1], M. Scott[1], S. Kartiko[1], M. Yen[1], M. Merriweather[1], R.H. Finnell[1,2].* 1) CEGM, IBT, Texas A&M HSC, Houston, TX; 2) TIGM, Houston, TX.

Neural tube defects (NTDs) are disabling birth defects and they are second only to cardiac defects among major congenital malformations. Spina bifida (SB) is a NTD that has a defect at the lumbosacral region where the spinal cord is dysplastic and the overlying spinal column is absent. In the US, the prevalence rate of SB is about 1 in 1000. Up till now, researchers have relied upon mouse NTD models to understand the mechanism of NTD development but many models suffer from partial penetrance of the phenotype or poor recapitulation of either the complex genetics or the phenotype in human NTDs. In our search for models with a SB, we identified a mouse mutant that has a severely dilated posterior neural tube (NT) and more importantly, it exemplifies many features of human SB. The mutant was generated via gene trapping and the trapped gene was Fkbp8, which belongs to the immunophilin family functioning in immunoregulation, protein folding, and trafficking. The gene trapped allele has a complete SB penetrance and is not embryo-lethal. The dilated posterior NT is evident at E10.5 and the NT is closed; however, cross-sectioned NT revealed that the thickness is only 25-30% of that in controls. These mutants have NT patterning defects and increased cell death at the ventral NT. Examination of sectioned spinal cord in mid-gestation fetuses revealed differentiation and DRG defects. At late-gestation fetuses, the skin-covered lesion contains protruded neural tissue and possibly spinal fluid. Alizarin staining of E18.5 nullizygotes revealed vertebrae abnormalities mostly in the thoracic-lumbar region. These mice are identical in size to their control littermates at birth and they survive many weeks post-partum though they develop lower body paralysis. Folate or inositol treatment did not rescue the SB phenotype in the nullizygotes. We are analyzing the phenotype at the cellular and molecular levels, and identifying FKBP8 interactors which might function to pattern the NT. This mouse represents an excellent SB model which will provide further insight into the etiology of human NTDs.

947/F

Creation of a humanized *IKBKAP* mouse that models a tissue-specific human splicing defect. *R.S. Shetty[1, 2], M.M. Hims[1, 2], Y.T. Chen[1, 2], J. Mull[1, 2], M. Leyne[1, 2], L. Liu[1, 2], J. Pickel[3], S.A. Slaugenhaupt[1, 2].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

The importance of proper mRNA splicing is evidenced by the fact that 20-30% of all disease-causing genetic mutations result in splicing defects. It has been demonstrated that the plasticity of the splicing reaction can be exploited for the development of therapeutics that modify mRNA splicing efficiency. In familial dysautonomia (FD), a severe neurodegenerative disorder, all patients have an intronic splice site mutation in the *IKBKAP* gene, which results in tissue-specific skipping of exon 20. Although FD is a recessive disease, homozygous mutant cells express wild-type *IKBKAP* transcripts, and are therefore capable of producing normal IKAP protein. The relative amount of wild-type and mutant *IKBKAP* mRNAs varies between tissues, with the lowest levels of wild-type *IKBKAP* production in tissues from the nervous system. These observations suggest that the cellular level of IKAP protein in neurons falls below the threshold level required for normal development and maintenance. In order to study the tissue-specificity of the splicing defect, as well as to test potential therapeutic agents targeted at mRNA splicing, we created several transgenic mouse lines expressing either human wildtype *IKBKAP* or FD (IVS20+6T>C) *IKBKAP* from a human BAC. We show that the presence of the FD mutation in the BAC causes mis-splicing of human *IKBKAP* in mice, and that the efficiency of exon inclusion varies in a tissue-specific manner that closely models that seen in FD patients. The splicing defect is most pronounced in neuronal tissues, proving that the cellular mechanism governing the observed tissue-specific splicing is conserved. These transgenic mice provide the perfect model for pre-clinical testing of therapeutic agents that have been previously shown to improve normal *IKBKAP* splicing *in vitro*. Further, it provides a unique model for unraveling the complexities of tissue-specific alternative splicing.

948/F

Phenotyping a *Magel2* knockout mouse: a model for Prader-Willi Syndrome. *E.M. Kwolek, R.E. Mercer, J.M. Bischof, R. Wevrick.* Dept. of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

First described in 1956, Prader-Willi Syndrome (PWS) is a polygenetic condition resulting from the loss of paternal expression of imprinted genes located at 15q11-15q13. While multiple genes have been implicated in PWS, the specific role of each gene is unknown and further investigation is warranted. PWS is one of the most common syndromic causes of obesity and major diagnostic criteria include neonatal hypotonia and failure to thrive, developmental delays, hypogonadism, dysmorphic facial features, hyperphagic behaviors that ultimately lead to obesity, and abnormal sleep and respiration. One of the genes identified within the implicated chromosomal region is *MAGEL2* - the expression of which is limited to the paternal allele as a result of its imprinted nature. This gene is developmentally regulated and expression in embryos is mostly in the hypothalamus and to a lesser extent in other nervous system tissues. In adults *Magel2* is expressed almost exclusively in the hypothalamus - specifically to the suprachiasmatic nucleus, which modulates circadian rhythm in mammals.

We have developed a *Magel2*-null knockout mouse to study the impact of loss *Magel2* expression in an *in vivo* system. Like neonates with PWS, the knockout mice are underweight compared to their wild-type counterparts prior to weaning; they ultimately gain more weight and have higher adiposity during adulthood than control littermates. Behavioral analyses show that *Magel2*-null mice have decreased basal activity and interruption of the wild-type circadian rhythm. We now report that the *Magel2*-null mice have abnormal locomotor function and reproductive deficits that worsen with ageing. As increased anxiety, repetitive behavior, and learning deficits are cardinal features of PWS, we are examining these traits in adult *Magel2*-null mice. This research aims to provide an appropriate in vivo model for studying Prader-Willi syndrome and the impact of *Magel2* dysregulation in hypothalamic dysfunction.

.

949/F

Expression of the nuclear receptor *Nr1d1* in the retina and its role in the regulation of photoreceptor development. *N.J. Mollema[1], M. Gaule[1], N.B. Haider[1,2].* 1) Genetics, Cell Biology and Anatomy. University of Nebraska Medical Center, Omaha, NE; 2) Ophthalmology and Visual Sciences. University of Nebraska Medical Center, Omaha, NE.

Mutations in human Nr2e3 result in Enhanced S-Cone Syndrome and mutations in mouse *Nr2e3* cause the retinal degeneration observed in *rd7* mice. The *rd7* mutant mouse is characterized by a unique retinal degeneration resulting from an over production of blue cone photoreceptor cells. To better understand the processes regulating photoreceptor development, we identified genes that are misexpressed in the *rd7* retina. We determined that the nuclear receptor *Nr1d1* is both misregulated in *rd7* retinas and is a target of *Nr2e3*. While a previous report identified *Nr1d1* as a cofactor of *Nr2e3*, little is known regarding the role of *Nr1d1* in photoreceptor development. In this study we determined the temporal expression profile of *Nr1d1* throughout retinal development and in its expression in the mature retina. We further determined the sub-cellular localization of *Nr1d1* in the developing and mature retina and its co-localization with other retinal proteins. We used *in vivo* chromatin immunoprecipitation (ChIP) to evaluate whether targets of *Nr2e3* are also regulated by *Nr1d1*. Our studies demonstrate a novel role for *Nr1d1* to function in the same transcriptional network as *Nr2e3* to regulate photoreceptor development.

950/F

Enhanced accumulation of Aβ in neurons in autism. *W. Brown, I. Kuchna, K. Nowicki, J. Wegiel, T. Wisniewski, J. Wegiel.* NYS Inst Basic Research, Staten Island, NY.

Studies of amyloid β in neurons has revealed the absence of, or only minimal, intraneuronal Aβ immunoreactivity in normal brains. The appearance of intraneuronal Aβ immunoreactivity has been suggested as a sign of neuronal pathology leading to fibrillar plaque formation in the brains of people with AD. However, detection of variable Aβ immunoreactivity in neurons in our 32 brains of control children, adults and aged subjects indicates that amyloid beta immunoreactivity is not a predictor of Alzheimer's disease (Wegiel et al., 07). We performed immunocytochemistry for intraneuronal Aβ immunoreactivity in 10 brains of subjects with autism. Remarkably, this revealed very strong Aβ immunoreactivity in 50% of the brains, including one 5-year old child and 4 adults of 20, 23, 52, 62 years of age. Also, numerous diffuse plaques were in the amygdala and neocortex in the 52 year-old subject. In five other children and adults the levels of intraneuronal Aβ were similar to those detected in control brains. Striking differences in Aβ immunoreactivity were observed in different neuronal populations, including very strong Aβ immunoreactivity in neurons in the granule layer of the dentate gyrus in the hippocampal formation, in the globus pallidus and in Purkinje cells. Aβ in neurons in control and autistic subjects was mainly a product of α- and β-secretases (17-40/42 aa). Our observations are in accord with biochemical studies of sera showing that in children with severe autism and aggression the level of secreted APP is higher than in children with mild autism, and much higher than in normal children (Sokol et al., 2006). We suggest an increased α-secretase (anabolic) pathway of APP processing exists in some autistic subjects that results in excessive intraneuronal accumulation of Aβ. The link between the clinical phenotype and pattern of APP processing suggests there are functional consequences of an abnormal accumulation of Aβ in autism.

Tissues provided by: Harvard Brain Tissue Resource Center, Brain Tissue Bank for DD of U of Md., & ATP. Supported by R01HD43960, PO1HD35897, & NAAR-AS.

951/F

DJ-1 gene confers susceptibility to Parkinson's disease. *G. Annesi, P. Tarantino, I.C. Cirò Candiano, E.V. De Marco, F. Condino, F.E. Rocca, G. Provenzano, S. Carrideo, G. Nicoletti, F. Annesi.* Inst Neurological Sci, National Research Council, Cosenza, Italy.

DJ-1 mutations cause autosomal recessive early-onset parkinsonism, dystonia in rare families (PARK7). In the present study we investigated whether an 18 bp insertion/deletion variant and single nucleotide polimorphisms (SNP) within the DJ-1 gene are associated with Parkinson's disease. We analyzed 297 patients with sporadic Parkinson and 290 unrelated healthy subjects from the same geographical area. Genomic DNA was extracted by standard method. The study of DJ-1 variants involved two phases. In the first phase, we identified a subsample of 30 unrelated control subjects with an age, gender, and ethnicity distribution similar to the cases. We determined the DJ-1 gene sequence in this subsample and identified 20 polymorphisms across a full length of the gene. Five SNP and an 18 bp insertion/deletion provided informative (minor allele frequencies >25%) , were easy to assay using multiplex SNaPShot, and had genotype frequencies consistent with Hardy-Weinberg equilibrium expectation. In the second phase, we genotyped the full sample of PD patients and unrelated control subjects of these polymorphisms.We constructed all possible locus haplotypes as defined by the five SNP and 18 bp I/D variant and studied their association with PD for the analyses. We performed score tests and determined pvalues. Haplotype analysis was performed using Haplowiev 3.2 and UNPHASED. We observed associations overall the six polymorphisms. There were significant differences in the genotypes or allele frequencies between the two groups PD and control subjects. Each marker, taken individually, did show association to PD. In our haplotypes we observed significant differences in the inferred haplotype frequencies of PD and control subjects ($p<0.001$). Our data show that in the southern Italy population, genetic variability within the DJ-1 is highly associated with susceptibility to idiopathic PD. This is in contrast with three previous findings, that did not show an association between DJ-1 polymorphisms and PD.

952/F

Expression patterns of organic cation/carnitine transporter family in adult murine brain: Implications for brain development. *A. Lamhonwah[1,2,3], C. Hawkins[2,3], L. Mai[1,2,3], C. Tam[1], J. Wong[1], I. Tein[1,2,3].* 1) Division of Neurology,Dept Pediatrics, Hosp Sick Children, Toronto, ON, Canada; 2) Dept of Pathology,Hosp Sick Children, Toronto, ON, Canada; 3) Dept of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON Canada.

Objective: To characterize expression patterns of mOctn1, -2 and -3 in murine brain. Methods: We applied our transporter-specific antibodies to mOctn1,-2 and -3, followed by 20 antibody and DAB peroxidase detection to adult murine brain sections counterstained with hematoxylin. Results: All 3 transporters showed strong expression in the external plexiform layer of olfactory bulb and in olfactory nerve, the molecular layer and neuronal processes of input fibres extending vertically in motor cortex, in the dendritic arborization of cornu ammonis and dendate gyrus, neuronal processes in arcuate nucleus, choroid plexus cells, and neuronal cell bodies and dendrites of cranial nerve nuclei V and VII. In the cerebellum, all three were strongly expressed in dendritic processes of Purkinje cells, but Octn1 and -2 were expressed more strongly than Octn 3 in Purkinje cell bodies. In spinal cord, Octn1,-2 and -3 were prominent in axons and dendritic end-arborizations of spinal cord neurons in ascending and descending white matter tracts, whereas Octn3 was also strongly expressed in anterior horn cell bodies. Conclusions: hOCTN2 deficiency presents with carnitine-responsive cardiomyopathy, myopathy and hypoglycemic, hypoketotic coma with strokes, seizures and delays. In mouse, Octn1,-2 and -3 are expressed in a CNS pattern suggestive of roles in modulating cerebral bioenergetics and in acetylcholine generation for neurotransmission in olfactory, satiety, limbic, memory, motor and sensory functions. This distribution may play a role in the pattern of neurological injury that occurs in hOCTN2 deficiency during catabolic episodes of encephalopathy which may manifest with cognitive impairment, hypotonia and seizures.

953/F

Genetic analysis of the human striatum-enriched CalDAG-GEFI gene with Japanese schizophrenia patients. *H. Mitsuyasu[1,2], H. Kawasaki[1], L. Gotoh[1], Y. Kobayashi[1], N. Oribe[1], A. Takata[1], S. Kanba[1].* 1) Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan; 2) Dept Psychiatry, Kyushu Kousei Nenkin Hospital, Kitakyushu, Japan.

We previously reported the isolation of striatum-enriched novel guanine nucleotide exchange factor (GEF) that has both calcium (EF-hand) and diacylglycerol (DAG) binding domains (CalDAG-GEFI) and the presence of its orthologue, CalDAG-GEFII. These genes are part of the novel second-messenger regulated GEF gene family whose GEF activities are regulated by the binding of second-messenger molecules such as cAMP, calcium and DAG (Kawasaki et al., 1998). Both CalDAG-GEFI and CalDAG-GEFII mRNAs are expressed almost exclusively in the brain and hematopoietic system (Kawasaki et al., 1998). It is shown that CalDAG-GEFI proteins are localized at the synaptic terminals of GABAergic output neurons in the striatum. Since the dysregulation of the striatal GABAergic output neurons are implicated in the pathophysiological mechanisms of schizophrenia, CalDAG-GEFI gene can be a good candidate for molecular studies of schizophrenia. In this study, we analyzed single nucleotide polymorphisms (SNPs) of the CalDAG-GEFI gene with Japanese schizophrenic patients (n=193) diagnosed based on DSM-IV criteria and control subjects (n=242). We amplified total 18 exons and their flanking regions of the CalDAG-GEFI gene. Out of 18, 16 fragments could be amplified and were screened by DHPLC. Three fragments showed heteroduplex elution pattern. Four SNPs were identified and were confirmed by direct sequencing method. All subjects were genotyped with four SNPs. Genotype and allele frequencies and linkage disequilibrium were calculated. We performed association study between the schizophrenia patients and the controls using single marker analysis and haplotype analysis with the four SNPs. There was no significant difference regarding the allele and genotype frequencies. There was no significant difference in haplotype frequencies. We could not find any significant differences between schizophrenia and controls in this study. All subjects were given informed consent based on the ethical regulations of Kyushu University.

954/F

Multiple candidate gene analysis identifies *CALB1(calbindin1)* as a susceptibility gene for sporadic Parkinson's disease. *I. Mizuta[1,2], W. Satake[1], T. Tsunoda[3], M. Watanabe[4], A. Takeda[5], K. Hasegawa[6], M. Yamamoto[7], N. Hattori[8], M. Murata[9], T. Toda[1,2].* 1) Div Clinical Genetics, Osaka Univ Grad Sch Med, Suita, Osaka, Japan; 2) CREST, JST, Saitama; 3) SNP Res Center, RIKEN, Yokohama; 4) Dept Neurol, Univ Tsukuba, Tsukuba; 5) Div Neurol, Dept Neuroscience, Tohoku Univ Grad Sch Med, Sendai; 6) Dept Neurol, Sagamihara National Hosp, Sagamihara; 7) Dept Neurol, Kagawa Prefectural Central Hosp, Takamatsu; 8) Dept Neurol, Juntendo Univ Sch Med, Tokyo; 9) Dept Neurol, Musashi Hosp, NCNP, Tokyo, Japan.

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra of the midbrain. Our recent case-control association study of 268 SNPs in 121 candidate genes identified α-*synuclein (SNCA)* as a definite susceptibility gene for sporadic Parkinson's disease (PD) ($P=1.7\times10^{-11}$). To find other susceptibility genes, additional 34 SNPs of 17 genes were included in the screen. Of 302 SNPs in total, excluding SNPs in *SNCA*, those in *NDUFV2, FGF2, CALB1 and B2M* showed significant association ($P < 0.01$, 882 cases and 938 control subjects). Association analysis of these SNPs was replicated in another sample panel (521 cases and 1003 control subjects). Only the SNP *rs1805874* in *calbindin1 (CALB1)* showed significance again ($P=7.1\times10^{-5}$, recessive model). Next, statistical combinational effect of *CALB1* and *SNCA* was analyzed. In homozygotes of PD-associated allele of *SNCA*, *rs1805874* revealed no significance, however, in the others, *rs1805874* showed significant association with PD ($P=8.7\times10^{-5}$, χ^2 statistics). Logistic regression analysis showed the significant additional effect of the two SNPs (combined $P=8.9\times10^{-17}$), however, showed little significant interaction ($P=0.05$). CALB1 is a calcium-binding protein that is widely expressed in neurons. It is known that there is a relative sparing of the CALB1-positive dopaminergic neurons in PD brains compared with CALB1-negative ones. Taken together, our genetic analysis suggests that *CALB1* is associated with PD independently of *SNCA*.

955/F

Parkin mutation analysis in patients with sporadic early-onset Parkinson's disease. *G. Provenzano[1], F. Annesi[1], M.T. Pellecchia[2], F.E. Rocca[1], E.V. De Marco[1], D. Civitelli[1], P. Tarantino[1], P. Barone[2], L. Morgante[3], M. Zappia[4], G. Annesi[1].* 1) Inst Neurol Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Department of Neurological Sciences, University Federico II, Napoli, Italy; 3) Department of Neuroscience, Psychiatry and Anesthesiology, University of Messina, Italy; 4) Clinica Neurologica I, Department of Neuroscience, University of Catania, Italy.

Mutations in the parkin gene (PARK2) are responsible of familial autosomal recessive early onset (≤ 45 years) Parkinson's Disease (EOPD) and sporadic EOPD. Recently, a novel parkin mutation, consisting of a deletion of the promoter and exon 1 of parkin, was described in a family with autosomal recessive EOPD and in an isolated case with EOPD. The promoter region is shared by parkin and the neighboring parkin coregulated gene (PACGR). The aim of this study is to perform mutational analysis of the coding regions of the parkin gene in sporadic EOPD and subsequently to investigate whether rearrangements within both the shared promoter region and the parkin gene are present in the patients with only one or no mutations. A total of 53 index cases with sporadic EOPD from Southern Italy were screened for parkin mutation. DNA was exstracted from peripheral blood and each exon of parkin was amplified and sequenced. Absolute quantification was perfomed by real time PCR 7900 HT-SDS, using TaqMan® probes for exons 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and shared promoter region. For exon 1 we utilized MGB (Minor Groove Binder) probes. Among 53 patients screened for parkin mutations, 8 carried single heterozygous mutations, 4 had simple homozygous mutations, 1 was a compound heterozygous and 40 had no mutations. Gene dosage experiment failed to reveal an exonic rearrangement of the parkin gene in patients with single heterozygous mutations. In our study the gene dosage of core parkin promoter is still in progress. The recent discovery of parkin promoter deletions has not only extended the spectrum of mutations in this gene but has also proved that deletions affecting the promoter should be looked for in patients with a single or no parkin mutation.

956/F

G-463A myeloperoxidase polymorphism and Parkinson's disease. *F.E. Rocca[1,2], P. Tarantino[1], E.V. De Marco[1], D. Civitelli[1], I.C. Cirò Candiano[1], S. Carrideo[1], F. Annesi[1], G. Provenzano[1], V. Greco[1], V. Scornaienchi[1], G. Nicoletti[1,2], G. Annesi[1]*. 1) Inst Neurological Sci, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a loss of substantia nigra pars compacta (SNpc) dopaminergic neurons, and can be modeled by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A recent study shows that myeloperoxidase (MPO), a key oxidant-producing enzyme during inflammation, is upregulated in the ventral midbrain of human PD and in MPTP mice. Moreover was suggested that inhibitors of MPO may provide a protective benefit in PD. A functional G-463A polymorphism (SNP) in the promoter of the MPO gene is associated with a number of diseases with inflammatory components. In the present study we investigated the association of this SNP with Parkinson's disease. We analyzed 233 PD cases and 100 controls. All patients were screened for this SNP in the MPO gene promoter by combination of PCR and RFLP analysis. The PCR product was digested with AciI restriction enzyme. We did not find significant differences in allele or genotype distribution between PD cases and controls (p=1,00). The MPO gene encodes an antimicrobial enzyme that produces oxidative free radicals. It is normally not present in brain tissue but is expressed under pathological conditions. The signals responsible for the induction of this expression in the brain have not been elucidated. Genetic findings show that the less common A allele decreases myeloperoxidase expression, apparently by destroying a binding site for the transcription factor. The reactive oxygen species play an important role in PD and individual susceptibility to PD may be modulated by G-463A MPO SNP. The precision of our study is limited by small number of controls. Our preliminary data will be completed by increasing the number of the controls analysed to provide a more powered study. If we confirm the present results, we will successively performed an haplotype analysis based on polymorphic markers in the MPO gene promoter, to examine the involvement of a more extended region.

957/F

Angiogenin gene mutations in a large cohort of Italian ALS patients. *C. Gellera[1], C. Colombrita[2], N. Ticozzi[2], B. Castellotti[1], A. Ratti[2], C. Bragato[1], F. Taroni[1], V. Silani[2]*. 1) Department of Biochemistry and Genetics, Fondazione IRCCS - Istituto Neurologico Besta, Milano, Italy; 2) Department of Neuroscience, University of Milan - IRCCS Istituto Auxologico Italiano, Milano, Italy.

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive and ultimately fatal neurodegenerative disorder. Most ALS are sporadic (SALS), and ≈ 10% of them are familial (FALS). Mutations in SOD1 gene account for about 20% of familial ALS cases. Mutations in alsin, VAPB, SETX, and dynactin genes are rare. Angiogenin (ANG) is a novel candidate gene for the pathogenesis of ALS. It is an angiogenic factor up-regulated by hypoxia highly expressed in motor neurons. Missense mutations in ANG gene have been identified in Northern Europe patients both in SALS and FALS patients. A significant allelic association with the rs11701 SNP has also been described in the Irish and Scottish ALS cases. We screened 738 Italian ALS patients (605 sporadic and 132 familial) and 517 controls for ANG gene coding region, 5'UTR and 3'UTR sequences. We used both DHPLC and direct sequencing procedures. We found no association with the previously reported rs11701 SNP in our ALS population. We identified seven different mutations in 14 patients (8 SALS and 6 FALS). We found six novel heterozygous mutations, three of which (M-24I, F-13S, P-4S) were located in the ANG signal peptide region, two (V113I, H114R) in the mature protein and one (+5C>T) in the 3'UTR region. These mutations were not present in the control population. The previously described I46V mutation was detected in six patients (0.8%) and in one healthy control (0.2%). We also found a new synonymous substitution (G20G) in two ALS patients, but not in controls. We found a statistically significant difference in the mutational frequency between ALS patients and controls (1.9% vs 0.2%; p=0.014). The frequency of mutations was 4.5% in FALS and 1.3% in SALS. Our results provide evidence that variations in ANG gene also occur in the Italian ALS patients. ANG may have an important role as a risk factor for ALS although functional evidences of ANG mutations are still missing. These data further support a possible link between ALS disease and angiogenic factors.

958/F

Alpha synuclein in familial Parkinson's disease and Lewy body dementia. *V. Greco, E.V. De Marco, F.E. Rocca, P. Tarantino, F. Annesi, D. Civitelli, G. Provenzano, V. Scornaienchi, I.C. Cirò Candiano, S. Carrideo, G. Nicoletti, G. Annesi.* Inst of Neurol Sciences, National Research Council, Mangone, Cosenza, Italy.

Alpha-synuclein has been implicated in the pathology of certain neurodegenerative diseases, including Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Alpha-synuclein is the major component of the filamentous Lewy bodies and Lewy neurites that define these diseases at a neuropathological level. Missense mutations (A30P and A53T) in alpha-synuclein gene cause familial forms of PD and DLB. Recently, a third missense mutation (E46K) in alpha-synuclein gene was described in an inherited form of DLB. The aim of this study was to evaluate the role of E46K mutation as a risk factor in DLB and in familial PD. We analysed the E46K mutation in seventeen sporadic DLB patients and thirty-seven familial PD patients. The clinical diagnosis of DLB was based on the criteria proposed by an international consortium on DLB that include the presence of cognitive decline plus at least two of the following: spontaneous parkinsonian symptoms and signs, visual hallucinations and fluctuations in consciousness. PD patients were diagnosed according to UK Brain Bank criteria. We conducted a genetic analysis by standard PCR and restriction digestion method. None of the subjects examined had the E46K mutation of the alpha-synuclein gene. The E46K mutation was identified in affected members of a Spanish family with autosomal dominant DLB and parkinsonism. This mutation substitutes the glutamic acid with the lysine in a much conserved area of the protein, causing likely severe disturbance of protein function. Moreover the A53K alpha-synuclein mutation was found in an elder case with DLB from Greece. These cases suggest that E46K and A53T mutations should be considered in the differential diagnosis of DLB. We did not found the E46K mutation in patients with DLB or in familial PD. These results do not support a role for this mutation in our patients with DLB or familial PD, in agreement with the emerging consensus that mutations in the alpha-synuclein gene are associated with PD in few families worldwide.

959/F

***DPYSL2*, a candidate schizophrenia (SZ) susceptibility gene on 8p.** *Y.P. Liu[1], L.L. Zhang[1], P-L. Chen[1], D. Avramopoulos[1], V.K. Lasseter[2], M.D. Fallin[3], J. McGrath[2], P. Wolyniec[2], G. Nestadt[2], K.Y. Liang[4], A. Pulver[2], D. Valle[1]*. 1) Institute of Genetic Medicine; 2) Department of Psychiatry, Johns Hopkins University School of Medicine; 3) Department of Epidemiology; 4) Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health.

Previously, in a genome-wide linkage scan of 54 European Caucasian (EC) multiplex families, we reported a SZ susceptibility locus on 8p22-p21 (~25.5 Mb) with a maximum NPL of 3.64 at D8S1771 (p=.0001) (Blouin *et al.* 1998). Additional STRP genotyping for these families supported a dominant model (LOD=4.10) peaking at D8S1048 (26.9 Mb) (Pulver, unpublished data). A follow up candidate gene SNP fine mapping study found that a gene in this region, *DPYSL2*, involved in axonal growth, showed positive association in both SZ and bipolar type 1 (BP1) in Ashkenazi Jewish (AJ) trios and cases (Fallin *et al.*, 2005). Previous work by others showed positive association of a SNP in the 3' UTR of *DPYSL2* with SZ and studies of SZ brains showed a > 5-fold reduction in *DPYSL2* protein. We have followed these observations up with extensive sequencing of *DPYSL2* exons (14), proximal promoter and cNCS (4) in the region in AJ (48) and EC (96) controls, SZ (48 AJ, 48 EC) and BP1 (48 AJ) individuals. We identified 4 coding SNPs, none of which were associated with SZ or BP1 in our sample but we found 3 SNPs (rs367948, rs400181, rs445678) in the promoter region that were highly associated with SZ in the EC sample (p=0.007) but not in the others. To test the functional consequences of these SNPs, we generated luciferase reporter constructs containing 521bp of the human *DPYSL2* proximal promoter sequences, differing only at these 3 SNPs, and transfected them into 293, pc12 and neuro2a cells. We found modest reductions in promoter function with the risk alleles for the 3 SNPs but none that reached statistical significance. We conclude that *DPYSL2* is an excellent candidate gene for both SZ and BP1 but that further sequencing and expression studies (in progress) are necessary to either confirm or reject a role in these disorders.

960/F

The Role of Neuroligin Genes in Autism Spectrum Disorder. *K. Meltz Steinberg, M.E. Zwick.* Department of Human Genetics, Emory University, Atlanta, GA.

Neuroligins are cell adhesion molecules important in the post-synaptic density. A number of recent studies identified mutations in the X-linked genes neuroligin 3 (NLGN3) and 4X (NLGN4X) that contribute to autism spectrum disorder (ASD), while other studies have failed to replicate these findings. In order to reconcile these results while identifying all rare and common genetic variation that may contribute to ASD, we are comprehensively resequencing the NLGN3 and NLGN4X genes in individuals from families with two or more affected male sibpairs from the Autism Genetic Resource Exchange (AGRE). Affected male sibpairs were chosen based upon sharing identical markers near NLGN4X (DXS9895 and 9902) with each other as well as with their mother. One male from each sibpair was selected for resequencing. The fathers of the affected males were selected as controls. A total of 314 affected and 314 control males are being resequenced. We designed a high density oligonucleotide resequencing array (RA) containing all of the unique sequences from NLGN3 and NLGN4X. Additionally we included the exon sequences of the neurexin-1beta (NRXN1b) and SHANK3 genes, autosomal genes that encode proteins that bind to neuroligins. Our Microarray-based Genomic Selection (MGS) protocol is being used to isolate target DNA from each patient sample. SNPs identified in our study are partitioned into functional classes (UTR, silent, replacement, intron, intergenic) and compared within and between these classes. Additionally, by using population genetics tests we are able to identify those rare SNPs in replacement sites that are most likely to cause functional changes in the protein. We are also able to identify if changes in conserved non-coding sequences that may be functional using comparative genomics. Data will be presented from the initial 115 affected-control dyads.

961/F

Screening for 7q11.2 duplications in individuals with Autism Spectrum Disorder. *P. Malenfant[1,5], X. Liu[2,5], Y. Qiao[3,4,5], MJ. Hildebrand[4,5], M. Hudson[2,5], E. Rajcan-Separovic[3,5], MES. Lewis[4,5], JJA. Holden[1,2,5,6]*. 1) Dept Physiology; 2) Dept Psychiatry, Queen's University, Kingston, K7L 3N6; 3) Dept Pathology; 4) Dept Medical Genetics, University of British Columbia, Vancouver, BC, V6H 3N1; 5) Autism Research Program, Ongwanada, Kingston, ON, Canada, K7M 8A6; 6) ASD-CARC: www.autismresearch.ca.

Genetic factors are recognized to play a major role in the etiology of Autism Spectrum Disorders (ASDs). There are several reports of genomic rearrangements in individuals with ASD, some identified in more than one individual. In most cases, however, they are identified in only one or a few cases. Using CGH microarrays, we have identified a duplication (dup) on chromosome 7q11.2 in an individual with confirmed ASD, severe expressive language deficit (apraxia), intellectual disability (ID) and minor craniofacial dysmorphism. Real-time qPCR localized the breakpoints within the flanking low-copy repeats (~72.2Mb and ~73.8Mb) that predispose to the genomic instability underlying the 7q11.2 deletion (del) observed in the majority of Williams-Beuren Syndrome cases (WBS). A total of 798 individuals with an ASD and 192 controls were screened for the presence of the dup 7q11.2 rearrangement, using probes specific for ELN and CYLN2, no additional instances were found in either group. There are several previous reports of individuals with dup of the WBS locus, most of which present with language delay and ID and in at least one case, with autism (Depienne et al, 2007). Additionally, Edelmann et al. (2007) reported an atypical del of the WBS region which had a 400kb overlap with the dup reported herein in a patient with autism. Although additional similar dups were not identified in our ASD population cohort, the dup found in our patient and in a few cases reported by others may signal a smaller refined chromosomal region for study by positional candidate gene. Consequently, additional linkage analysis may yield positive results. Three genes located within the duplicated interval, STX1A, CYLN2 and GTF2i were selected based on their function and genomic location for linkage analysis in 725 families, for which results will be presented.

962/F

Admixture may modulate risk for psychiatric disorders. *X. Luo[1,2], L. Zuo[1,2], H.R. Kranzler[3], R.F. Anton[4], R.A. Rosenheck[1,2], H.P. Blumberg[1], J. Covault[3], D.S. Charney[5], D.P. van Kammen[6], L.H. Price[7], J. Lappalainen[1,2], M.D. Shriver[8], M.B. Stein[9], J. Cramer[1,2], J. Krystal[1,2], J. Gelernter[1,2].* 1) Dept Psychiatry, Yale Univ Sch Medicine, New Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) Dept Psychiatry, Univ CT Sch Med, Farmington, CT; 4) Inst Psychiatry, Med Univ S. Carolina, Charleston, SC; 5) Dept Psychiatry, Mount Sinai Sch Med, New York, NY; 6) Clin Dev, ACADIA Pharm., San Diego, CA; 7) Dept Psychiatry, Brown Univ, Providence, RI; 8) Dept Anthropology, Penn State Univ, Univ Park, PA; 9) Dept Psychiatry, Univ CA, La Jolla, San Diego, CA, USA.

The admixture of ethnic populations in America may have important consequences with respect to the risk for psychiatric disorders, as it appears to do for other medical disorders. The present study aimed to investigate the role of admixture in risk for several psychiatric disorders in European-Americans (EAs) and African-Americans (AAs). A total of 3870 subjects (3119 EAs, 673 AAs, and 78 West Africans) were included, including healthy controls and subjects with substance dependence (SD), including alcohol dependence (AD), cocaine dependence, and opioid dependence, social phobia, affective disorders (AFD), and schizophrenia. The degree of admixture for each subject was measured by analysis of a set of ancestry-informative genetic markers using the program STRUCTURE, and was compared between cases and controls. We found that the degree of admixture in AAs was higher than in EAs. In EAs, the degree of admixture (with African ancestry) was significantly lower in patients with SD (mainly AD) than in controls (p=0.009 for SD; p=0.008 for AD), but suggestively higher in patients with AFD than controls (p=0.057). In AAs, the degree of admixture (with European ancestry) was significantly higher in patients with schizophrenia than in controls (p=0.015). These findings suggest that admixture may modulate risk for psychiatric disorders. Admixture may decrease risk for SD and AD by decreasing the rate of inbreeding, and increase risk for AFD and schizophrenia via environmental factors or underlying disease loci in an overdominant manner.

963/F

The tau H2 haplotype contributes to susceptibility to Parkinson disease in a Southern Italian population. *D. Civitelli[1], G. Annesi[1], P. Tarantino[1], E.V. De Marco[1], F. Annesi[1], G. Nicoletti[1,2], F. Condino[1], I.C. Ciró Candiano[1], S. Carrideo[1], F.E. Rocca[1], V. Scornaienchi[1], V. Greco[1], G. Provenzano[1], A. Quattrone[1,2].* 1) Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, Department of Medical Sciences, University Magna Graecia, Catanzaro, Italy.

The microtubule-associated tau protein is involved in common neurodegenerative pathways and a number of association studies have been conducted to clarify the role of the tau gene in neurodegenerative diseases, including Parkinson's disease (PD). Several polymorphisms localized along the entire tau gene length are inherited in complete linkage disequilibrium, forming two distinct extended haplotypes designated H1 and H2, respectively. Recombination between these two haplotypes is rare. The H1 haplotype appears to be related to an increased risk for progressive supranuclear palsy, cortical degeneration, frontotemporal lobar degeneration syndromes and primary progressive aphasia. A contribution of the H1 haplotype to PD susceptibility was also suggested. Here, we assessed the distribution of the tau haplotypes in a group of 262 sporadic PD patients from Southern Italy and in 197 healthy controls from the same area. We reconstructed tau haplotypes by genotyping three SNPs (BanII in exon 3, MspI in exon 9, AluI in exon 11). Moreover, we tested a GT dinucleotide polymorphism located in intron 9 and inherited as allele 0 (11 repeats), allele 1 (12 repeats), allele 2 (13 repeats), allele 3 (14 repeats) and allele 4 (15 repeats). Of interest, we found a significant overrepresentation of the H2 haplotype in PD patients (OD 2.26 - 95% CI 1.64-3.18). Despite PD is not primarily considered a tauopaty, the tau gene could play a role in PD pathogenesis. The significant overrepresentation of the H2 haplotype in the examined PD patients suggests that the this haplotype is a risk factor for PD in our sample. Thus, southern Italian population appears to be different from most of other Caucasian populations in which the tau H1 haplotype contributes to susceptibility to PD.

964/F

Genome-wide association studies on Multiple system atrophy (MSA). *Y. Nakahara[1,2], Y. Momose[1,2], Y. Takahashi[1], J. Goto[1,2], S. Tsuji[1,2].* 1) Department of Neurology, Univ. of Tokyo, Tokyo, Japan; 2) JAMSAC (Japan Multiple System Atrophy research Consortium).

Background: Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder characterized by various combinations of autonomic failure, cerebellar symptoms, parkinsonism and pyramidal signs. Although the discovery of alpha-synuclein has been identified as a major component of the glial cytoplasmic inclusions (GCIs), a pathologic hallmark for MSA, the etiologies of MSA remain to be elucidated. To obtain clues as to the genetic factors for MSA, we have conducted genome-wide association studies on MSA cases and controls.Design/Methods: We have established a consortium focusing on multiple system atrophy (JAMSAC; JApan MSA Research Consortium), to obtain longitudinal clinical information and genomic DNA. We genotyped 166 patients with MSA and 95 neurologically normal controls, using Affymetrix Gene Chip® Human Mapping 500K Array Set.Results: Genotype data (334,278 SNPs) fulfilling the following conditions are processed for statistical analyses; the call rate exceeding 0.9 in MSA patients and controls, p value for Hardy-Weinberg equilibrium exceeding 0.01. The number of the SNPs with significant p values of $\chi 2$ test are as follows; ($p<0.05$: 14,362 SNPs, $p<0.01$: 3,196 SNPs, $p<0.001$: 396 SNPs, and $p<0.0001$: 46 SNPs). To identify susceptibility genes for MSA further replication with independent data set will be required.

965/F

Genomic copy number variations as a basis of genetic susceptibility for Amyotrophic Lateral Sclerosis (ALS). *J.H. Veldink[1], H.B. Blauw[1], M.A. Van Es[1], C. Saris[1], C. Sabatti[2], L.H. Van den Berg[1], R.A. Ophoff[1,2].* 1) Department of Neurology, Rudolf Magnus Institute of Neuroscience and Complex Genetics Section, University Medical Center Utrecht, Netherlands; 2) Department of Human Genetics, University of California, Los Angeles, USA.

Genomic copy number variations (CNVs) are abundant source for genetic variation in the human genome and have been associated with genomic disorders as well as complex human traits. ALS is a devastating disease characterized by progressive degeneration of motor neurons in brain and spinal cord leading to muscle weakness. We and others have shown that CNV in the Survival of Motor Neuron gene (SMN) is associated with the severity/susceptibility to ALS. In order to investigate the role of CNVs in ALS genome-wide, we have developed a CNV detection script for high-density SNP data. We genotyped 450 patients with ALS and 450 healthy sex- and age-matched controls using the Illumina HumanHap300 BeadChip. Two observations are relevant to the detection of CNVs: the logR ratio (= SNP intensity), and the B allele frequency (= SNP genotype). Criteria in our script were set based on 1,000 visually scored CNVs. We identified 1144 CNVs in patients with ALS and 1184 in controls (in total 2328) with 36% (n=833) not present in the online database. Duplications outnumbered deletions (1416 versus 868 hemizygous deletions). We identified 382 CNVs that were uniquely observed in controls and 407 that were only present in ALS patients. Although differences were found in a few common CNVs with regard to their frequency between patients and controls, none were statistically significant. To achieve a more comprehensive and objective way of CNV detection, we developed an HMM-based algorithm. Prior probabilities were estimated using an external dataset (HapMap) and 245 true positive CNV findings (HumanHap300 BeadChip). This enabled us to describe the emission and transition probabilities for our HMM. Using this approach we designed an operational algorithm and are currently further optimizing our model. Our efforts will lead to improved detection of CNVs using genome-wide SNP data and enable us to study the impact of CNVs on disease susceptibility.

966/F

HLA Genetics of multiple sclerosis in Israeli Arab populations. *T. Paperna[1], G. Benedek[2], A. Miller[1], I. Lejbkowicz[1], C. Brautbar[2], J.R. Oksenberg[3], S. Israel[2], Israeli MS Genetics Group.* 1) Medicine, Technion, Haifa, Israel; 2) Hadassah Med. Ctr. Jerusalem, Israel; 3) University of California, San Francisco, CA.

Genetic studies of multiple sclerosis (MS) highlighted the HLA region as associated with disease risk, although specific genes have not yet been identified. Expanding analyses to previously under-studied populations, specifically, populations with high genetic homogeneity may reveal and strengthen associations with specific genes. Israeli Arabs, including Christians, Druzes and Muslims, have maintained endogamy over generations, suggesting their possible advantage for genetic studies. The aim of this study was to examine the association of HLA genes with MS in Israeli Arab populations, and characterize disease phenotypes. DNA samples from 96 Arab MS patients and 114 unrelated, ethnicity matched healthy individuals were genotyped for HLA class I (HLA A and B) and II (DRB1 and DQB1). Case control analyses to compare allele distribution were done. Disease characteristics including age of onset, disease type, severity, and rate of progression were compared between the different sub-ethnicities. DRB1 allele frequencies differed significantly between Muslims, Druzes, and Christians. Global testing of cases versus controls revealed a significant difference only in Christians (p=0.027), likely due to DRB1*03. However, in contrast to previous reports, here DRB1*03 was negatively associated with disease (17.7% in controls vs. 2.6% in MS, p=0.003) with a clear dose effect (p for trend= 0.014). In Muslims and Druzes, the pattern for DRB1*03 is reversed, consistent with data from other populations. We were not able to detect association with DRB1*15 previously reported as a susceptibility allele. For DQB1, HLA A and HLA B, we currently could not identify allele associations. Differences in disease characteristics between the sub-ethnicities were observed, suggesting genetic heterogeneity may underlie the phenotypes. In conclusion, genetic studies in Israeli Arab populations suggest new allele associations with MS risk compared to other populations. Further research in these populations is ongoing.

967/F

Association study between the monoamine oxidase A gene (*MAOA*) and schizophrenia: a meta-analysis. *D. Li[1,2,3], L. He[3,4].* 1) Laboratory of Statistical Genetics, Rockefeller University, New York, 10021, NY, USA; 2) Bio-X Center, Shanghai Jiao Tong University, Shanghai 200030, China; 3) Institute for Nutritional sciences, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; 4) NHGG Bio-X Center, Shanghai Jiao Tong University, Shanghai 200030, China.

The human monoamine oxidase A gene (*MAOA*), located on Xp11.23-11.4, has attracted considerable attention as a candidate gene for schizophrenia based both on its chromosomal position and its enzyme function as a key factor in neurotransmitter catabolism pathways. A number of independent studies have attempted to find evidence of association between *MAOA* and schizophrenia, however studies to date have reported inconsistent findings regarding the association of the variable number tandem repeat (VNTR) and T941G polymorphisms, possibly reflecting inadequate statistical power and the use of different populations and methodologies. Therefore we undertook a meta-analysis to establish a comprehensive relationship between the two polymorphisms and schizophrenia across international populations. We have combined all the published case-control and family-based studies using multiple research methods and models. For both allelic and genotypic analyses, the current study investigated global studies as well as sub-studies grouped according to variables including ethnicity (European and Asian ethnic populations) and gender. However, we found no evidence of significant association with the two schizophrenia susceptibility polymorphisms. No publication bias or heterogeneity was found in any of the combined studies (No $P(T)$ or $P(Q) < 0.1$).

KEY WORDS: chromosome Xp11; linkage disequilibrium; Single Nucleotide Polymorphism (SNP); psychosis.

968/F

Association between FGF20 and Parkinson's disease and Genome-wide association study using 27,158 microatellite by The Japanese PD Susceptibility Gene Consortium.

W. Satake[1, 2], I. Mizuta[1, 3], Y. Hirota[1], A. Oka[4], M. Watanabe[5], A. Takeda[6], K. Hasegawa[7], S. Sakoda[2], M. Yamamoto[8], N. Hattori[9], M. Murata[10], H. Inoko[4], T. Toda[1,3]. 1) Div Clinical Genetics, Osaka Univ Grad Sch Med; 2) Dept Neurol, Osaka Univ Grad Sch Med; 3) CREST, JST; 4) Dept Mol Life Sci, Tokai Univ Sch Med; 5) Dept Neurol, Univ Tsukuba; 6) Div Neurol, Tohoku Univ Grad Sch Med; 7) Dept Neurol, Sagamihara National Hosp; 8) Dept Neurol, Kagawa Prefectural Central Hosp; 9) Dept Neurol, Juntendo Univ Sch Med; 10) Dept Neurol, Musashi Hosp, NCNP, Japan.

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. A genetic association between the fibroblast growth factor 20 (FGF20) gene and PD has been found by the pedigree disequilibrium test. However, this association was not replicated by a case-control association study. In order to clarify the association between FGF20 and PD, we attempted to replicate this association by a case-control association study using a large number of Japanese samples (1388 patients and 1891 controls). rs1721100 exhibited a significant difference in allele C versus G (P=0.0089), and in genotype CC+CG versus GG (P=0.0053). These results suggest that FGF20 is a susceptibility gene for PD in the Japanese population. Moreover, to identify another susceptibility genes for PD, we performed three step-wise association studies with DNA samples of 624 PD patients and 624 controls (1st screening,124 samples each, 2nd 250 each, 3rd 250 each) using the pooled DNA method and 27,158 microsatellite markers(MS) throughout the genome, and 280 MS showed associations throughout all three screenings. We have been confirming the associations of these MS using individual samples of 624 PD patients and 624 controls (Tier 1). We have performed individual genotyping of 164 markers with Tier 1 samples, and 24 markers still showed an association ($p < 0.01$), of which 7 markers showed a stronger association ($p < 0.001$). In parallel, we have been genotyping samples of 779 PD patients and 1,217 controls (Tier 2) on these markers. Genes in linkage disequilibrium with these markers may be associated with the pathogenesis of PD.

969/W

Screening Program for Connexin 26 and Connexin 30 Genes in 648 Institutionalized Deafness Population in Colombia. *M. Olarte[1,2], M. Gómez[1], N. Gelvez[1], S. Flórez[2], D. Medina[2], C. Varón[3], N. Garcia[1], L. Morales[1], I. del Castillo[4], F. Moreno[4], M.L. Tamayo[1,2].* 1) Inst de Genetica Humana, Univ Javeriana, Bogota 1, Colombia; 2) Fundación Oftalmológica Nacional, Bogotá DC, Colombia; 3) Fundación Oftalmológica de Santander, Clínica Ardila Lulle (FOSCAL). Bucaramanga, Santander. Colombia; 4) Unidad de Genética Molecular, Hospital Ramon y Cajal, Madrid, España.

Hearing loss affects one of 1000 children. In developed countries, half of the hearing loss is due to genetic causes. The screening program was performed in order to establish the frequency of mutations in Connexin 26 (Cx26) and Connexin 30 (Cx30) genes. We visited institutions for deafness in 11 cities of Colombia. A total of 648 individuals with non-syndromic deafness were selected. The population was classified in two groups: 39.2% (254/648) as recessive nonsyndromic hearing loss (RNSHL) and the remaining 60.8% (394/648) as sporadic cases with unknown cause. The molecular studies including the PCR-RFLP for the 35delG mutation in Cx26 gene; a specific multiplex PCR for the deletions (del(D13S1830) and del(D13S1854)) in Cx30 gene and automatic sequencing of Cx26 gene. We identified in total, 22 mutations in the Cx26 gene, corresponding to 16,2% of cases (105/648). Among these, 5 were new mutations. The most frequent mutations were S199F (42,3%) and 35delG (41%). In Connexin 30 gene, we identified the two studied deletions in 6 cases (0.9%); among these, 5 were compound heterozygotes Cx26/Cx30. Interestingly, the frequency of S199F mutation in our nonsyndromic deaf population is higher than reported in other studies, being the most frequent in our population. The 35delG mutation was found in similar frequency to other reports.

970/W

A targeted *Coch* missense mutation: a knock-in mouse model for DFNA9 late-onset hearing loss and vestibular dysfunction. *N.G. Robertson[1], S.M. Jones[2], T.A. Sivakumaran[1], A.B.S. Giersch[1], S.A. Jurado[1], M.C. Liberman[3], S.F. Maison[3], C.E. Miller[3], C.C. Morton[1].* 1) Dept OB/GYN & Pathology, BWH & Harvard Med School, Boston, MA; 2) Dept Communication Sciences & Disorders, East Carolina Univ, Greenville, NC; 3) Dept Otology & Laryngology, Mass Eye & Ear Infirmary, Eaton-Peobody Lab, Harvard Med School, Boston, MA.

Mutations in *COCH*, expressed at high levels in the inner ear, are etiologic for the late-onset hearing loss and vestibular dysfunction at the DFNA9 locus. To date, 11 mutations (10 missense and one in-frame deletion) have been found in *COCH*. To develop an animal model for DFNA9, we created a $Coch^{G88E/G88E}$ knock-in mouse. By RT/PCR and immunohistochemistry, we confirmed successful transcription and translation of the mutated Coch RNA and protein products. Vestibular evoked potential (VsEP) thresholds were analyzed using a two factor ANOVA (Age X Genotype) revealing significant main effects for age and genotype. VsEP thresholds at all ages tested (11-19 months) were elevated compared to wild-type littermates. At 19 months, one of four homozygous $Coch^{G88E/G88E}$ mice had no measurable VsEP. Age-related changes were also observed for both wild-type and affected mice; however, the affected homozygotes clearly showed elevated thresholds at every time-point tested. ABR threshold measurements at 11 months were identical at all frequencies for all genotypes. At 19 months, ABR thresholds for the $Coch^{G88E/G88E}$ mice were elevated by as much as 15 dB relative to age-matched wild-type; however, a two factor ANOVA was not significant. DPOAE amplitudes at 19 months were slightly reduced compared to controls, which were generally consistent with the elevated ABR thresholds. These results suggest that vestibular function, particularly gravity receptor function, is affected beginning as early as 11 months when cochlear function appears to be normal, and increases with age. Preliminary histological evaluation shows some degeneration of the type IV fibrocytes of the spiral ligament. Initial transcriptional profiling experiments have been performed and will be followed up further using both our own cDNA arrays and Affymetrix arrays.

971/W

Mixed Inbred $Gne^{M712T/M712T}$ Mice Show Increased Survival, Attenuated Kidney Disease, and Altered NeuGc/NeuAc Profile. *D. Darvish[1, 2], Y. Valles[1], S. Darvish[1, 2], J. Orozco[2], O. Scremin[2], G. Lawson[3], B. Darvish[2].* 1) HIBM Research Group, Inc, Encino, CA; 2) VA Greater Los Angeles(VA-GLA / UCLA), Los Angeles, CA; 3) UCLA, Los Angeles, CA.

Recessive form of Hereditary Inclusion Body Myopathy (HIBM, IBM2 - MIM:600737) is an adult onset muscle wasting disorders that is caused by hypomorphic GNE, the rate-limiting enzyme of sialic acid biosynthesis. Unlike human patients, mice bearing the $Gne^{M712T/M712T}$ genotype in B6 background strain suffer severe glomerular hematuria, incomplete podocyte development, and do not survive beyond the first few days of life. We back-crossed heterozygous mice ($Gne^{M712T/+}$) of B6 strain with FVB strain mice. In FVB;B6 mixed inbred background (N1), the homozygous mice show attenuated glomerular disease and survive longer (n=12, 18.2±13.3 weeks). Wildtype, heterozygous, and homozygous mice were used for phenotype analysis. Functional motor evaluation included Rotarod treadmill, exercise induced creatine kinase elevation, and grip strength. Large difference in strength or endurance, which could be attributed to the genotype of the mice, was excluded in all tests. Laboratory workup included complete blood count (CBC), chemistry panel, and liver enzymes. The only notable abnormality was that homozygous mice show increased BUN (45.2±18.6mg/dL, ref range 12-28). Histology shows varying levels glomerular disease in every homozygous mouse without exception. Sia levels, normalized to protein concentration, were measured in liver, kidney, muscle, and serum. Paradoxically, the homozygous mice showed increased total Sia levels in serum (2x control). Additionally, the NeuGc:NeuAc ratios were slightly shifted in homozygous mice, which seems to be attributed to higher levels of NeuGc in muscle and higher levels of NeuAc in serum. Although kidney disease is attenuated and survival is improved in mixed inbred background (FVB;B6), $Gne^{M712T/M712T}$ mice do not show reduced muscle strength/endurance. Increase in serum Sia levels may be caused by altered glomerular filtration. This paradoxical increase in serum Sia may contribute to Sia pools of muscle, and exert a potential beneficial effect.

972/W

Hyperglycosylation of mutations in GABRB3 polypeptide in childhood absence epilepsy. *M. Tanaka[1,2], M.T. Medina[3], R.M. Duron[3], R.H. Castro[4], I.J. Martinez[5], I.P. Castroviejo[6], M.J. Salas[2], M.E. Alonso[5], J.N. Bailey[2,7], D. Bai[2,8], A.V. Delgado–Escueta[2,8], R.W. Olsen[1].* 1) Dept Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Epilepsy Center of Excellence VA GLAHS & UCLA, Los Angeles, CA; 3) Neurology, National Autonomous University, Tegucigalpa, Honduras; 4) University of Sonora, Hermosillo, Mexico; 5) Neurology, National Institute of Neurology & Neurosurgery, Mexico City, Mexico; 6) The Hospital of Lapaz, Madrid, Spain; 7) Semel Institute for Neuroscience & Brain Behavior at UCLA Los Angeles, CA; 8) Dept Neurology David Geffen School of Medicine at UCLA, Los Angeles, CA.

Childhood absence epilepsy (CAE) accounts for 10 to 12% of epilepsy under 16 years of age. Because transmission disequilibrium test showed possible genetic association between $GABA_A$ receptor β3 subunit gene (GABRB3) and CAE, while GABRB3 deficient mice shows absence like features including EEG characteristics and pharmacological response and because atypical absences in Angelman syndrome correlate with chromosome 15q11-13 deletions which includes GABRB3, we screened for mutations in GABRB3 in 48 probands and families with CAE. One heterozygous missense mutation (P11S) in exon 1a segregated with CAE in two unrelated multiplex multigeneration Mexican families and another heterozygous missense mutation (S15F) was present in a singleton from Honduras (mutations absent in 440 controls). G32R missense mutation in exon 2 was present in 3 affecteds of one Honduras family (mutations absent in 200 controls). We studied functions and possible pathogenicity by expression of mutations in HeLa cells using Western blots and an *in vitro* translation/translocation system. Expression levels were not different from controls, but all mutations showed hyperglycosylation in the *in vitro* translation/translocation system with canine microsomes. We suggest that the gain in glycosylation affects maturation of the β3 subunit protein and trafficking from endoplasmic reticulum to cell surface. The resulting malfunction of $GABA_A$ receptors which contain the β3 subunit in turn produces absence attacks.

973/W

Characterization of the structural aberrations and mutations of the RCCX module in patients with Congenital Adrenal Hyperplasia and Ehlers-Danlos syndromes. *W. Chen[1], J. Yang[1], M. Berk[2], C. VanRyzin[2], D. Merke[2], N.B. McDonnell[1].* 1) Lab Clinical Investigation, NIA/NIH, Baltimore, MD; 2) NICHD/NIH, Bethesda, MD.

The gene2 encoding 21-hydroxylase (CYP21A2) and Tenascin-X (TNXB) are located within the HLA complex on chromosome 6, in a region of high gene density termed the RCCX module. The region has multiple pseudogenes as well as tandem repeat sequences that promote misalignment during meiosis leading to complex gene rearrangements, deletions and gene conversion events. CYP21A2 mutations cause Congenital Adrenal Hyperplasia (CAH) and TNX deficiency has been proposed as a cause of hypermobile Ehlers-Danlos syndrome (EDS). We investigated the structure of the RCCX module in a cohort of CAH patients seen at the National Institute of Child Health and Development, and in Ehlers-Danlos patients seen at the National Institute on Aging. Southern blotting, PCR-based detection of deletions, and direct sequencing of exons of interest were utilized. A novel heterozygous 30 kB TNXB deletion that did not extend into CYP21A2 was found in a family with hypermobile form of EDS. CYP21A2 deletions were detected in 30% of the subjects in the CAH cohort, 25% of those subjects had a deletion extending into TNXB. Unusual haplotypes including three CAH probands with triplication of CYP21A2, and a sibling pair with a deletion of TNXB and triplication of CYP21A2 were identified through Southern Blot analysis. A novel mutation in a CAH family with joint hypermobility was detected in exon 8 of TNXB (c.G3452>A Arg1151His). This mutation involves the highly conserved fibronectin type III repeat in TNXB which is known to be involved in collagen fibrillogenesis, and was not seen in control subjects.

974/W

Phenotypic analysis of the *Crtap-/-* mice: the first animal model for recessive osteogenesis imperfecta. *R. Morello[1], J. Lennington[2], S. Kakuru[1], M. Jiang[1,4], Y. Chen[1,4], D. Keene[3], B. Lee[1,4].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 3) Shriners Hospital for Children, Portland, OR; 4) Howard Hughes Medical Institute, Houston, TX.

We recently described the skeletal phenotype of the *Crtap-/-* mice consisting in a severe osteochondrodysplasia, with low bone mass, kyphosis and shortening of the long bones. Crtap interacts with prolyl 3-hydroxylase 1 (P3h1) and is essential for proper type I and II collagens post-translational modification. The phenotype of *Crtap-/-* mice closely resembles that of OI patients and mutations in the *CRTAP* gene cause recessive osteogenesis imperfecta (OI). Here we further the phenotypic analysis of our mutant mice to obtain a better understanding of the Crtap role during development and adulthood. Adult *Crtap* null mice showed marked craniofacial abnormalities, consisting in shortening and compression of the anterior portion of the cranium, and increased skin laxity compared to WT littermates as revealed by a decreased thickness of the dermis accompanied by a decreased matrix density with collagen fibrils of increased diameter, as seen in Ehlers Danlos syndrome. Histological analyses of organs showed abnormal lung morphology, with alveolar dilatation associated with thinning of the alveolar walls, a feature seen in Marfan syndrome. Moreover, the kidney glomerular basement membrane appeared to have an expanded lamina lucida and reduction of the lamina densa with mesangial proliferation. These data suggest CRTAP may exert more widespread effects on collagen homeostasis including types V in skin and type IV in kidney. The lung phenotype raises the question of whether this unique post-translational modification may also effect TGFb signaling. Finally characterization of the cartilage dysplasia in the *Crtap-/-* mice identified an increase in chondrocyte proliferation in the zeugopod growth plates of E15.5 *Crtap* null mice compared to controls, though this was not noted in the stylopod. These studies point to the requirement for 3-prolyl-hydroxylation in the widespread regulation of collagen structural and signaling function.

975/W

Molecular events underlying the genesis of Cerebral Cavernous Malformations. *A.L. Akers[1], R. Shenkar[2], E.W. Johnson[3], I.A. Awad[2], D.A. Marchuk[1].* 1) Mol Genet & Microbio, Duke University, Durham, NC; 2) Div of Neurosurgery, Northwestern University, Chicago, IL; 3) PreventionGenetics, Marshfield, WI.

Cerebral cavernous malformations (CCM) are vascular lesions of the brain consisting of closely-packed, grossly-dilated vessels within a matrix of connective tissue. Radiological analysis has shown the lesions develop from small dilated vessels into complex, multicavernous lesions with a propensity for bleeding often leading to seizures and/or hemorrhagic stroke. CCM may occur sporadically or may be inherited as an autosomal dominant disorder due to mutation in one of three genes, *CCM1*, *CCM2*, or *CCM3*. While the causative genes have been identified, the molecular events initiating lesion formation have yet to be elucidated. We and others have hypothesized that CCM lesion genesis occurs due to second-site somatic mutations following a Knudsonian two-hit model. Support for this hypothesis comes from epidemiological evidence; sporadic patients typically develop single CCM lesions, while multiple lesions are observed in patients with familial CCM. The neural predilection for CCM limits accessibility of human tissue to surgically resected specimens which are typically very late stage, bleeding lesions; thus, severely limiting molecular analyses at the earliest stages of lesion development. We have created mouse models for *Ccm1* and *Ccm2* that in conjunction with MRI technology, allow us to identify and dissect CCM lesions at the earliest stages of genesis. Using laser capture microscopy, we have micro-dissected the vascular components from these lesions and have amplified DNA for both loss-of-heterozygosity and somatic mutation analyses. From MRI-identified, bulk lesion tissue both DNA and RNA have been isolated and are being examined. The nature of the knockout mutation and assay design allows for specific amplification of the wildtype allele at the cDNA level. While in the first lesion examined no consistent sequence changes have been identified, additional lesions are being analyzed. We are similarly examining a series of late-stage human lesions resected from *CCM1* patients. Both of these studies are ongoing and we will report the results of our investigation.

976/W

Progressive mitochondrial degeneration leads to neuropathology of the somatosensory-motor system in the Harlequin mouse: a model for mitochondrial respiratory chain complex I defect. *V. El Ghouzzi[1], Z. Csaba[2], P. Olivier[1], C. Verney[1], P. Rustin[1], P. Gressens[1].* 1) INSERM U676, Dept of Pediatric Neurology, Hosp. Robert Debre, Paris, France; 2) Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Dept of Human Morphology and Developmental Biology, Budapest, Hungary.

Apoptosis-inducing factor (AIF) is a mitochondrial protein which acts as a promoter of cell death after its release from mitochondria and translocation to the nucleus in response to apoptotic stimuli, but its mitochondrial function in non-apoptotic cells is unclear. The recent discovery that AIF deficiency compromises oxidative phosphorylation (OXPHOS) and that Harlequin (Hq) mice, where AIF is downregulated, develop a severe mitochondrial complex I (CI) deficiency has uncovered a mitochondrial function for AIF and pointed out the Hq mice as a natural model of the most frequent OXPHOS disorders. However, the brain phenotype reported to date specifically involves the cerebellum whereas human CI deficiencies often manifest as complex multifocal neuropathologies. To evaluate whether this model can be used as a valuable tool to study CI-deficient disorders, the whole brain of Harlequin mice was investigated during the course of the disease. Neurodegeneration was not restricted to the cerebellum but progressively affected thalamic, striatal and cortical regions as well. Strong astroglial and microglial activation with extensive vascular proliferation was observed by 4 months of age in thalamic, striatal and cerebellar nuclei associated with somatosensory-motor pathways. At 2 months of age, degenerating mitochondria were observed in most cells in these structures, even in non-degenerating neurons, a finding that indicates mitochondrial injury to be rather a cause than an effect of neuronal cell death. Thus, mitochondrial degeneration precedes neuropathological signs in Hq mice and leads to progressive multifocal neuropathology of the somatosensory-motor system, a phenotype much wider than previously described, resembling histopathological features of devastating human neurodegenerative mitochondriopathies associated with CI deficiency.

977/W

Loss of *Tsc2* in radial glia models the brain pathology of tuberous sclerosis complex in the mouse. *M.J. Gambello, J. McKenna III, S. Way.* Dept Pediatrics, Univ Texas Medical Sch, Houston, TX.

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by mutations in either the *TSC1* or *TSC2* gene, which encode the proteins hamartin and tuberin. Substantial morbidity and mortality are caused by brain lesions such as tubers, subependymal nodules, and other neuronal heterotopias. These lesions are associated with seizures, autism, and other developmental disabilities. The histology of TSC brain lesions suggests that they are developmental lesions resulting from abnormalities in cell growth, migration and differentiation. Consequently a current hypothesis of TSC brain pathogenesis is that loss of function of either TSC1 or TSC2 in neural progenitor cells initiates developmental neuropathology. At the cellular level, loss of function of either gene causes activation of the insulin signaling/mTOR pathway, leading to increased translation, cell growth and proliferation.

In the developing cortex, radial glia were thought to function mainly as a scaffold for migrating neurons. Recent data suggest, however, that radial glia are neuroglial precursors, contributing to the majority of cells in the cerebral cortex. Given this progenitor function of radial glia, we hypothesized that loss of function of TSC1 or TSC2 in radial glial cells might be an initiating event in TSC brain pathogenesis. To test this hypothesis we used a conditional disruption of the *Tsc2* gene and an *hGFAP-Cre* mouse that expresses Cre in radial glial progenitors. Mice deleted for *Tsc2* in radial glia develop megalencepahly, hydrocephalus, and die between 3 and 4 weeks of age. Their brains demonstrate cortical lamination defects, hippocampal heterotopias, and giant, dysplastic neurons. These histologic abnormalities are accompanied by activation of the mTOR pathway and are similar to human lesions. These results establish the novel concept that loss of function of Tsc2 in radial glial progenitors is an initiating event in the development of TSC brain lesions. This model will be useful to study TSC brain pathophysiology, test potential therapies, and identify other genetic pathways that are altered in TSC.

978/W

Central nervous system impairment in mouse saposin C deficiency. *G.A. Grabowski, H. Ran, M. Zamzow, B. Quinn, Y. Sun.* Div Human Genetics, Cincinnati Childrens Hosp, Cincinnati, OH.

Saposin C has unique activation and proteolytic protective functions for acid β-glucosidase (GCase), the enzyme that cleaves glucosylceramide, a glycosphingolipid in the lysosomal degradation pathway. Saposin C as well as saposins A, B and D is processed from a common precursor, prosaposin. The few patients with saposin C deficiencies developed a Gaucher-like phenotype due to diminished glucosylceramide cleavage activity. To explore the *in vivo* effects of saposin C, saposin C null mice (C-/-) were generated by introducing a point mutation into the cysteine codon in exon 11 of prosaposin locus. Prosaposin and saposin A, B and D were expressed at normal levels whereas saposin C protein was not detected in C-/- mice. Inclusions in dorsal root ganglion and loss of Purkinje cells were evident at 25 weeks. The neurological phenotype in C-/- mice developed at about 1 year and those mice exhibited wobbly walking and weakness of the hind limb. Activated microglial cells and astrocytes were present in thalamus, brain stem and hind brain, demonstrating restricted regional proinflammatory response in C-/- mice. Deficiency of saposin C resulted in decreases of *in vitro* GCase activity in liver, kidney and brain due to the decrease in proteolytic structure of the wild type GCase. No storage cells or glucosylceramide were found in visceral organs in C-/- mice. These results support the notion that saposin C has a predominant function in the central nervous system. The further elucidation of saposin C's *in vivo* function will advance the study of glycosphingolipid storage diseases.

979/W

Highly mutable CCGCGG interruptions in expanded CTGCAG repeat tracts increase penetrance in SCA8 families. *Y. Ikeda[1], M.L. Moseley[1], J. Nielsen[2], L. Hasholt[2], A. Nørremølle[2], T. Bird[3], J.W. Day[1], L.P.W. Ranum[1].* 1) Inst. of Human Genet., Univ. of Minnesota, Minneapolis, MN; 2) Dept. of Cellular and Mol. Medicine, Univ. of Copenhagen, Denmark; 3) Dept. of Neurology, Univ. of Washington, Seattle, WA.

Spinocerebellar ataxia type 8 (SCA8) is a dominantly inherited neurodegenerative disorder caused by a CTGCAG repeat expansion bidirectionally expressed as both a polyglutamine protein and a non-coding CUG transcript. We have investigated interrupting trinucleotides within the repeat as modifiers that could explain the puzzling degree of reduced penetrance in SCA8. All expansion chromosomes in affected individuals (n=12) from a branch of the MN-A family, the largest SCA8 family known, contain variable numbers of CCGCGG interruptions within the CTGCAG tract. In contrast, all expansion chromosomes from a non-penetrant branch of the MN-A family have pure repeats, suggesting CCGCGG interruptions influence disease penetrance in this family. Analysis of an additional 31 SCA8 families show CCGCGG interruptions in 6 families including a three generation Danish family with 6 affected members. The number of CCGCGG interruptions within the repeat tract show dramatic variation between members of both the MN-A and Danish families (1-11 CCGCGGs). Additionally, CCGCGG interruptions were found at a higher frequency in families with multiple (6/13) versus single affected family members (1/19) (p=0.015). Analysis of additional relatives of the sporadic case with interruptions show that expansion alleles in the unaffected father and sibling had no interruptions. Additional studies in cells expressing interrupted vs. pure polyQ ATXN8 protein show that expression of ATXN8 with these arginine encoding interruptions in the polyQ tract changes the aggregation and solubility properties of the protein resulting in more 1C2 inclusions and shifted gel mobility. In summary, penetrance differences in humans indicate that CCGCGG interruptions increase the risk of developing ataxia and additional work is needed to determine if this effect is mediated by changes in the mutant protein or by changes in the toxicity of the mutant transcripts.

980/W

Molecular screening of FMR1 mutation among autism and mental retardation patients in China. *W. Ju[1], X-Z. Wang[2], N. Zhong[1,2].* 1) Dept Human Genetics, New York State Inst Basic Res, Staten Island, NY; 2) Peking University Center of Medical Genetics, Beijing, China.

Fragile X syndrome (FXS) is the most common inherited form of mental retardation. It is resulted from an unstable expansion of which consequently silences FMR1 gene expression. Our earlier study has determined that FXS accounts for 3.2% of the Chinese mental retarded population. Recently, FMR1 mutation has been found in clinically diagnosed autistic patients. In this report, we present a pilot study of molecular screening of FMR1 mutation in a subset of Chinese autistic MR patients. A total number of 323 DNA samples, including 195 samples from autism patients (183 males and 12 females) and 128 samples from mental retardation (MR) patients (114 males and 14 females), were studied for FMR1 mutations. Among the samples screened, 311 (124 MR and 187 autism) were shown to be normal and 12 (4 MR and 8 autism) were found to carry expanded mutant CGG repeats at FMR1 gene. Our pilot study provided us a feasibility to conduct a larger size of autism samples.

981/W

Generation of a knock-in spartin (Spg20) mouse, a model for motor neuron degenerative disease. *H. Patel, A.H. Crosby.* Medical Genetics, St George's, University of London, London, United Kingdom.

We have previously shown that mutations in spartin underlie a form of motor neuron degenerative disease, the cardinal features of which are spastic paraparesis, dysarthria, distal amyotrophy, mild developmental delay and subtle skeletal abnormalities. The condition is at high frequency amongst the Old Order Amish where a founder 1bp (1110delA) exon 4 deletion mutation is responsible; this mutation results in the substitution of the following 29 amino acids and truncation of the protein by 268 residues (fs369-398X399). In order to learn more about this condition, we have generated a mouse knock-in model of murine spartin that closely represents the human mutation. Unfortunately, as mouse spartin exon 4 is variably spliced, we introduced into the targeting vector a 1bp 1102delA exon 4 deletion that closely corresponds to the human mutation in parallel with two stop codons in exon 5 to ensure the premature termination of both possible spartin transcripts. We have obtained heterozygous animals which are being inter-crossed to generate constitutive homozygous 1102delA *Spg20* knock-in mice. Initial assessment of the breeding program is presented. This knock-in mouse will provide a valuable animal model allowing us to investigate the underlying pathogenic processes of motor neurone degenerative disease.

982/W

Candidate Gene Selection and Mutation Screening of Adult-Onset Primary Open Angle Glaucoma (POAG) at the GLC1B, GLC1C and GLC1H Loci. *M. Sarfarazi[1], J. Aragon-Martin[1,2], R. Sharafieh[1,2], T. Rezaie[1], A. Child[2].* 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT; 2) Department of Cardiac and Vascular Sciences, St. George's University of London, London, U.K.

Glaucoma is a blinding condition that affects millions of people worldwide. Of the 20 loci published so far, only 4 of their defective genes (CYP1B1, MYOC, OPTN, WDR36) have been identified. In this study, we used published information on Linkage Mapping, mRNA expression, Microarray Data, known Biological Function or Predicted Biochemical Pathways of candidate proteins and prioritized a selected group of genes for specific screening at the GLC1B (2cen-q13), GLC1C (3q23) and GLC1H (2p16) loci. The GLC1B region contains over 220 genes, of which we previously excluded 20 of them. Bioinformatic, Genomic Convergence and Proteomic Streamlining methods prioritized 23 of these genes as the most promising candidates. We screened and excluded 7 new genes (TGOLN2, MAT2A, ST3GAL5, BCL2L11, NCK2, UNC50, FHL2) from this region. Only 3 non-synonymous, non-disease causing variations were observed in TGOLN2 (R259W, F453L) and FHL2 (R88K). Likewise, we screened 10 genes (ACPL2, ZBTB38, RASA2, RNF7, GRK7, ATP1B3, TFDP2, GK5, XRN1 and ATR) from the GLC1C locus and identified a total of 90 DNA variations. Seven non-disease causing alterations were observed in ZBTB38 (P300A, S319A, N617D), GRK7 (E443G, P460T) and ATR (M211T, R2606Q). For the GLC1H locus, we previously screened and excluded 29 genes. In this study, a total of 10 new genes were prioritized from a list of over 61 possible candidates. Six of these genes (RPS27A, EFEMP1, USP34, PSME4, PAPLOG and RTN4) were screened and excluded. Altogether, 72 genes were screened from these 3 published POAG regions. Screening of other prioritized genes is currently in progress. The overall linkage data suggest that GLC1B, GLC1C, and GLC1H loci are physically located within regions of 6.66, 1.32 and 10.90 Mb, respectively. The strategy used in this study will facilitate gene selection for detailed mutation screening at these 3 known POAG-linked loci. Supported by EY-009947 and M01RR-06192.

983/W

The diverse phenotypic spectrum associated with fibulin-4 mutations. *A. De Paepe[1], L. Ades[2], M. Renard[1], K. Wettinck[1], B. Callewaert[1], P. Coucke[1], B. Loeys[1].* 1) Medical Genetics, Univ Hosp Gent, Belgium; 2) University of Sidney, Australia.

Heritable diseases of elastic fiber formation affect multiple organ systems and are associated with several genes coding for elastin (ELN), fibrillin-1 and -2 (FBN1/2), fibulin-5 (FBLN5), GLUT10 (SLC2A10), TGFbeta receptor 1 or 2 (TGFBR1/2). Recently, fibulin-4 (FBLN4) was linked to autosomal recessive cutis laxa. In one patient presenting with cutis laxa, multiple fractures, hernias, emphysema, generalized tortuosity with aneurysms and joint hypermobility a homozygous FBLN4 missense mutation (p.E57K) was identified. In this study, we characterize the clinical and molecular spectrum associated with FBLN4. We performed direct sequencing of FBLN4 in two cohorts of patients: group I of 15 patients with cutis laxa (negative for ELN and FBLN5) and group II of 38 patients with arterial tortuosity, stenosis and aneurysms (negative for SLC2A10, FBN1 and TGFBR). In group I, no FBLN4 mutations were found but in group II, three homozygous FBLN4 missense mutations were identified. The first mutation replaces the exact same glutamine from the EGF consensus sequence as the previously reported p.E57K but in a different cbEGF domain, whereas the others affect amino acids in the first cbEGF domain and the fibulin-type module. The latter mutations were absent in 200 control chromosomes. All patients present with a vascular phenotype but quite diverse in nature. The first proband has a history of extreme arterial tortuosity and massive aneurysms of the aorta, pulmonary artery and major abdominal arteries necessitating surgery at young age (2.5 m). She did have a smooth, velvety skin but no cutis laxa. The second patient presented with premature coronary disease at age 46, necessitating bypass surgery. At that time, a thickened aortic wall was noticed. Her history is otherwise significant for atrophic scars and keloid formation. The last patient presented with joint hypermobility, ascending aortic aneurysm and tortuosity. No other skin, lung or bone abnormalities were seen. Histology of aortic biopsies showed disrupted elastic fibers and increased deposition of glycosaminoglycans.

984/W

FXTAS: a descriptive study of premutation carriers from fragile X families. *E.G. Allen[1], J. Juncos[2], M. Rusin[1], G. Novak[1], D. Hamilton[1], L. Shubeck[1], R. Letz[3], S.L. Sherman[1].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Neurology, Wesley Woods Center, Emory University School of Medicine, Atlanta, GA; 3) Department of Behavioral Science and Health Education, Rollins School of Public Health, Emory University, Atlanta, GA.

We are conducting a study to further examine the symptoms, penetrance, and risk factors associated with the tremor/ataxia syndrome (FXTAS) among carriers of premutation alleles of the FMR1 gene. Our study population includes all siblings of premutation carrier males over the age of 50 identified through a survey of families with fragile X syndrome. We conducted a comprehensive battery of tests including a medical history, a neuropsychological test battery, and quantitative neurological assessment. Within the neurological assessment, we use a series of tests to obtain objective, quantitative measures of key features observed in FXTAS cases to date: postural or intention tremor and postural stability. We have obtained these measures on 25 control males (mean age=64.9), 56 premutation males (mean age=64.4), 18 control females (mean age=67.6) and 15 premutation females (mean age=64.1). Subjects were scored as expressing the above phenotypes if they scored greater than one standard deviation above age-adjusted standards. Male premutation carriers showed a significantly increased incidence of tremor compared to male controls (66.0% compared to 33.3%; p= 0.008). In addition, premutation carrier males showed a significantly increased frequency of ataxia compared to controls (61.1% compared to 22.2%; p=0.007). These phenotypes were not significantly increased among female premutation carriers. Among males, a significant association was seen between repeat size and VIQ and PIQ when adjusting for age at testing and education. When premutation carriers were divided into those that show motor symptoms and those that do not, only premutation carriers with motor symptoms were significantly different from controls for these IQ measures. Premutation carriers with motor symptoms also showed some deficits in visual scanning and attentional abilities.

985/W

Genotype-phenotype correlations in spinal NF. *L. Messiaen[1], T. Callens[1], J.B. Williams[1], D. Babovic-Vuksanovic[2], S. Huson[3], E. Legius[4], R. Mac Gardner[5], I. Pascual-Castroviejo[6], S. Plotkin[7], G.B. Schaefer[8], M. Wilson[9], B. Korf[1].* 1) Genetics, Univ Alabama, Birmingham, AL; 2) Medical Genetics, Mayo Clinic, Rochester, MN; 3) Medical Genetics, St Mary's Hospital, Manchester, UK; 4) Human Genetics, Catholic University Leuven, Belgium; 5) Genetic Health Services, Royal Children's Hospital, Melbourne, Vic, Australia; 6) Univ Hospital La Paz, Madrid, Spain; 7) Neurology, MGH, Boston, MA; 8) Univ Nebraska Medical Ctr, Omaha, NE; 9) Clinical Genetics, Children's Hosp Westmead, Sydney, Australia.

We studied 22 adult patients with multiple spinal dorsal root neurofibromas with or without additional massive involvement of peripheral nerve sheaths, but with very few, if any, other clinical symptoms of *NF1*. Twelve of the patients did not fulfill NIH criteria. Classic NF1-associated skin findings that lead to a diagnosis in childhood (CAL-spots and freckling) or adolescence (cutaneous neurofibromas) were typically absent. As a consequence, patients came to clinical attention in adulthood, when tumors had become symptomatic. A different *NF1* mutation was identified by comprehensive *NF1* analysis in 19/22 unrelated cases. No *NF1* mutation was found in 2 sporadic and one non-founder patient, indicating that genetic heterogeneity may exist. The mutational spectrum identified in patients with spinal NF differed highly significantly from the unbiased spectrum as characterized in our cohort of 1500 unrelated patients. In patients with spinal NF, no *NF1* total gene deletions, frameshift or nonsense mutations, accounting for 56% of mutations in our cohort, were found and an overrepresentation of missense (5/19) and splice mutations (14/19) was observed. Importantly, 5 of the splice mutations would escape detection using gDNA-based exon-by-exon screening, as they are caused by creation of a splice site several hundred bp deep into the large *NF1* introns. Two other splice mutations mimick a nonsense (W2054X) or silent mutation (Q2267Q) at the gDNA level and would get misclassified or not recognized as pathogenic in the absence of an RNA-based approach. The subtle changes in the *NF1* gene observed in this cohort of spinal NF patients may hold key insights into the tumor-suppressor roles of NF1.

986/W

Comprehensive genetic analysis of Caucasian patients with oculocutaneous albinism and autosomal recessive ocular albinism. *R. Spritz, S. Hutton.* Hum Med Gen Prog , Univ Colorado Hlth Sci Ctr, Aurora, CO.

Oculocutaneous albinism (OCA) is a group of congenital hypopigmentary disorders that can result from mutations in at least 16 different genes, four of which are associated with classical OCA. Autosomal recessive ocular albinism (AROA) is a clinically mild variant of OCA1 and OCA2. Of the four genes associated with classical OCA, at least 211 different pathologic mutations have been reported in *TYR* (OCA1), 70 in *OCA2*, 5 in *TYRP1* (OCA3), and 26 in *SLC45A2* (OCA4). Furthermore, some patients with clinical "OCA" have mutations in a Hermanksy-Pudlak syndrome (HPS) gene, *HPS1*, and others thought clinically to have HPS have mutations in *OCA2*. Thus, molecular analysis is essential for accurate diagnosis.

To establish relative prevalence of different OCA types and different gene mutations in one large group of patients, we carried out DNA sequence analysis of the *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *HPS1*, and *HPS4* genes, and *SILV* (a candidate OCA gene) in 155 unselected, unrelated non-Hispanic Caucasian patients; 118 with classical OCA and 37 with AROA. Of the OCA patients, 84 (71%) proved to have OCA1, 22 (19%) had OCA2, none had OCA3, and 7 (6%) had OCA4; 5 (4%) had no apparent pathological mutations in any of the genes studied. Of the AROA patients, 20 (54%) had pathological mutations in *TYR*, 3 (8%) had mutations in *OCA2*, 2 (5%) had novel variants in *TYRP1* (OCA3) (although these were not definitively pathological), 2 (5%) had pathological mutations in both *TYR* and *OCA2*, and 10 (27%) had no apparent pathological mutations in any of the genes studied. Overall, we found 61 different *TYR* mutations, 11 different *OCA2* mutations, 2 possible *TYRP1* mutations, and 12 different *SLC45A2* mutations. Among OCA patients, no gene mutations were common. However, of the 22 AROA patients with mutations in *TYR*, 21(95%) were compound heterozygotes for a severe OCA1 mutation and the common R402Q variant that is associated with reduced tyrosinase activity.

Our findings thus show that, among both patients with OCA and AROA, OCA1 is most frequent, with a specific type of high-risk genotype common among patients with AROA.

987/W

Molecular pathogenesis of isolated multiple cutaneous neurofibromas in segmental NF. *J.B. Williams[1], O. Maertens[2], T. Callens[1], B. Yuan[1], A. Carroll[1], F. Mikhail[1], A. Theos[3], B. Korf[1], L. Messiaen[1].* 1) Genetics, UAB, Birmingham, AL; 2) Medical Genetics, Ghent University Hospital, Belgium; 3) Dermatology, UAB, Birmingham, AL.

The large size of the *NF1* gene and its many pseudogenes, the complex interactions between cell types and the *NF1* haploinsufficient state of all cells in the body complicate the elucidation of the biological framework underlying the development of the wide range of neurofibromatosis type 1 (NF1)-related symptoms and complications. Determining the timing during development and the cell-type disturbed by the constitutive and somatic *NF1* mutations will be critical for understanding the phenotype in NF1 and its alternate forms. We and others have recently shown that a second *NF1* hit in specific cell types within the lesion is associated with CAL-spots, neurofibromas, gastrointestinal tumors, JMML and tibial dysplasia. We studied a 50-yo woman with a segmental phenotype consisting of ~50 cutaneous neurofibromas (diameter 3-7mm) restricted to the R-ear, cheek and neck in the absence of any other signs of disease. No *NF1* mutation was found in her blood lymphocytes. Accurate diagnosis of segmental NF was established through *NF1* comprehensive mutation analysis on various cell types cultured from 8 cutaneous neurofibromas. An identical first hit mutation (*NF1* total gene deletion, type 1) as well as a different second hit mutation was present in all 8 Schwann cell (SC) cultures, selectively cultured in the absence of forskolin ($SC^{-/-}$). Interestingly, the neurofibromas arose within the background of predominantly *NF1* wild-type cells: the total gene deletion was absent in the fibroblasts and only present in ~10% of the Schwann cells from the $SC^{+/-}$ cultures, indicating the abundance of $NF1^{+/+}$ cells in the neurofibroma environment. The data indicate that the clinical phenotype of segmental NF in this patient is due to a post-zygotic first-hit *NF1* mutation in a neural-crest derived SC precursor, followed by different *NF1* second hit mutations at a later point in time/development. The strategy proposed allows a more accurate diagnosis and genetic counseling in patients with segmental and/or mosaic *NF1*.

988/W

Comprehensive evaluation of the Bardet-Biedl syndrome type 1 (BBS1) M390R mouse model. *R.E. Davis[1], K. Rahmouni[1], K. Agassandian[1], R. Swiderski[1,2], R.F. Mullins[1], A.R. Philp[1,2], C.C. Searby[1,2], D.Y. Nishimura[1,2], M.P. Andrews[1,2], B. Yang[1], M.D. Cassell[1], E.M. Stone[1,2], V.C. Sheffield[1,2].* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder resulting in obesity, retinopathy, polydactyly, cognitive impairment, congenital heart defects, as well as renal and reproductive tract abnormalities. Patients with this disorder exhibit variable expressivity. Twelve BBS genes have been identified to date. The most frequent BBS variation, the *BBS1* M390R missense mutation, is implicated in ~20% of cases. Our laboratory has developed a *Bbs1* M390R knock-in mouse model. $Bbs1^{M390R/M390R}$ mice manifest cardinal features of the human phenotype including photoreceptor cell death, male infertility and obesity. Here we demonstrate additional aspects of the $Bbs1^{M390R/M390R}$ mouse phenotype including olfactory deficits, social dominance defects and ventriculomegaly. We found that obesity in $Bbs1^{M390R/M390R}$ mice is associated with hyperphagia, decreased locomotor activity and high circulating levels of the adipocyte-derived hormone leptin. To assess the relative contributions of hyperphagia and decreased energy expenditure to the obesity associated with the *Bbs1* M390R mutation, we performed a pair-feeding study and found that hyperphagia contributes significantly to the obesity of $Bbs1^{M390R/M390R}$ mice. We also found that the hyperleptinemia observed in $Bbs1^{M390R/M390R}$ mice is associated with leptin resistance, as systemic and direct central neural injection of leptin failed to decrease body weight and food intake in $Bbs1^{M390R/M390R}$ mice. Furthermore, evaluation of $Bbs1^{M390R/M390R}$ brains using transmission electron microscopy shows abnormalities in ependymal cell motile (9+2) cilia and primary (9+0) cilia of the hypothalamus. These data verify the efficacy of the $Bbs1^{M390R/M390R}$ knock-in mouse model for further elucidation of the BBS obesity phenotype and suggest a connection between obesity and cilia abnormalities in the brain.

989/W

Further delineation of regions of dosage imbalance in rearrangements of 1p36: patient with choanal atresia, cataracts, severe mental retardation. *E. Chen[1,2], X. Li[1], E. Obolensky[2].* 1) Kaiser Permanente, San Jose, CA; 2) and Oakland, CA.

The 1p36 deletion syndrome is recognized as the most common terminal deletion syndrome. However, few cases of 1p36 duplications have been reported and genotype-phenotype correlations are emerging. One case of isolated duplication 1p36.3 has been associated with growth delay, metopic synostosis, blepharophimosis, ASD, rectal stenosis, 2-3 toe syndactylies, mild delays. It has been proposed that a deleted chromosome 1 undergoes multiple breakage-fusion-bridge cycles and inversions to produce a chromosome arm with a complex rearrangement. A region of overlap is thought that, when triplicated is associated with craniosynostosis, and when deleted is associated with large, late-closing anterior fontanels. Overexpression or haploinsufficiency of MMP 23A and B genes has been proposed as possible candidate genes. We describe a male with an apparently de novo cytogenetically visible duplication of 1p36.31p36.33. FISH studies show that the critical region for the 1p36 deletion syndrome (p58) is duplicated. D1Z2 and TEL1p probes are also duplicated. He has congenital cataracts, blepharophimosis, choanal atresia, dysmorphisms, transient hypogammaglobulinemia, severe growth and developmental delays, but no craniosynostosis. Targeted BAC aCGH (Signature Genomics) detected a duplication and a deletion in the 1p36.3 region. BAC whole genome array (UCSF, 1.4 Mb resolution) detected an additional duplication in the 1p36.11-p36.33 region. Oligonucleotide aCGH (Agilent Technologies, 44K) further defined three regions of rearrangements: a duplication of distal 1p36.32-p36.33, a deletion of adjacent 1p36.32p36.32, and an 11 Mb duplication of proximal 1p35.3-p36.21. The distal duplication region contains the putative gene for epilepsy, KCNAB2, and the MMP 23A and B genes, whereas the proximally duplicated region contains putative genes for congenital cataracts. This is the first patient with choanal atresia, transient hypogammaglobulinemia of infancy, and congenital cataracts associated with 1p36 duplication/deletion. Comparison of our data with other studies will provide insights into genotype-phenotype correlations, gene dosage, and positional effects.

990/W

Molecular analysis of the CYP1B1 gene in congenital glaucoma Brazilian patients. *M.B. Melo[1,3], M.D. Paolera[2], C. Caixeta-Umbelino[2], N. Kasahara[2], M.N. Rocha[3], F. Richeti[3], C.A. Longui[3], G.V. Almeida[2], R. Cohen[2], C. Mandia Jr.[2], V.P. Costa[4], J.P. Vasconcellos[4].* 1) CBMEG, University of Campinas, Campinas, São Paulo, Brazil; 2) Department of Ophthalmology, Santa Casa de São Paulo, São Paulo, Brazil; 3) Laboratory of Molecular Medicine, Santa Casa de São Paulo, São Paulo, Brazil; 4) Department of Ophthalmology, University of Campinas, São Paulo, Brazil.

Purpose. Primary congenital glaucoma (PCG) is a severe form of glaucoma, which has its onset from neonatal period to three years of age and when hereditary is transmitted as an autosomal recessive trait with variable penetrance. Three loci have been described, but only one gene was identified, CYP1B1, located in the GLC3A locus, on chromosome 2p21. The aim of this study was to screen PCG Brazilian patients and their parents for mutations in the CYP1B1 gene. Methods. Thirty three PCG patients from 30 different families were evaluated through direct sequencing of the CYP1B1 gene coding regions and intron/exon boundaries. Results. Mutations were detected in 9 of 30 unrelated patients, a prevalence of 30%. Ten different mutations were observed, three of which, to our knowledge, are being reported for the first time. A deletion at exon 2, 4635delT, that leads to a stop codon at aminoacid 277 was observed in two unrelated patients. In three brothers (two twins), two other new alterations were described in heterozygosity, 4523delC in exon 2, leading to a stop codon at aminoacid 243 and a T to A point mutation in exon 3, at position 7970, changing a leucine for a glutamin (L378Q). Four patients were compound heterozygous, 2 were homozygous and in three only one mutation was detected. The previously reported polymorphisms 3793T to C, R48G, A119S, L432V, D449D and N453S were also identified in our patients. Conclusions. This work reports a 30% prevalence of CYP1B1 mutations in PCG Brazilian patients, describing three new different alterations related to the disease. Taken together, the two studies involving Brazilian patients reflect the genetic heterogeneity of the disease in this population and open possibilities to further analysis in other candidate loci.

991/W

Identification of four novel mutations determined EDA gene as one of the major defects for sporadic non-syndromic oligodontia. *S-J. Song[1,2], D. Han[2], M. Yan[1,2], H-L. Feng[2], N. Zhong[1,2,3].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Peking University Health Science Center, Beijing, China; 3) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Recently mutations in the EDA gene have been shown to result in non-syndromic hypodontia in two families inherited in an X-linked recessive manner. It is noteworthy that all affected males exhibited oligodontia (congenital absence of six or more permanent teeth, third molars excluded) in these families. To determine the prevalence of EDA mutations in sporadic non-syndromic oligodontia, we investigated 14 unrelated male probands and their familial members. Mutation screening of the EDA gene was performed by direct sequencing of eight PCR fragments, which spun the entire exons and intron-exon junctions with more than 100 bp. Analyses of the complete coding region of the EDA gene identified four novel missense mutations, Ala259Glu, Arg289Cys, Arg334His and Thr338Met. These mutations account for 37% (5 out 14) of probands we studied, indicating that EDA is a major gene involved in genetic defects of sporadic congenital oligodontia. Our results provided evidence that for non-syndromic sporadic oligodontia, genetic defects in EDA gene should be considered in order to facilitate clinical diagnosis and genetic counseling.

992/W

Integrating representation of variation at NCBI: RefSeqGene, OMIM, GeneReviews, dbGaP and dbSNP. *D. Maglott, J. Paschall, L. Phan, G. Yu, Y. Shao, L. Forman, K. Pruitt, M. Feolo, S. Sherry.* Natl Ctr Biotechnology Info, NIH/NLM, Bethesda, MD.

Integrating and reporting information about molecular variation is a critically important task advancing understanding of biomedical data for researchers, clinicians and patients. As the number of research and clinical tests expands, so too does the need to make it easy to determine (1) whether information about a specific variant has been reported previously, (2) how often a variant has been identified, (3) in what populations or genetic backgrounds a variant has been observed, (4) where public information can be found about a variant, (5) laboratories known to test for a variant, and (6) current understanding of a variant's clinical significance. One step to facilitate this process is the generation of stable, gene-specific genomic reference sequences, termed RefSeqGene. Having a single, well-defined genomic coordinate system as a reference standard, independent of chromosome re-assemblies, provides a common currency for inter-group communication about the sequences being tested, especially when a gene has multiple splice variants or frequent mutations in non-transcribed regions. RefSeqGene accessions (format NG_000000.0) are being established in collaboration with gene-specific authorities to complement the existing RefSeq mRNAs so often used to report mutations. These sequences are being used within NCBI to increase explicit connections among multiple resources including dbGaP, dbSNP, Entrez Gene, GeneReviews, OMIM, and PubMed.

This presentation will summarize current progress in improving access to gene-specific variation data at NCBI. New tools to facilitate data submission, new viewers, and new mutation reports will be reviewed.

993/W

Molecular alteration in exon 28 of VWF gene from Patients with von Willebrand Disease. *R. Peñaloza[1], H. Benitez[2], V. Rojas[1], F. Salamanca[1].* 1) UIM Genetica Humana, Instituto Mexicano del Seguro Social, Mexico City; 2) Servicio de Genética, Hospital de Pediatría, CMN SXXI, IMSS.

von Willebrand disease (VWD) is the most frequent inherited coagulopathy in humans, it is expressed as mucocutaneous bleeding of variable intensity. The origin is due an alteration in VWF gene (mutations), that produces changes in the multimerizable protein. It normal function is to induce platelet adhesion to vascular endothelium when tissue damage is present, and carry and protect factor VIII in serum. The aim of the present work was to analyze the molecular alterations in exon 28 of VWF gene from ten families with VWD. METHODS: DNA from peripheral blood was obtained by standard methods, previous informed consent. Exon 28 of VWF was amplified by PCR method, purified and directly sequenced, after labeled by Big Dye (Applied Biosystem, USA). RESULTS. We found alterations in five families, four mutations have been previously informed in other populations, and a new mutation (insT3706) was found in a patient and her mother. This alteration produces a modified protein, shorter than normal. CONCLUSION. It is important to realize the molecular study in order to establish a correct diagnosis, treatment and rightful genetic counseling.

994/W

***FOXC1*/*PITX2* mutations and copy number changes in a Belgian-Dutch cohort of patients with Axenfeld-Rieger malformations.** *B.N. D'haene[1], F. Meire[2], T. de Ravel[3], I. Casteels[4], B.P. Leroy[1,5], P. Kestelyn[3], A.S. Plomp[6], M. Joosten[7], A. De Paepe[1], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University Hospital, Belgium; 2) HUDERF, Brussels, Belgium; 3) Center for Human Genetics, Catholic Leuven University, Belgium; 4) Dept of Ophthalmology, Catholic Leuven University, Belgium; 5) Dept of Ophthalmology, Ghent University Hospital, Belgium; 6) Dept of Medical Genetics, AMC, Amsterdam, The Netherlands; 7) Clinical Genetics, University Rotterdam, The Netherlands.

Axenfeld-Rieger (AR) malformations comprise a spectrum of rare autosomal dominant congenital structural malformations of the anterior eye segment. A primary purpose of this study was to determine the prevalence of disease-causing *FOXC1*/*PITX2* mutations and copy number changes in a Belgian-Dutch cohort of patients with AR malformations. A second goal was to evaluate the contribution of three candidate genes: *FIBULIN-4*, *P32* and *FOXC2*. Thirty-six probands with AR, mainly of Belgian-Dutch origin, were examined for copy number changes of *FOXC1*/*PITX2* with MLPA and screened for subtle *FOXC1*/*PITX2* mutations by sequencing. Array CGH with a tiling BAC array for the *FOXC1* region was carried out for patients with a known *FOXC1* deletion and for all patients without identified mutation. In addition mutation screening of *FIBULIN-4*, *P32* and *FOXC2* was performed in the latter group. This revealed no disease-causing mutations so far. In conclusion, a disease-causing genetic defect was found in 44% of the probands in this study. The majority of these are *FOXC1* mutations (69%) and one third *PITX2* mutations (31%). Thirty-eight percent of these defects are genomic rearrangements: 5 *FOXC1* deletions and 1 *PITX2* deletion. Our data sustain a major role of the *FOXC1*/*PITX2* genes in the molecular pathogenesis of the AR spectrum in the Belgian-Dutch population. Screening of other candidate genes is currently ongoing.

995/W

Deletions of Tenascin-X are associated with joint hypermobility and features of Ehlers-Danlos syndromes in a cohort of Congenital Adrenal Hyperplasia (CAH) patients. *N.B. McDonnell[1], W. Chen[1], B.F. Griswold[1], A.C.M. Smith[3], M. Berk[2], C. VanRyzin[2], D. Merke[2].* 1) LCI, NIA/NIH, Baltimore, MD; 2) NICHD/NIH, Bethesda, MD; 3) NHGRI/NIH, Bethesda, MD.

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is thought to be the most common autosomal recessive disorder, leading to cortisol deficiency, with or without aldosterone deficiency (salt wasting), and androgen excess (virilization). The gene encoding 21-hydroxylase, CYP21A2, is mapped to the short arm of chromosome 6 within the HLA complex, in a region of high gene density with multiple pseudogenes. This region, termed the RCCX module, has tandem repeat sequences that promote misalignment during meiosis leading to gene rearrangements, deletions and gene conversion events. Flanking CYP21A2 is the gene encoding tenascin-X (TNX), an extracellular matrix protein that is highly expressed in connective tissue. Homozygous TNX deficiency has been proposed as a cause of autosomal recessive form of Ehlers-Danlos syndrome (EDS) characterized by hypermobile joints, stretchy skin and easy bruising and hypermobility type EDS has been linked to heterozygosity for TNX mutations. A high incidence of joint hypermobility was noted in a large cohort of CAH patients with genetically confirmed CAH due to 21-hydroxylase deficiency seen at the National Institute of Child Health and Development. Molecular investigations of the RCXX module were initiated to analyze the presence of TNX deletions and gene conversion events, utilizing Southern blotting and PCR approaches. Out of 96 probands with CAH and 83 family members, 12 subjects were found to be heterozygous for a non functional TNX gene conversion product resulting from a 30 kb deletion. All of these persons also had deletions of the CYP21A2 gene. Results suggest that 6-7% of persons affected with CAH may also have TNX deletions leading to joint abnormalities. CYP21A2 deletions were detected in 30% of the chromosomes in the cohort, and amongst those subjects, 11% also had a TNX deletion. Further studies are underway to better define the clinical, molecular and biochemical aspects of this novel CAH-TNX (CAH-X) Contiguous Gene Deletion Syndrome.

996/W

Genotype/Phenotype Correlations in Vascular Ehlers-Danlos Syndrome. *J. Yang[1], B.F. Griswold[1], W. Chen[1], C.A. Francomano[2], N.B. McDonnell[1].* 1) LCI, NIA/NIH, Baltimore, MD; 2) GBMC, Baltimore, MD.

Vascular Ehlers-Danlos syndrome is a hereditary disorder of connective tissue caused by mutations in COL3A1, encoding procollagen III. The clinical features include skin fragility, easy bruising and bleeding, joint hypermobility, bowel or uterine rupture, arterial dissections and aneurysms. The life expectancy is significantly reduced. Analysis of phenotype/genotype correlations with COL3A1 mutations can be helpful for anticipatory guidance and counseling for affected families. Thirteen affected families were identified at the National Institutes of Health, and COL3A1 gene was sequenced. The mutations identified included substitution mutations leading to the critical glycine residues in the collagen helix, splice site mutations, small deletions that disrupt the alignment of collagen trimers, as well as premature termination codons that lead to haploinsufficiency. The exon skipping mutations were associated with severe skin features and scarring, while the haploinsufficency mutations were found to lead to a relatively milder phenotype with later age of onset of complications. Glycine substitution mutations were associated with a higher incidence of aneurysms and variable skin features. Maternal death due to uterine rupture had not occurred in the probands or affected family members in our cohort.

997/W

Mutations in the noncoding regions of *ACVRL1* and *ENG* in Hereditary Hemorrhagic Telangiectasia. *K. Damjanovich[1], H. Escobar[2], J. McDonald[1,3], F. Gedge[1], L.-S. Chou[1], P. Bayrak-Toydemir[1,4].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) DNA Sequencing and Genomics Core Facility, University of Utah, Salt Lake City, UT; 3) Dept. of Radiology, University of Utah, Salt Lake City, UT; 4) Dept. of Pathology, University of Utah, Salt Lake City, UT.

Hereditary Hemorrhagic Telangiectasia (HHT) is a vascular dysplasia characterized by arteriovenous malformations and telangiectasia. The majority of the HHT cases are caused by mutations in the coding regions of activin A receptor type II-like (*ACVRL1*) and endoglin (*ENG*) genes. However, approximately 20% of the HHT cases do not have mutations in the coding regions of either gene that can be detected by sequencing or deletion/duplication testing by multiplex ligation probe amplification (MLPA). We had 28 HHT patients whom we were unable to find the causative mutations in the coding regions of *ACVRL1* and *ENG*. Locus specific linkage analysis with the families of two of these patients suggested linkage to *ACVRL1* locus and one family had linkage to the *ENG* locus. This prompted us to screen for the noncoding regions of the HHT genes in our cohort. We sequenced the 5' and 3' untranslated regions (UTRs) and introns of *ACVRL1* and *ENG*. Based on studies in mice we selected critical regions of the 5' UTR of both genes. We sequenced about 9kb from the 5'UTR region, two 600 bp segments, up to 8 kb upstream of the *ENG*, and the entire 3' UTR of both genes. We sequenced all the introns of both genes. For the 4 large introns in *ENG*, we used a mutation screening protocol based on heteroduplex formation followed by targeted sequencing. For unreported sequence variants found, we sequenced the available family members to determine the segregation with the disease or 100 chromosomes from healthy individuals. We will report our findings and discuss the role of noncoding region mutations of *ENG* and *ACVRL1* genes in HHT. Some of these mutations are candidates for future studies to explore their disease causing potential. In addition, by determining the sequences of critical importance, our results will enhance our knowledge of the normal *ACVRL1* and *ENG* function.

998/W

Glycine N-methyltransferase-/- mice develop chronic hepatitis and glycogen storage disease in liver. *Y.M. Chen[1], S.P. Liu[1], Y.S. Li[1], Y.J. Chen[2], E.P. Chiang[3], A. Li[4], Y.H. Lee[5], T.F. Tsai[2], M. Hsiao[6].* 1) Division of Preventive Medicine, Institute of Public Health, School of Medicine, National Yang-Ming University, Taipei 112, Taiwan; 2) Faculty of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei 112, Taiwan; 3) Department of Food Science and Biotechnology, National Chung Hsing University, Taichung 402, Taiwan; 4) Department of Pathology, Veterans General Hospital, Taipei 112, Taiwan; 5) Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan; 6) Genetics Research Center, Academia Sinica, Taipei 115, Taiwan.

Glycine N-methyltransferase (GNMT) affects genetic stability by regulating DNA methylation and interacting with environmental carcinogens. To establish a Gnmt knockout mouse model, two lambda phage clones containing a mouse Gnmt genome were isolated. At 11 weeks of age, Gnmt-/- mice had hepatomegaly, hypermethioninaemia, and significantly higher levels of both serum alanine aminotransferase and hepatic S-adenosylmethionine. Such phenotypes mimic patients with congenital GNMT deficiency. Real-time PCR analysis of 10 genes in the one carbon metabolism pathway revealed that 5,10-methylenetetrahydrofolate reductase, S-adenosylhomocysteine hydrolase (Ahcy), and formiminotransferase cyclodeaminase (Ftcd) were significantly down-regulated in Gnmt-/- mice. Results from pathological examinations indicate that 57.1% (8/14) of the Gnmt-/- mice had glycogen storage disease (GSD) in their livers. Focal necrosis was observed in male Gnmt-/- livers, while degenerative changes were found in the intermediate zones of female Gnmt-/- livers. In addition, hypoglycemia, increased serum cholesterol, and significantly lower numbers of white blood cells, neutrophils, and monocytes were observed in the Gnmt-/- mice. Real-time PCR analysis of genes involved in the gluconeogenesis pathways revealed that the following genes were significantly down-regulated in Gnmt-/- mice: fructose 1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphate transporter (G6PT). The Gnmt KO mice model is useful for studies of the pathogenesis of congenital GNMT deficiency, the role of GNMT in GSD as well as in liver tumorigenesis.

999/W

Shwachman-Diamond syndrome - a Human *Minute*? *S. Zhang[1,2], G. Otulakowski[3], J. Zhong[2], O. Gan[4], J. Yuan[5], C. Guidos[5], J.E. Dick[1,4], J.M. Rommens[1,2].* 1) Dept of Molecular & Med Genetics, Univ of Toronto; 2) Prog in Genetics & Genome Biol, Hosp Sick Children; 3) Prog in Physiology & Experimental Medicine, Hosp Sick Children; 4) Div of Cell & Molecular Biol, University Health Network; 5) Dept of Immunology, Univ of Toronto; Prog in Developmental & Stem Cell Biol, Hosp Sick Children, Toronto, ON Canada.

Shwachman-Diamond syndrome is a multi-system disorder caused by mutations in *SBDS*. Clinical features include failure to thrive, exocrine pancreatic dysfunction as well as haematological and skeletal abnormalities. Patients that carry two early truncating alleles have not been described and mice that are homozygous for null alleles ($Sbds^{-/-}$) exhibit embryonic lethality prior to E6.5. We have generated a R126T missense disease allele on the prediction that the mutation is hypomorphic in nature. $Sbds^{R126T/wt}$ mice were found to develop normally and show no disease phenotypes, in accordance with the recessive inheritance of SDS. However, both $Sbds^{R126T/R126T}$ and $Sbds^{R126T/-}$ mice exhibit marked size reduction, and die at birth. The growth difference becomes apparent in the mid-fetal period with noted delay or abnormalities of major organs including the skeleton, brain and lung. Hematopoiesis is also disturbed. Comparable deficiencies were noted overall, but the $Sbds^{R126T/-}$ embryos were consistently more severely affected than $Sbds^{R126T/R126T}$ embryos. Investigations of mouse embryonic fibroblasts indicated an impairment of protein translation capacity in mutant cells, as well as slow growth and cell cycle defects. Polysome profiles generated by sucrose gradient centrifugation of cell extracts were also abnormal, exhibiting marked reductions in 80S peaks as well as an increase in the ratio of 40S to 60S subunit peaks. These cellular deficiencies together with the developmental delay and poor growth emphasize the severe consequences of loss of Sbds. These findngs are reminiscent of the classic *Minute* mutations that have been described in *Drosophila* and indicate that SDS is a translation insufficiency syndrome.

1000/W

Novel mouse model for nonsense mutation bypass therapy shows geneticin generates a dramatic multi-day response. *C. Yang[1], J. Feng[1], W. Song[1], J. Wang[1], B. Tsai[1], Y. Zhang[1], K. Hill[1,4], P. Margaritis[2], K. High[2,3], S. Sommer[1].* 1) Beckman Res Inst, Molec Gen, City of Hope, Duarte, CA; 2) Department of Pediatrics, University of Pennsylvania School of Medicine and Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; 3) Howard Hughes Medical Institute, Philadelphia, PA, USA; 4) Department of Biology, the University of Western Ontario, London Ontario Canada N6A 5B7.

Aminoglycosides can suppress nonsense mutations and are the prototypic agents for translational bypass therapy (TBT). Initial results demonstrate the need for more potent drugs and for an in vivo model system for quantitative assessment of successful translational bypass. Herein, we devise an in vivo system for quantitating TBT in the presence and absence of nonsense-mediated decay and we use the system to show that treatment with geneticin can elicit a multi-day in vivo response. Application of the system reveals that geneticin is much more efficacious in vivo than gentamicin. After two doses of geneticin, residual factor IX (F.IX) antigen can be detected after three weeks. These data demonstrate the utility of the mouse system for evaluating nonsense suppressors in vivo. In addition, this model may be helpful for testing inhibitors of nonsense-mediated decay, as a combination of geneticin and a decay inhibitor therapy may produce a therapeutic response in the R29X models. Furthermore, geneticin, its metabolites or better tolerated analogues should be evaluated as a general multi-day treatment for severe genetic disease due to nonsense mutation.

1001/W

GJB2 mutation study in Korean patients with hearing loss. *H. Kim[1], Y.H. Choung[2], J.A. Yang[1], J.H. Hong[1], S.Y. Jeong[1].* 1) Dept. of Medical Genetics, Ajou Univ. Sch. of Medicine, Suwon, Korea; 2) Dept.of Otolaryngology, Ajou Univ. Sch. of Medicine, Suwon, Korea.

Congenital hearing loss occurs in approximately 1 in 1,000 live births, over 50% of these cases being hereditary. About 70-80% of genetic deafness is nonsyndromic: autosomal-recessive (80%), autosomal-dominant (20%), X-linked (1%), and mitochondrial (<1%). Mutations in the GJB2 gene, which has a single coding exon encoding for the gap-junction protein connexin-26, a member of the large family of connexins, are the major cause of nonsyndromic autosomal-recessive (DFNB1) and sporadic deafness (as much as 50% of such cases in many populations). GJB2 mutations also cause dominant nonsyndromic sensorineural hearing loss (DFNA3). Several heterozygous GJB2 mutations located in a particular domain of the protein (first extracellular domain) have been reported to segregate with autosomal-dominant hearing loss in a small number of families. Mutations in the GJB2 gene are also responsible for syndromic forms of hearing loss. In this study, we screened total 48 unrelated Korean patients with congenital nonsyndromic (46 cases) and syndromic (2 cases) hearing loss for GJB2 mutation. All subjects were diagnosed to have hearing loss at the Otolaryngology clinic in Ajou University Hospital. Three different mutations, p.E47K, p.T123N, and c.235delC, and three polymorphisms, p.V27I, p.E114G, and p.I203T, were found in the 23 nonsyndromic patients. The p.V27I allele has been reported to be a just sequence variant that is not responsible for hearing loss. But, we found that 20 patients out of 46 cases had this allele with (14 cases) or without the p.E114G allele. These findings suggest that the p.V27I allele in the GJB2 gene may involve in the phenotype of hearing loss. Two syndromic autosomal dominant hearing loss patients with palmoplantar keratoderma (PPK) and keratitis-ichtyosis-deafness syndrome (KID) were revealed to have the GJB2 mutations, p.D50N and p.R75W, respectively. Further study including pedigree analysis with familial mutation study will contribute to a better understanding the genetic and phenotypic heterogeneities of GJB2 mutations in Korean patients with hearing loss.

1002/W

Clinical and molecular genetics of Leber's congenital amaurosis (LCA): a multi-center study of Italian patients. *S. Banfi[1], C. Ziviello[1,2], F. Testa[3], S. Rossi[3], S. Signorini[4], S. Galantuomo[5], E.M. Valente[6,7], E. Rinaldi[3], F. Simonelli[3].* 1) Telethon Institute of Genetics and Medicine (TIGEM), Naples; 2) Institute of Genetics and Biophysics "A. Buzzati-Traverso", CNR, Naples; 3) Department of Ophthalmology, Second University of Naples, Naples; 4) Department of Child Neurology and Psychiatry of the IRCCS C. Mondino Foundation, Pavia; 5) Department of Ophthalmology University of Cagliari, Cagliari; 6) IRCCS CSS-Mendel Institute, Rome; 7) Department of Medical and Surgical Pediatric Sciences, University of Messina, Messina, Italy.

Leber's congenital amaurosis (LCA) is a group of hereditary retinal dystrophies characterized by severe loss of visual function early in life. We investigated the molecular basis of Leber's congenital amaurosis (LCA) in a cohort of Italian patients and we performed genotype-phenotype analysis. Overall, we carried out mutation analysis, by using an integrated approach including microarray and sequencing analyses, in 95 patients for nine LCA genes. Disease-causing mutations were identified in 28% of patients and twelve novel mutations were identified. Mutations occurred more frequently in the RPE65 (8.4%), CRB1 (7.4%), and GUCY2D (5.2%) genes. Mutations in CEP290 were found in only 4.2% of the patients analyzed. Overall, we found that RPE65 gene mutations represent a significant cause of LCA in the Italian population while GUCY2D and CEP290 mutations have a lower frequency in the Italian population, as compared to other reports. These findings confirm that the genetic epidemiology of LCA in Italy, similar to what has been observed for other forms of inherited retinal degenerations, is different from what reported in the U.S. and in other Northern European countries.

1003/W

Genetic Studies in a Colombian Family with Familial Exudative Vitreoretinopathy or Criswick-Schepens Disease. *N. Gelvez[1], J. Montoya[1], C. Varón[2], M. Gómez[2], J. Gómez[2], M. Jaramillo[2], M.L. Tamayo[1].* 1) Inst Genetica Humana, Univ Javeriana, Bogota, 1, Colombia; 2) Fundación Oftalmológica de Santander, Clínica Ardila Lulle (FOSCAL). Bucaramanga, Santander. Colombia.

The Familiar exudative vitreoretinopaty (FEVR) or Criswick-Schepens Disease is a genetic disorder of retinal vessel. The features of the disease can be variable even within the same family. It is bilateral, asymmetric and progressive with variable inheritance, being the Autosomal Dominant the most common. A complete ocular examination was practiced on 32 individuals belonging to a family with diagnosis of FEVR. After informed consent, DNA sample was taken for DNA extraction and the coding region of FZD4 gene was sequenced. Eleven individuals were defined as affected and the other 21 as non-affected. Autosomal Dominant inheritance was confirmed and the "1501delCT" mutation in the FZD4 gene was identified in all affected individuals. We confirmed the hypothesis that some non-affected relatives did not present partial manifestations of the disease. The findings of Angiography, Optical Coherent Tomography (OCT) and ocular ecography showed the peripheral retinal avascularization. We defined an Autosomal Dominant inheritance and the causal mutation in the FZD4 gene in this family. The molecular characterization of this family allows us to practice a complete genetic counseling in all evaluated individuals.

1004/W

Transgenic Mice for 4 bp Deletion Mutation in DLX3 Gene Cause Severe Taurodontism in Tooth Development. *T.C. Hart[1], S.K. Lee[1], J.Q. Feng[2], S.J. Choi[1].* 1) NIDCR, NIH, Bethesda, MD; 2) University of Missouri, Kansas City, MO.

Tricho-dento-osseous (TDO) syndrome is an autosomal dominant disorder characterized by anomalies in hair, tooth, and bone development. A 4 base-pair deletion mutation in the Distal-Less 3 (DLX3) gene is etiologic for the condition. To investigate the in vivo effect of mutant DLX3 (MT-DLX) on tooth development in TDO, we established transgenic (TG) mice expressing MT-DLX3 driven by a mouse 2.3 k bp type I collagen promoter. TG mice were fertile and body lengths were not significantly changed, however, body weights were reduced about 30% from age 6 weeks compared to wild-type littermates. Incisors in MT-DLX3 TG mice were yellowish and misshapen. High-resolution radiography of incisors and molars revealed an enlarged pulp chamber in the molars and incisors of MT-DLX3 TG mice, consistent with clinical findings in patients with TDO. Histochemical studies of teeth demonstrated that the polarization of odontoblasts in both molars and incisors were markedly disrupted in MT-DLX3 TG mice and dentin matrix mineralization was markedly reduced. Immunohistochemical studies revealed that differentiating odontoblasts, which were type I collagen positive, expressed both WT-DLX3 and MT-DLX3 protein. However, biglycan, decorin, and dentin sialophosphoprotein expression were dramatically restricted in the pre-dentine zone in MT-DLX3 TG mice. Backscatter and resin casted scanning electron microscopy demonstrated that dentinal tubule formation was totally ablated in MT-DLX3 TG mice. In vitro studies also revealed that the transduction of MT-DLX3 into odontoblastic MDPC-23 cells totally inhibited calcium deposition on the culture plate compared to those of WT-DLX3 or EV transduced cells, while the growth rate of those cells were not significantly different. These findings demonstrate that MT-DLX3 inhibits the dentin matrix mineralization in tooth development and causes severe taurodontism consistent with what is clinically seen in patients with TDO syndrome. This mice model mimicking TDO syndrome could be a valuable tool for the investigation of odontoblast biology in dentin mineralization.

1005/W

Genetic Analysis of Syndromic X-Linked Microphthalmia. *J.J. Johnston[1], E. Hilton[2,3], V. Kimonis[4], C. Schwartz[5], G.C.M. Black[2,3], L.G. Biesecker[1].* 1) NHGRI, NIH, Bethesda, MD; 2) St. Mary's Hospital, Manchester, UK; 3) Manchester Royal Eye Hospital, Manchester, UK; 4) Harvard Medical School, Boston, MA; 5) Greenwood Genetics Center, Greenwood, SC.

Lenz microphthalmia is inherited in an X-linked pattern and comprises microphthalmia, mental retardation (MR), skeletal and other anomalies. This disorder has been mapped to two loci, MCOPS1 (microphthalmia with associated anomalies) at Xq27-q28 and MCOPS2 at Xp11.4. A single mutation in the BCL-6 interacting corepressor, BCOR, on chromosome Xp11.4, was identified in the family used to map the MCOPS2 locus. Mutations in BCOR have also been identified in Oculofaciocardiodental syndrome (OFCD). OFCD is inherited in an X-linked pattern with apparent male lethality and comprises microphthalmia, congenital cataracts, radiculomegaly, and cardiac and digital abnormalities. Initial studies show BCOR to be the major gene for OFCD. We have continued to screen additional patients with Lenz (2), OFCD (10), microphthalmia with or without MR (7), and X-linked MR with eye abnormalities (25) to better understand the contribution of BCOR mutations to these phenotypes, and in the case of Lenz syndrome, to identify families that map to the MCOPS1 locus. Nine out of ten patients with OFCD have had loss of function mutations in BCOR and no mutations have been identified in individuals with non-Lenz microphthalmia or in those with X-linked MR with eye anomalies. The identical substitution found in the original MCOPS2 family, p.P85L, was identified in the Lenz syndrome proband from a second family. The other proband with Lenz syndrome did not have a mutation in BCOR and the family is currently being evaluated for linkage to the MCOPS1 locus. We hypothesize that this family maps to Xq27-q28 and we will incorporate their data into our current efforts to refine the mapping of MCOPS1 in two previously reported families. In summary, loss of function mutations that affect BCOR cause OFCD as demonstrated by the mutations identified in affected individuals. Furthermore, it appears that while alterations in BCOR may contribute to Lenz syndrome, they do not appear to contribute to non-Lenz microphthalmia or X-linked MR.

1006/W

Novel MFN2 mutations and phenotypic variability in patients with Charcot-Marie-Tooth disease type 2A. *M. Muglia[1], F. Boaretto[2], A. Martinuzzi[3], G. Vazza[2], L. Piva[2], A. Vettori[2], A. Patitucci[1], C. Bertolin[2], G. Siciliano[4], A. Quattrone[1,5], M.L. Mostacciuolo[2].* 1) ISN-CNR, Mangone Cosenza, Italy; 2) Department of Biology, University of Padova, Italy; 3) IRCCS E. Medea, Conegliano Research Center, Italy; 4) Dept. of Neuroscience, Neurological clinic, University of Pisa, Italy; 5) Department of Neurology, University Magna Graecia, Catanzaro, Italy.

Mutations in the MFN2 gene have been reported as the primary cause of Charcot-Marie-Tooth disease type 2. In our study we intend to better characterize the divergence of phenotypes associated with MFN2 mutations in order to explore possible genotype-phenotype correlations. A mutation screening of MFN2 gene has been performed on a cohort of CMT2 patients. We identified 4 MFN2 mutations,3 of them have not been reported before. The first one is a missense mutation leading an amino acid change in exon 11(p.A383V). Interestingly this mutation has been detected in two independent families originating from Southern Italy. Haplotype reconstruction evidenced a disease haplotype shared by both families, thus suggesting that the mutation may have been inherited from a common ancestor. Clinical and neurophysiological examinations showed an extremely variable expression in respect to age at onset and severity of symptoms. The second mutation is an amino acid change in exon 19 (p.A738V) and has been identified in a family with a severe CMT2 phenotype. The third one is a point mutation in intron 13 affecting the conserved consensus sequence of the donor splice site. The mutated allele generates an aberrant transcript that is likely translated in a truncated protein. This mutation seems associated to a late onset CMT2 phenotype with typical features, but two patients of the family experienced a rapid worsening of symptoms and died suddenly after few months. The last mutation is a substitution in exon 8 causing an amino acid change in the GTPase domain(p.R250Q). Although already reported, this mutation does not cosegregate with the disease in the family suggesting that other genetic factors may contribute to the disease in this family(Supported by a Telethon-grant to MM).

1007/W

Infantile spasms and seizure disorder associated with deletion of a 2.5 Mb interval of 7q11.23-7q21.11. *L.R. Osborne[1], J. Skaug[2], P. Kaplan[3], E.J. Young[1], M.L. Freckmann[4], M. Morimoto[5], S.W. Scherer[2].* 1) Medicine, University of Toronto, Toronto, Ontario, Canada; 2) Genetics & Genomic Biology, SickKids, Toronto, Canada; 3) Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Clinical Genetics, Sydney Children's Hospital, Randwick, NSW, Australia; 5) Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Deletion of a 1.55 Mb region of chromosome 7q11.23 results in Williams-Beuren syndrome (WBS), a disorder associated with cardiovascular symptoms, mild to moderate mental retardation and a variety of cognitive and behavioral deficits. Seizures are rare in the WBS population, but several individuals with larger deletions that extend toward the telomere have been reported to exhibit seizures in addition to WBS. As part of our ongoing study to relate genotype to phenotype at the WBS locus, we have used copy number variant analysis to define deletion breakpoints in subjects with deletions of the 7q11.23 region, distal to the WBS interval. We have mapped the extent of deletion in eleven new subjects with deletions of 7q11.23, identified through our study of WBS and through the continuous curation of our Chromosome 7 Annotation Project. DNA samples were genotyped with the Affymetrix GeneChip Human Mapping NspI Array, the scans were analyzed using dChip 2006 software and comparative intensity analysis performed. Eight of the subjects were hemizygous for the WBS region, and three had deletions that did not include the common WBS deletion region. Together with information from ten previously published subjects with deletions of 7q11.23, we have defined an approximately 2.5 Mb interval of 7q11.23-7q21.11 that is commonly disrupted in subjects with infantile spasms or seizure disorders, but is intact in subjects with no seizure symptoms. This common interval spans only four known genes, all of which are expressed in the brain and are therefore candidates for causing seizure symptoms. The identification of additional subjects with 7q11.23 deletions should allow further narrowing of the critical interval, with eventual identification of the causative gene.

1008/W

Molecular and cellular dissection of ABCA12: the major cause of Harlequin Ichthyosis. *A.C. Thomas, C. Sinclair, M. Patel, E.A. O'Toole, D.P. Kelsell.* Cutaneous Research, Queen Mary University of London, London, United Kingdom.

Harlequin ichthyosis(HI) is the most severe form of autosomal recessive congenital ichthyosis. Infants born with this skin condition have hard, thick skin covering most of their body as well as distortion of the lips, eyelids, ears and nose. Due to the impaired cutaneous barrier function, neonates struggle to control water loss, regulate temperature and are more susceptible to infection. Using SNP chip technology and subsequent sequencing, we have previously shown that mutations in the ABCA12 [(ATP)-binding cassette transporter] gene underlie HI and to date over 50 patients analysed have mutations in this gene. Additionally complex mutations, such as a heterozygous whole exon deletion and a multiple exon duplication, have been identified via CGH oligo array and multiplex PCR. The presence of these complex mutations shows the need for thorough investigation when considering pre-natal testing for HI. Our studies also show that there are ethnic-specific mutations in individuals of Pakistani, White British and Balkan origin. In order to elucidate the role of ABCA12 in epidermis, siRNA mediated knockdown was performed in keratinocytes. These cells were used to create 3D organotypic co-culture skin models that mirror many of the phenotypic changes observed in HI patient skin including abnormal lipid content and thickened epidermis. Evidence suggests ABCA12 is involved with lipid transport (Glucosylceramides) in the lamellar granule network of the skin. Additionally, our results from immunostaining experiments on HI skin and the skin model show that the programme of epidermal differentiation is severely impaired compared to control skin. Markers of late epidermal differentiation such as Keratin 2e, involucrin and transglutaminase appear in the lower and often basal layers of the skin suggesting loss of ABCA12 triggers early terminal differentiation but without the signals to form the cornified envelope. These data suggest the abnormal skin barrier function related to abnormal lamellar granule formation and subsequent abnormal lipid transport seen in HI skin may, in part, be due to the dysregulated keratinocyte differentiation programme driven by absent ABCA12.

1009/W

Genotype-phenotype correlation in adenylosuccinate lyase (ADSL) deficiency. *M. Zikanova, K. Mullerova, J. Krijt, S. Kmoch.* Institute for Inherited Metabolic Disorders, Prague, Czech Republic.

ADSL is enzyme acting in two pathways of purine nucleotide metabolism. Mutations in ADSL gene compromising the enzyme activity lead to hypotonia, seizures, psychomotor retardation and behavioral changes. Although spectrum and severity of clinical symptoms overlaps, three forms of ADSL deficiency - severe neonatal, severe childhood and mild myopathic - can be distinguished clinically based on onset and symptoms severity, and biochemically as severity of symptoms decrease with increased ratios of accumulating succinylpurines concentration in body fluids(SAdo/SAICAr ratio). Pathogenetic mechanisms underlying the phenotypic and biochemical heterogeneity remain unknown. We introduced a complex diagnostic system for ADSL deficiency based on metabolite profiling, enzyme activity measurement, mutation analysis and recombinant mutant protein characterization. So far we have analyzed 22 patients from 18 families (8 Czech, 7 Poland, 5 Germany and 2 US). We identified 16 ADSL mutations and cloned, expressed, purified and characterized catalytic properties of corresponding recombinant wild type and mutant ADSL proteins. We observed that residual enzyme activity, calculated as a mean of genotype corresponding homoallelic activities, correlates with severity of phenotype. However, all the active mutant enzymes displayed proportional decrease in activity towards both substrates and no ground for the varied SAdo/SAICAr ratio was found. As all the experiments have been performed on single isolated proteins, the situation does not reflected compound heterozygosity status found in most patients and thus the fact that two different mutant enzymes may form structurally and functionally unique tetrameric structures. This limitation may be partly overcome in complementation experiments. From the literature we therefore collected data on 57 ADSL patients and chose 17 patients with clinically different forms (4 severe neonatal, 6 severe childhood and 7 mild myopathic phenotypes) associated with extreme SAdo/SAICAr ratios. We cloned all 21 ADSL mutations involved in the selected cases and expressed mutant proteins which are currently being characterized.

1010/W

A novel mutation in TPM2 causes distal arthrogryposis type 2B in a Chinese family. *X. Zhao, X. Zhang.* Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

Distal arthrogryposes (DAs) are a group of clinically and genetically heterogeneous disorders, characterized by multiple congenital contractures of limbs. The characteristic primary limb malformations in DAs include bilateral and symmetric clenched fist, overlapping fingers, camptodactyly, ulnar deviation of fingers, and positional foot deformities such as talipes equinovarus. Ten different forms of DAs have been recognized and classified. The prototypic DA type 1 (DA1, MIM 108120) has no additional abnormalities. Among the other nine forms with additional features, DA type 2A (DA2A) has facial phenotypes including a very small orifice, H-shaped dimpling of the chin, prominent nasolabial folds, increased philtrum length, small nose, blepharophimosis, deep-sunken eyes with hypertelorism. Severe scoliosis may also be present in some cases. DA type 2B (DA2B, MIM 601680) has features intermediate between DA1 and DA2A. Besides limb phenotypes, it may have facial features like a triangular face, downslanting palpebral fissures and small mouth. Recently, mutations in the TNNI2, TNNT3, TPM2, MYH3 and MYH8 genes have been identified to be associated with DAs. Here we reported a Chinese DA family with 4 affected individuals in three generations. The proband showed notable DA phenotype combined with short stature and DA2B facial features. Two-point linkage analysis was first performed using microsatellite markers selected from the genomic regions adjacent to the TPM2, TNNI2/TNNT3, TNNC2 and MYH3 gene. A positive LOD score was obtained with the markers close to the TPM2 gene. Direct sequencing of the PCR-amplified DNA fragments spanning exon 1 to exon 11 of the TPM2 gene revealed in the proband a heterozygous missense mutation in exon 3, c.308A>G (pQ103R), substituting a highly conserved amino acid in the protein. This mutation was confirmed to cosegregate with the disease phenotype in the family but not detected in all unaffected individuals and 75 unrelated healthy controls. In summary, we have confirmed the link between the TPM2 gene and DA2B in a Chinese family.

1011/W

Understanding the molecular basis of mucolipidosis type IV. *G. Borsani[1], A. Benini[1], M. Beltrame[2], L. Calvarini[1], S. Molerí[2], S. Barlati[1], A. Bozzato[1].* 1) Department of Biomedical Sciences and Biotechnology, University of Brescia, Brescia, Italy; 2) Department of Biomolecular Sciences and Biotechnology, University of Milano, Milano, Italy.

Mucolipidosis type IV (MLIV, MIM 252650) is an autosomal recessive lysosomal storage disorder that causes mental and motor retardation as well as visual impairment. The lysosomal storage defect in MLIV is consistent with abnormalities of membrane traffic and organelle dynamics in the late endocytic pathway. MLIV is caused by mutations in the *MCOLN1* gene, which codes for mucolipin-1 (MLN1), a member of the large family of transient receptor potential (TRP) cation channels. Although a number of studies have been performed on mucolipin-1, the pathogenesis of MLIV is not fully understood. We are studying the molecular mechanisms underlying this disorder using a combination of experimental approaches. To identify genes that characterize pathogenic changes in mucolipidosis type IV, we compared the expression profiles of MLIV and normal skin fibroblasts cell lines using oligonucleotide microarrays. Genes that were differentially expressed in patients' cells were identified. 231 genes were up-regulated, and 116 down-regulated. This study allowed to evidence the modulation at the transcriptional level of a discrete number of genes relevant in biological processes which are altered in the disease such as endosome/lysosome trafficking, lysosome biogenesis, organelle acidification and lipid metabolism. The analysis of the Zv6 assembly of the zebrafish genome led us to the identification of *mcoln1*, the putative ortholog of *MCOLN1* in *Danio rerio.* Quantitative real-time PCR has been used to evaluate the expression of the gene at different developmental stages. Whole mount *in situ* hybridization analysis of zebrafish embryos allowed to determine the expression profile of zebrafish *mcoln1*. The subcellular localization of the encoded protein has been studied in human cell lines and compared to that of human mucolipin-1. The study of the phenotypic consequences of *mcoln1* gene knockdown using morpholino-based antisense oligonucleotides is currently in progress.

1012/W

***Bicc1* zebrafish model for Polycystic Kidney Disease (PKD).** *D.J. Bouvrette[1,2], V. Sittaramane[1,3], A. Chandrasekhar[1,3], E.C. Bryda[1,2].* 1) University of Missouri-Columbia, Columbia, MO; 2) Department of Veterinary Pathobiology; 3) Department of Biological Sciences.

Polycystic kidney disease (PKD) is a common genetic disorder with a prevalence of 1/500. There is no cure for PKD. Clinical manifestations include progressive cyst formation, renal enlargement, and ultimate progression into end-stage renal disease. The molecular mechanisms leading to cyst formation remain unclear. A defect in the *Bicaudal-C* (*Bicc1*) gene results in a PKD phenotype in the *j*uvenile *c*ongenital *p*olycystic *k*idney (*jcpk*) mouse model. The function of *Bicc1* is unknown; however, there is a high degree of conservation at both the nucleotide and amino acid levels across >9 species. **Statement of Purpose:** In this study, we use the unique characteristics of zebrafish to further investigate *Bicc1* function in the kidney. Early kidney development in zebrafish parallels that of mammals to the mesonephros stage. The zebrafish pronephros forms between 12-72 hours post fertilization (hpf) and can be visualized easily in the transparent embryos. **Methods/Results:** The expression of *Bicc1* in zebrafish was evaluated by RT-PCR and *in situ* hybridization, demonstrating that *Bicc1* is expressed throughout pronephros development. An antisense morpholino was used to knockdown *Bicc1* expression in zebrafish to examine the effects of loss of *Bicc1* function. Histological analyses of the resulting morphants reveal large, epithelial-lined cysts throughout the tubules of the pronephric kidney, closely resembling the cystic phenotype in the mouse. The morphant cystic phenotype was rescued with the addition of mouse *Bicc1* mRNA. **Conclusion:** These data provide convincing evidence that *Bicc1* has a similar functional role in both the mouse and zebrafish. This work supports the validity of using a zebrafish model to study *Bicc1* function in the kidney.

1013/W

Towards a zebrafish model of Spinocerebellar Ataxia Type 1: Cloning of the zebrafish Ataxin-1 and Ataxin-1 Like Homologs. *K.M. Carlson[1], S.C. Ekker[2], H.Y. Zoghbi[3], H.T. Orr[1].* 1) Lab Medicine & Pathology; 2) Genetics, Cell Biology & Deveopment, Univ of Minnesota, Minneapolis, MN; 3) Mol & Human Genetics, Pediatrics, Howard Hughes Medical Institute Baylor Col of Medicine, Houston, TX.

Spinocerebellar Ataxia-Type 1 (SCA1) is an autosomal dominant neurodegenerative disease resulting in a glutamine repeat expansion in ataxin-1 (ATXN1). Recently, an ATXN1 paralog, Ataxin-1 Like (ATXN1L), was described and shown to play a role in mediating SCA1 pathology. To further characterize the function of the ATXN1 gene family in both Purkinje cell development and SCA1 pathology, we have initiated the steps towards developing a SCA1 zebrafish model. To begin, we cloned the zebrafish homologs of both proteins. ATXN1 and ATXN1L contain a highly conserved AXH domain that is involved in protein/protein interactions. A search of the zebrafish genome using the hATXN1 AXH domain identified three putative zebrafish AXH domains that are greater than 60% identical to hATXN1 (Chr. 7, 16 and 19). The chr.19 and 16 domains are most similar to ATXN1 (82% and 78% identical respectively) while the chr. 7 domain is most similar to ATXN1L (83% identical). Following further sequence analysis, we isolated RNA from 24-hour zebrafish embryos and used RT-PCR to clone the complete coding sequence of the three zebrafish ATXN1 genes. Protein conservation and whole genome sequence alignment suggest that there are two hATXN1 homologs on chr. 19 and 16 in the zebrafish genome (zATXN1α and zATXN1β respectively) and one hATXN1L homolog on chr. 7 (zATXN1L). Overall, zATXN1α and zATXN1β are 46% and 35% identical to hATXN1, while zATXN1L is 40% identical to hATXN1L. A closer look at the ATXN1 homologs show that although they do not include a polyglutamine tract, other key protein features involved in SCA1 pathogenesis, including a nuclear localization sequence and phosphorylation site at S776, are conserved. We are currently characterizing the expression pattern of the ATXN1 gene family in the developing zebrafish embryo. Our data suggests that the zebrafish will be a useful model system for studying the developmental role of ATXN1 as well as further studying ATXN1-induced pathogenesis.

1014/W

Bardet-Biedl syndrome proteins are required for receptor localization to neuronal cilia. *K. Mykytyn[1,2], N.F. Berbari[1], J.S. Lewis[1], G.A. Bishop[3].* 1) Dept. of Pharmacology, The Ohio State University, Columbus, OH; 2) Dept. of Internal Medicine, Division of Human Genetics, The Ohio State Medical Center, Columbus, OH; 3) Dept. of Neuroscience, The Ohio State University, Columbus, OH.

Primary cilia are solitary appendages that are found on nearly all mammalian cells. They are thought to provide important cellular sensory and signaling functions. The importance of these organelles is highlighted by the fact that primary cilia dysfunction has been implicated in the pathophysiology of a number of human genetic disorders. However, the mechanisms underlying cilia dysfunction and their role in disease pathophysiology remain unclear. We have discovered that mouse models of Bardet-Biedl syndrome (BBS), a pleiotropic human genetic disorder whose etiology has been linked to cilia dysfunction, have defective localization of neuronal ciliary receptors in the brain. We find that neurons cultured from mice lacking the *Bbs4* gene lack ciliary localization of receptors and this localization can be corrected by Bbs4 overexpression. Our results indicate that BBS proteins are required for the localization of receptors to neuronal cilia. We hypothesize that BBS proteins function in the localization of ciliary signaling proteins and, in the absence of BBS proteins, ciliary signaling is disrupted. Importantly, this finding may represent the fundamental mechanism underlying the pathophysiology of the seemingly diverse BBS phenotypes, including obesity, cognitive deficits, renal cystic disease, and retinal degeneration. These results may provide important insights into the roles of ciliary signaling and the basis of disease in BBS and other ciliary disorders.

1015/W

An obese rat model with retinal degeneration. *V. Vasireddy[1], G.B. Reddy[2], M.N.A. Mandal[1], T. Mrudula[2], X. Wang[3], M.M. Jablonski[3], N.V. Giridharan[4], R. Ayyagari[1].* 1) Ophthalmology and Visual Scien, University of Michigan, Ann Arbor, MI; 2) Department of Biochemistry, National Institute of Nutrition,Hyderabad, India; 3) Ophthalmology, University of Tennessee Health Sciences Center, Memphis, TN; 4) National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderbada, India.

A strong association between obesity and ocular complications including retinal degeneration has been reported. The molecular basis through which obesity increases the risk of retinal degeneration is not yet known. We have identified a spontaneous obese rat model (WNIN/ob) and evaluated the retinal phenotype. Retinal morphology was studied by histology and ultrastructure analysis of retinal sections from 2 to 12 months old WNIN/Ob rat and lean littermate controls. Immunohistochemistry was performed using retinal cell specific marker antibodies. RNA from retina of 2 and 12 months old WNIN/Ob and its lean littermate rats was used for microarray analysis using Affymetrix Rat Genome 230 2.0 Gene Chip and expression of selected retinal genes was analyzed by real-time PCR analysis. No obvious change in retinal morphology was observed at 2 months age in obese rats as compared to its littermate lean controls. Onset of retinal degeneration appeared to be between 4-6 months of age. By 12 months age there were noteworthy changes in obese rat retina, particularly significant thinning of outer nuclear layer. Immunohistochemical analysis indicated photoreceptor degeneration, particularly rod cell loss, in obese rats. Gene expression analysis identified 429 genes that were differentially expressed in 12 months obese retina. Most of the down-regulated genes were found to be involved in phototransduction and the up-regulated genes are associated with apoptotic and stress response pathways. RT-PCR analysis of selected retinal genes validated the microarray data and further confirmed photoreceptor degeneration phenotype in WNIN obese rats. The spontaneous obese rats developed progressive retinal degeneration, with predominant rod cell loss at the early stages of degeneration.

1016/W

Characterization of glycosylation defects in muscular dystrophy. *S.E. Sparks[1, 2], E.P. Hoffman[2].* 1) Genetics & Metabolism, Children's Natl Med Ctr, Washington, DC; 2) Center for Genetic Medicine, Children's Research Institute, CNMC, Washington, DC.

Abnormal glycosylation of α-dystroglycan underlies the pathology of a group of muscular dystrophies known as the dystroglycanopathies. The clinical phenotype ranges from congenital onset of muscular dystrophy with CNS and eye involvement, to a later onset form of limb girdle muscular dystrophy, without any CNS or eye involvement. Deficiency of the dystroglycan protein is thought to be incompatible with life, as shown by dystroglycan knock out mice. There is no known disorder that is associated with dystroglycan deficiency. To date, six genes have been identified which alter the glycosylation pattern of α-dystroglycan, all of which are known or putative glycosyltransferases. However, with the anticipated 10-15 steps in the glycosylation of α-dystroglycan, there are more to be identified. An initial screening of 5000 muscle biopsy samples led to 176 samples from patients under 5 years of age who did not have the diagnosis of Duchenne muscular dystrophy (potential congenital muscular dystrophy patients with a glycosylation defect). Those with normal muscle histology and other confirmed diagnoses were excluded. Of the remaining 121 samples, 113 had clinical findings in 2 out of the following 4 criteria: involvement of the CNS, involvement of the eyes, muscle involvement demonstrated by elevated creatine kinase or EMG consistent with a myopathy, or muscle histology consistent with congenital muscular dystrophy. These 113 samples are being screened for a glycosylation defect. In those with a defect, molecular and biochemical characterization will be pursued. Furthermore, the natural history of these defects will be studied. In addition, the evaluation of clinical end points and biomarkers will be undertaken in hopes of investigating therapeutic options. Eventually, these initial studies on congenital muscular dystrophies will be broadened to include glycosylation analysis in unknown limb girdle muscular dystrophy muscle samples.

1017/W

The genetics of Oculocutaneous Albinism: molecular results in a European and African cohort. *C. Rooryck[1,2], F. Morice[2,3], V. Bubien[2], D. Lacombe[2], A. Taieb[3], B. Arveiler[1,2].* 1) Laboratoire Génétique Humaine, Université Victor Segalen, Bordeaux, France; 2) Service de Génétique Médicale, Hôpital Universitaire Pellegrin-Enfants, Bordeaux, France; 3) Service de Dermatologie Pédiatrique, Hôpital Universitaire Pellegrin-Enfants, Bordeaux, France.

Oculocutaneous albinism (OCA) is an autosomal recessive disorder characterized by skin, hair, iris and retina hypopigmentation. Four genes are involved in the four non syndromic OCA types: TYR at 11q14.3 in OCA1, OCA2 at 15q12 in OCA2, TYRP1 at 9p23 in OCA3, and SLC45A2 (MATP) at 5p13.3 in OCA4. We studied a cohort of 72 OCA patients from several European and African countries. Molecular exploration included deletion search by quantitative PCR and mutation screening by DHPLC and sequencing in the four genes. In the TYR gene, 8 novel point mutations were identified. One heterozygous deletion encompassing the whole gene was identified. OCA2 screening revealed 8 new point mutations. Novel intragenic deletions encompassing one or several exons were identified, and a deletion of the whole gene was found in a patient presenting both OCA and Angelman syndrome. We identified 4 new mutations in the TYRP1 gene, including two mutations in a Caucasian patient. MATP screening revealed a higher frequency of mutations than ever described in the literature, since 5 different mutations were identified, including four new ones, in patients from different countries. This study allowed us to reevaluate the worldwide frequency of mutations in each OCA gene. Among the mutated alleles, mutations in TYR were predominant (44%), then OCA2 (38%) MATP (13%) and TYRP1 (5%). 70% of patients were either compound heterozygotes or homozygotes, 24% presented only one mutation and 6% had no mutation identified. These observations lead to two hypotheses: the missing mutations are in unexplored regions of the genes (such as intronic or regulatory elements) or in other genes that have not been identified so far. Two patients presented three mutations in two different genes suggesting possible triallelic inheritance.

1018/W

Deficiency of Arid4a and Arid4b alters histone modifications, impairs genome stability, and induces acute myelogenous leukemia. *M. Wu[1], K. Eldin[2], A.L. Beaudet[1].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pathology, TEXAS Children Hospital, Houston, TX.

Arid4a and Arid4b are two related Arid (AT-rich interaction domain) family genes, previously known as retinoblastoma-binding protein 1 (Rbbp1) and Rbbp1-like protein 1 (Rbbp1l1), respectively. Recently, we have reported a murine model for Arid4a and Arid4b deficiency. Studies indicated Arid4a and Arid4b function in the regulation of genomic imprinting through controlling epigenetic modifications. Here, using the murine model system, we found that Arid4a-deficient mice displayed ineffective myeloid hematopoiesis. Analysis of histone modifications in Arid4a-/- bone marrow cells characterized the role of Arid4a in controlling H4K20 trimethylation and H3K9 trimethylation at heterochromatic regions. We further combined deficiency of Arid4a and Arid4b in mice and found that the Arid4a-/-Arid4b+/- mice frequently developed acute myelogenous leukemia (AML). Analysis of mouse embryonic fibroblasts (MEFs) demonstrated genomic instability caused by the Arid4a and Arid4b mutations. We also found that Arid4a together with Arid4b serve an unanticipated function for normal development of male germ cells, in which deficiency of these two genes led to spermatogenic failure observed in meiotic spermatocytes and during the maturation of postmeiotic haploid spermatids.. Our findings define molecular mechanisms for the Arid4a and Arid4b genes in myeloid homeostasis, and assign their roles in normal mammalian development.

1019/W

Linkage of gene for extreme obesity in genetic isolate. *E.I. Rogaev[1,2,3], Y.K. Moliaka[1], O.V. Plotnikova[1], V.A. Nikishina[1], V.A. Koshechkin[4], E.K. Ginter[5].* 1) Brudnick Neuropsychiatric Research Institute UMASS MS, Worcester, MA; 2) Research Center of Mental Health RAMS, Moscow, Russia; 3) Vavilov Institute of General Genetics RAS, Moscow, Russia; 4) Peoples' Friendship University of Russia, Moscow, Russia; 5) National Research Center for Medical Genetics, RAMS, Moscow, Russia.

During an epidemiologic screen we described unique isolated population in Central Asia characterized by high endogamy and accumulation of three independently inherited diseases: cataract, hypertension and extreme obesity with hyperphagia. Obese individuals are characterized by excessive appetite and food intake persistent since infancy or early childhood. Familial and segregation analysis demonstrated autosomal-recessive inheritance and virtually complete penetrance of the obesity gene The samples collected from selected families were used for gene mapping and mutation screening. The STR markers selected randomly across human autosomal chromosomes and from candidate-loci linked to obesity in humans and mice were initially used for genome scan and paternity and maternity analysis. The linkage analysis provided evidence for a linkage to chromosomal locus 7q32. The analysis showed at least three haplotypes for the five STR markers on mutated chromosome persisting in this population.

The data demonstrated the evidence for linkage of the obesity associated with extreme hyperphagia to locus on chromosome 7 and suggested strong candidate-gene for the obesity in this population. The genotyping data suggest also that the obesity mutation affects puberty but does not disrupt fertility in males. Supported by NIDDK 1R01 HD045570-01.

1020/W

Dysregulation of sodium channel β4 expression in the striatum of Huntington Disease transgenic mice. *F. Oyama[1], H. Miyazaki[1], M. Kurosawa[1], T. Kaneko[2], N. Nukina[1].* 1) Lab Structural Neuropathology, RIKEN Brain Science Inst, Saitama, Japan; 2) Department of Morphological Brain Science, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Sodium channel β4 (β4), a recently identified auxiliary subunit of the voltage gated-sodium channels, is significantly downregulated in the striatum of Huntington Disease (HD) model mice and patients. We examined β4-expressing neurons in striatum using in situ hybridization with β4 probe, followed by immunohistochemistry with anti preproenkephalin (PPE) or anti preprotachykinin A (PPTA) and found that β4 mRNA is expressed in two groups of striatal neurons projecting to globus pallidus (marker protein: PPE) and substantia nigra (marker: PPTA). TaqMan RT-PCR analysis indicated that both β4 and PPE mRNAs are preferentially decreased in striatum at a presymptomatic stage of HD mice, while PPTA mRNA and its peptide are unaltered even at the symptomatic stage. These results indicate that there is a difference in downregulation of mRNA and its product among striatal projection neuron proteins.

1021/W

Spectrum of NPHP6 (CEP290) Mutations in Leber Congenital Amaurosis and Delineation of the Associated Phenotype. *I. Perrault[1], N. Delphin[1], S. Hanein[1], S. Gerber[1], J.-L. Dufier[2], O. Roche[2], H. Dollfus[3], A. Munnich[1], J. Kaplan[1], J.-M. Rozet[1].* 1) Genetics Dpt & Research Unit INSERM U781, Hopital Necker, Paris, France; 2) Ophthalmology Dpt, Hopital Necker, Paris, France; 3) Ophthalmology Clinic, Hopitaux Universitaires de Strasbourg, France.

Purpose: Mutations in the NPHP6(CEP290)gene account for Joubert and Senior-Loken syndromes and Leber congenital amaurosis (LCA). LCA patients were reported to carry an intronic mutation resulting in an aberrantly spliced transcript and low levels of wild-type transcript believed to explain the absence of cerebellar and renal involvement in LCA patients. The aim of the present study was to give the survey of NPHP6 mutations in our series. Methods: 192 unrelated LCA cases were screened for the frequent intron 26 mutation(c.2991+1655A>G)as well as the 53 coding NPHP6 exons. Results: Mutations were identified in 38/192 LCA families (38/38 of European descent). All mutations but two were either non-sense, frameshift or splice-site changes. The common NPHP6 intronic mutation accounted for 33/76 of all disease alleles. Twelve unrelated LCA cases did not carry this common intronic mutation, ten of which, at least, harboured two mutations expected to truncate the protein. Whatever their genotype, all patients but three had a visual acuity <1/20, salt and pepper aspect of the retina with macular degeneration in the first decade progressing to a typical aspect of RP at the end of the second decade onwards, a severe hyperopia (+6D or more) and a slight photoaversion. Conclusions: We confirm the high frequency of NPHP6 mutations in LCA (19.8%) as well as that of the c.2991+1655A>G mutation (43% of disease alleles). We suggest that a significant fraction of LCA families segregate two null alleles questioning the relevance of the assumption according to which the retinal-restricted phenotype in LCA could be due to a residual NPHP6 activity. Indeed, Joubert syndrome was excluded by cerebral MRI in all patients presenting with developmental delay. Finally, we show that all patients of our series are affected with the cone-rod subtype of the disease whatever their NPHP6 genotype.

1022/W

The molecular etiology of Stargardt disease in Newfoundland. *A.K. Sheaves, J.S. Green, T.L. Young.* Faculty of Medicine, Human Genetics, Memorial University, Health Sciences Centre, St. John's , NL, Canada.

Stargardt disease is an autosomal recessive genetic disorder causing central vision loss often beginning in the first decade of life and is the most common form of juvenile macular degeneration. Mutations in the *ABCA4* gene cause Stargardt disease, with more than 400 reported mutations worldwide. The *ABCA4* protein is a component of the visual phototransduction cascade and absence or deficiencies in this protein lead to the death of retinal pigment epithelium and photoreceptor cells. Individuals with Stargardt disease are often compound heterozygotes for different disease-causing mutations. Other inherited eye disorders can be caused by *ABCA4* mutations, including cone-rod dystrophy, retinitis pigmentosa, and possibly age-related macular degeneration.

There are 34 families with Stargardt disease in Newfoundland and Labrador (NL). The only known mutation associated with Stargardt disease in this population is a homozygous c.5714+5G→A splice site mutation. The primary objective of this study is to identify all mutations in the *ABCA4* gene responsible for causing Stargardt disease in the NL population and to determine the genetic epidemiology and genotype-phenotype correlations of the *ABCA4* mutations in the NL population. Haplotype analysis will be performed to associate demography with particular mutations, which can be used to assess distant genealogical connections between families.

So far, the homozygous c.5714+5G→A mutation has been verified in a 3-generation family with 3 affected probands, and also found in the homozygous state in an additional 3 families. Additional SNPs found to date include a nonsense mutation (c.2564G→A), four missense mutations (c.3322C→T, c.3323G→A, c.4139C→T, c.4163T→C), and several known polymorphisms. While the cause of Stargardt disease has been explained in several of the families, continued sequencing of the *ABCA4* gene is needed to understand the full spectrum of disease-causing mutations in the NL population.

1023/W

Dissecting the role of Ofd1 in limb development and skeletal patterning. *S.B. Bimonte[1], L.Q. Quagliata[1], R.T. Tammaro[1], B.F. Franco[1,2].* 1) Telethon Institute of Genetics and Medicine, TIGEM, Naples, Italy; 2) Department of Pediatrics, Federico II University of Naples, Italy.

OFD type 1 syndrome is a genetic disorder characterized by oral, facial and digital anomalies, due to dysfunction of primary cilia. We have generated a mouse model for OFD1 syndrome. Ofd1Δ4-5/+ females displayed craniofacial abnormalities and a skeletal phenotype which included polydactyly of both limbs and shortened long bones. To bypass the problem of the embryonic male and perinathal female lethality, we have developed a mouse model in which the Ofd1 gene has been specifically inactivated in the limbs by crossing the Ofd1fl with the CrePrx1 transgenic mice, which specifically express the Cre recombinase in the early limb bud mesenchyme. Ofd1Δ4-5/+;CrePrx1 and Ofd1Δ4-5;CrePrx1 mutants were viable and displayed only limb and skeletal abnormalities. The strongest phenotype was observed in Ofd1Δ4-5;CrePrx1 males. Malformations include severe polydactyly with 7 to 9 digits and lack of normal digit identity, shortening of bones, fusion of synovial joints, disorganization of the growth plate and delay in endochondral ossification. Whole mount RNA in situ studies on limb buds from Ofd1Δ4-5/+;CrePrx1 and Ofd1Δ4-5;CrePrx1 embryos at E11.5 revealed that the domain of expression of Hoxd12, Hoxd13, Gremlin and dHand was abnormally extended anteriorly in the limb buds of the male mutants starting from E11.5. Western blotting analysis indicates a reduction of the Gli3R (Gli3-83) repressor form suggesting that Ofd1, as already shown for IFT proteins, is implicated in the control of the transcription activities of Gli proteins. Preliminary analysis of mutants indicate deregulation of Ihh, an increase of chondrocytes proliferation, absence of an organized growth plate, reduction in the bone mineralization and lack/reduction of bone collar. Our data indicate that Ofd1 plays an important role in the determination of the correct digit number and could be involved in the chondrocytes differentiation from hypertrophic chondrocytes to osteoblasts and in the periosteal ossification. Our experiment are now aimed to understand which are the molecular steps underlying these functions.

1024/W

Identification of novel mutations in NEMO in a cohort of Incontinentia Pigmenti. *F. Fusco[1], A. Pescatore[1], M. Paciolla[2], F. Ottobre[1], M. D'Urso[1], M.G. Miano[1], M.V. Ursini[1].* 1) Dpt Human Molecular Genetics, IGB-ABT-CNR, Naples, Italy; 2) University of Basilicata, Potenza, Italy.

Incontinentia Pigmenti (IP) is an X-linked dominant disease caused by mutation in the NEMO gene located in the Xq28 chromosomal region. NEMO encodes for a key subunit of the crucial IKK regulatory complex required for the activation of NF-kB pathway. Therefore, the remarkably heterogeneous and often severe clinical presentation reported for IP is due to the pleiotropic role of this transcriptional signalling pathway. Previous reports from us and from others have demonstrated that >70% of the IP cases are due to a recurrent exon 4-10 genomic rearrangement in the NEMO gene. Beside the NEMO rearrangement, about 39 small mutations (missense, frameshift and nonsense mutations) scattered all along the NEMO gene, have been reported. We will present an update and a report of 10 novel small mutations in NEMO that we identified in a cohort of IP patients from Europe and Mediterranean area. In this cohort, we confirm that the recurrent exon 4-10 NEMO rearrangement accounts for 70% of cases and we will present genotype-phenotype correlation, obtained applying a phenotype score based on clinical features of IP. The updated distribution of all the small mutations associated to IP along the NEMO gene highlights a secondary hot spot mutation in a conserved exon/domain of NEMO and reveals that all small alterations found in IP are private mutations. Furthermore, we found an unexpected high incidence of sporadic cases (about 65%). In summary, those observations might aid in determining the molecular basis of IP disease and also genetic counselling in patients with IP will benefit from an updated analysis of NEMO mutations.

1025/T

Investigation of genetic variants within candidate genes of the TNF signalling pathway on the response to anti-TNF agents in a UK cohort of RA patients. *J. Bowes[1], C. Potter[1], K. Hyrich[1], BRAGGSS[2], A. Morgan[3], A.G. Wilson[4], J. Isaacs[5], J. Worthington[1], A. Barton[1].* 1) ARC-EU, University of Manchester, UK; 2) Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; 3) University of Leeds, UK; 4) University of Sheffield, UK; 5) Newcastle University, UK.

Purpose: To investigate genetic variants within candidate genes of the tumour necrosis factor (TNF) signalling pathway in determining response to anti-TNF therapy in a UK population of rheumatoid arthritis (RA) patients.

Methods: A panel of single nucleotide polymorphisms (SNPs) were selected to span six candidate genes (DUSP1, HRB, IKBKAP, MAP3K1 MAP3K14 and TANK). Pairwise tag SNPs were selected from the HapMap phase II. Samples from RA patients (n=642) treated with anti-TNF agents (Etanercept, Infliximab and Adalimumab) were recruited by a UK-wide, multi-centre collaboration and genotyped on a Sequenom MassARRAY® platform. Linear regression was performed to determine if candidate SNPs could predict the response to anti-TNF therapy at six months, defined as the absolute change in disease activity score (DAS28). The regression model was adjusted for baseline DAS28, HAQ score and concurrent DMARD therapy. SNPs demonstrating genotypic association ($p<0.05$) were analysed under additional genetic models.

Results: A total of 71 SNPs were genotyped in 630 patient samples. Linear regression analysis identified two tag SNPs associated with treatment response in the cohort. Further analysis revealed stronger associations under a dominant genetic model. Carriage of the minor allele of rs96844 (MAP3K1) was associated with improved treatment response (genotypic $p = 0.037$, dominant $p = 0.011$). Carriage of the minor allele of rs4792847 (MAP3K14) was associated with a reduced treatment response (genotypic $p = 0.044$, dominant $p = 0.016$).

Conclusion: Association was found to two SNPs in candidate genes of the TNF signalling pathway in a large cohort of UK RA patients receiving anti-TNF therapy. These findings will be explored further when more samples from the on-going collection become available.

1026/T

Atlantoaxial dislocation is associated with MTHFR (C677T) polymorphism. *M. Pradhan[1], S. Agarwal[1], S. Behari[2].* 1) Dept Of Medical Genetics, SGPGIMS, Lucknow, UP, India; 2) Dept of Neurosurgery, SGPGIMS, Lucknow, UP, India.

Atlantoaxial dislocation (AAD) has a high incidence in the Indian subcontinent. Depending upon the type of defect this can be either reducible or irreducible type. AAD has been detected as early as 5 yrs of age and it's presence since birth cannot be ruled out. Hence, this congenital defect which is more common in this part of the world; may be due to nutritional deficiency during intrauterine life in genetically predisposed individuals. We looked into the MTHFR gene polymorphism (C677T and A1298C) in 75 patients and 60 matched controls. CT genotype frequency of MTHFR 677C→T polymorphism in AAD (OR 3.00, 95 % CI 1.28-7.14; $p =0.005$) as well as in the irreducible subgroup (OR 2.81, 95 % CI, 1.17-6.86; $p =0.01$) was significantly higher than controls. The frequency of T alleles was also higher (25.3%) in AAD compared to the control group [15%] (OR 1.92, 95% CI 0.97-3.37; $p = 0.053$). There was no association of A1298C polymorphism in MTHFR gene in any of the group. Hence, it can be predicted that C677T polymorphism in MTHFR gene plays an important role in causation of AAD. Widespread use of periconceptional folic acid has resulted in reduced occurrence of this disease in those part but still seen in areas where it is not being practiced.

1027/T

Genome-wide association of bronchodilator response in asthma. *A.A. Litonjua[1,2], K.G. Tantisira[1,2], J.A. Su-Lasky[3], A. Murphy[1,2], R. Lazarus[1,2], B. Klanderman[1,2], C. Lange[3], E.K. Silverman[1,2], S.T. Weiss[1,2].* 1) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Rationale: Short acting inhaled β_2 agonists are one of the most widely used classes of drugs for the treatment of asthma. However, a substantial proportion of asthmatics do not have a favorable response to these drugs, and identifying genetic determinants of drug response may aid in tailoring treatment for individual patients. Methods: We conducted a genome-wide association analysis using the Illumina HumanHap550 BeadChip in 209 children (randomized to the beta agonist only arm) and their parents participating in the Childhood Asthma Management Program (CAMP). A total of 534,290 autosomal SNPs met quality thresholds (MAF >0, completion rate >90%, <1% Mendelian inconsistency, <0.1% discordancy among replicates) and were included in the analysis. We screened the association of these SNPs with bronchodilator responsiveness (BDR) over the four years of the trial, in a family-based screening algorithm implemented in PBAT that ranked SNPs in order of statistical power. The algorithm screens using the between family-component of the variance in order to identify candidate SNPs with the greatest power for association. Results: We identified a SNP in chromosome 18q21.32 that met the strict screening criteria for association with BDR in a recessive model. Replication genotyping of this SNP in three additional asthma clinical trials is ongoing and will be presented at the meeting. Conclusions: We have identified a novel SNP that determines BDR in a genome-wide association study. Replication of this finding is currently being performed in three separate asthma clinical trials. This work is supported by U01 HL065899 and PO1 HL083069 from the National Heart, Lung and Blood Institute, NIH.

1028/T

Genome-wide Association Identifies a Novel Asthma Pharmacogenetic Locus. *K.G. Tantisira[1], A. Murphy[1,2], A.A. Litonjua[1], J. Lasky-Su[1,2], R. Lazarus[1], B. Klanderman[1], E.K. Silverman[1], C. Lange[2], S.T. Weiss[1].* 1) Channing Laboratory, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; 2) Harvard School of Public Health, Boston, MA.

Introduction: Pharmacogenetic identification of loci influencing response to medications has been largely limited to candidate gene approaches. We hypothesized that genome-wide association testing would permit the rapid identification of novel pharmacogenetic genes associated with response to inhaled corticosteroids in asthma. **Methods:** Using genotype data from the Illumina HumanHap550 BeadChip, we performed a genome-wide association screen on 118 Caucasian trios taking inhaled corticosteroids as part of the Childhood Asthma Management Program clinical trial. A total of 534,290 autosomal SNPs met quality thresholds (MAF >0, completion rate >90%, <1% Mendelian inconsistency, <0.1% discordancy among replicates) and were included in the analysis. We applied the PBAT screening algorithm (Ionita-Laza et al., 2007) to family-based association tests using principal components methodology (FBAT-PC; Lange et al., 2004) to screen for association with 6 lung function measures taken over 16 months. After ranking SNPs in order of statistical power, SNPs were formally evaluated by FBAT testing using a weighted hypothesis-testing approach. **Results:** One SNP, rs2978473, met the strict screening criteria. This SNP maps to the mitochondrial solute carrier protein (MSCP) gene, a gene that has not been previously associated with asthma or corticosteroid response. **Conclusion:** A novel asthma pharmacogenetic locus for inhaled corticosteroid response has been identified via FBAT screening of genome-wide association data. Formal replication studies in other asthma clinical trial populations are underway.

Funding: NIH K23:HG3983, U01:HL65899, P01:HL83069.

1029/T

Influence of FCGR3A-V212F and TNFRSF1B-M196R genotypes in patients with rhumatoid arthritis treated by Infliximab therapy. *B. Arveiler[1], C. Rooryck[1], T. Barnetche[1,2], C. Richez[2], A. Laleye[1], T. Schaeverbeke[2].* 1) Human Genetics Laboratory, Université Victor Segalen Bordeaux 2, Bordeaux, France; 2) Department of Rheumatology, University Hospital Pellegrin, Bordeaux, France.

Rheumatoid arthritis (RA) is a complex, polygenic disease of unknown aetiology, with prevalence estimates of 0.25 to 0.5% in French population. Anti-TNFα therapies are widely used in rheumatoid arthritis (RA) patients. Despite their efficacy has been clearly proven, some discrepancies were observed in the treatment response with still 40% of non-responders patients. The aim of this study is to determine whether two functional single-nucleotide polymorphisms: V212F in the FCGR3A, and M196R in the TNFRSF1B genes correlate with rheumatoid arthritis susceptibility and response to anti-TNFα therapy. The population study included a French cohort of 78 RA patients and 70 healthy controls. Allele and genotype frequencies were compared between patients and controls, according to their response to infliximab therapy, using the American College of Rheumatology response criteria (OR=4.58, IC95%= [1.67-12,8], p=7.10-4). No association was found between these two SNPs and RA susceptibility. However, a significant correlation was found between 196R allele carriers and low response to infliximab therapy. This is the first report of a statistically significant association between the TNFRSF1B-M196R SNP and response to infliximab in a French cohort. Larger studies are needed to confirm the relevance of this association.

1030/T

Association of RF and anti-CCP positivity, but not carriage of shared epitope or *PTPN22* susceptibility variants, with response to anti-TNF treatment in RA. *A. Barton[1], K.L. Hyrich[1], BRAGGSS[2], A. Morgan[3], A.G. Wilson[4], J. Isaacs[5], J. Worthington[1], C. Potter[1].* 1) University of Manchester, UK; 2) Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; 3) University of Leeds, UK; 4) University of Sheffield, UK; 5) Newcastle University, UK.

Purpose: To determine whether rheumatoid factor (RF), anti-CCP antibodies, or carriage of shared epitope (SE) and *PTPN22* susceptibility variants predict response to anti-TNF therapy in a large UK cohort of patients with rheumatoid arthritis (RA).

Methods: UK-wide multi-centre collaborations were established to recruit 642 patients treated with anti-TNF drugs for RA (46% received Infliximab, 43% Etanercept and 11% Adalimumab). Serum RF, anti-CCP antibody and SE status were determined using commercially available kits. *PTPN22* R620W genotyping was performed using a Sequenom MassArray® platform. Linear regression analyses were performed to investigate association between these 4 factors and drug response at 6 months, defined as the absolute change in disease activity score (DAS28). Analyses were performed in the entire cohort and also stratified by anti-TNF agent.

Results: Eighty nine per cent of patients tested positive for RF and 82% positive for anti-CCP. Patients who tested negative for RF had a 0.48units greater mean improvement in DAS28 compared to RF positive patients (95% CI: 0.08, 0.87, p=0.02). A better response was also seen among patients who tested negative for anti-CCP (Coef: 0.39, 95% CI: 0.07, 0.71, p=0.02). Upon stratification, association of both antibodies was restricted to the Infliximab-treatment group. No association was demonstrated between drug response and SE or *PTPN22* carriage.

Conclusion: The presence of RF or anti-CCP antibodies was associated with a reduced response to anti-TNF drugs as a whole and Infliximab, in particular. However, the presence of these antibodies only accounts for a small proportion of the variance in treatment response. It is likely that genetic factors will contribute to treatment response but these do not include the 2 genes known to confer susceptibility to RA.

1031/T

Association between *TNF receptor 2* gene polymorphisms and anti-TNF treatment response in a large cohort of patients with rheumatoid arthritis. *C. Potter[1], J. Bowes[1], K.L. Hyrich[1], BRAGGSS[2], A. Morgan[3], A.G. Wilson[4], J. Isaacs[5], J. Worthington[1], A. Barton[1].* 1) University of Manchester, UK; 2) Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; 3) University of Leeds, UK; 4) University of Sheffield, UK; 5) Newcastle University, UK.

Purpose: To investigate association between response to anti-TNF therapy and genetic variation in the gene encoding the type 2 TNF receptor (*TNFR2*) in a large UK cohort of patients with rheumatoid arthritis (RA).

Methods: UK-wide multi-centre collaborations were established to recruit a cohort of 642 patients treated with anti-TNF drugs for RA (46% received Infliximab, 43% Etanercept and 11% Adalimumab). Pairwise tagging and random single nucleotide polymorphisms (SNP) spanning the *TNFR2* gene were identified from the phase II Hapmap dataset (www.Hapmap.org). Genotyping was performed using a Sequenom MassArray® platform. Linear regression was performed to investigate association between SNPs and response to anti-TNF therapy at 6 months, defined as the absolute change in disease activity score (DAS28), under a genotypic model.

Results: Twenty SNPs were successfully genotyped and conformed to Hardy-Weinberg expectations. Associations of borderline significance were demonstrated between drug response and 3 SNPs, mapping to the promoter and 5' region of the *TNFR2* gene (rs520916: p=0.04, rs652625: p=0.04, rs3766730: p=0.04). Strong linkage disequilibrium was exhibited between two of these SNPs (rs520916-rs652625: r^2=0.72) but not the third (rs520916-rs3766730: r^2=0.01, rs652625-rs3766730: r^2=0.02). No association with the remaining SNPs was demonstrated. In particular, a tagging marker for a potentially functional polymorphism (M196R, rs1061622) mapping to exon6 of the gene did not demonstrate association with drug response.

Conclusion: Association between 2 independent effects within the *TNFR2* gene and anti-TNF treatment response was demonstrated in a cohort of patients with RA. These findings require replication in other series and, if confirmed, further fine mapping to identify the causal variant.

1032/T

Association between a polymorphism in PDE10A and bone mineral density. *L.S. Wood[1], A.B. Seymour[1], E.H. Pickering[2], D.S. Lee[2], P. Banerjee[3].* 1) Pharmacogenomics/Translational and Molecular Medicine, Pfizer, Groton, CT; 2) Statistics, Pfizer, Groton, CT; 3) Translational and Molecular Medicine, Pfizer, New York, NY.

Osteoporosis is a complex disorder that involves a decrease in bone mineral density (BMD). A number of candidate genes have been implicated in osteoporosis but to identify other targets for therapeutic modulation of the disease we investigated 219 SNPs from 31 candidate genes to determine if any were genetically associated with BMD. The SNPs were initially examined in 368 healthy post-menopausal Caucasian women. The model for the initial analysis of the data (adjusted for age, years post-menopause and BMI) was run on all the markers and the results were sorted by the interaction p-value (marker with BMI). A total of 63 SNPs showed association with BMD (q-value = 0.5) and were then tested in a second population that contained 688 healthy post-menopausal women and 863 osteopenic women. One SNP, rs4709081, in the gene PDE10A, showed consistent association in both data sets. The expected positive correlation between BMD and BMI for subjects with the "CC" rs4709081 genotype (15% of the population studied) does not hold. One possible implication is that the "CC" individuals could be more susceptible to fractures as their BMI increases. The SNP rs4709081 is located in the first intron of PDE10A and is of unknown function, most likely serving as a surrogate for the causal variant.

1033/T

In-vivo genetic screen for functionally active nuclear import inhibitors. *J. Rosenbluh, A. Loyter.* Biological Chemistry, The Hebrew University of Jerusalem,Israel Jerusalem, Israel.

Trafficking of proteins into the cell nucleus is an energy dependent process which involves binding of nuclear localization signals (NLS) within the protein to cellular receptors (kariopherins). Upon binding the kapiopherin-protein complex is transported through the nuclear pore complex. Interestingly, the NLS signal has been found to be of diverse sequence namely, proteins contain many different sequences with no apparent consensus sequence. Many pathogenic proteins such as viral or oncogeneic proteins function in the cell nucleus and thus contain a functional NLS. Inhibiting nuclear translocation of pathogenic proteins may serve as a novel mechanism for therapy. We have designed a yeast screening system which enables to detect in-vivo functionally active nuclear import inhibitors. This assay is based on the use of a LexA DNA binding domain, Gal4 activation domain fusion protein which does not contain an NLS. Only when a protein with a functionally active NLS is added the fusion protein can be imported to the nucleus and activate LexA regulated genes. Using this system in a yeast strain which contains a LexA-Ura3 regulated gene allows activation of the Ura3 gene only if the construct is translocated to the nucleus. Supplementing the media with the Ura3 toxin 5-FOA results in survival only of yeast cells that the fusion protein is not imported to the nucleus. To summarize in the above described method only if a nuclear import inhibitor exists the yeast cell can grow on media containing 5-FOA. A random peptide library embedded in a scaffold protein was used to screen for nuclear import inhibitors of the viral HIV-1 Tat protein. The Tat protein is an essential HIV protein required for viral transcription. Five peptides were found that inhibit to various extends Tat nuclear import, when tested these peptides were able to inhibit Tat also in different assays. Thus these peptides are a potential lead compound for development of anti-HIV drugs.

1034/T

Sherpa - A Bioinformatics Quality Control and Assurance Tool to Analyze Genotyping Data across Multiple Genomic Platforms. *T. van Rooij[1], C. Beck[1], M. Blazejczyk[2], W. al Abed[1], N. Gaudreault[1], P. Guelpa[1], A. al Mallah[1], I. Mongrain[1], Y. Renaud[1], M.S. Phillips[1].* 1) Genome Quebec and Montreal Heart Institute Pharmacogenomics Centre, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec.

The Pharmacogenomics Centre currently develops pharmacogenomic content on multiple genomic technologies. There is a current need for results that combine output from several genomic and proteomic technology platforms. Sherpa is an in-house developed software tool that links outputs from different instruments and creates a single, easily interpretable interface. It integrates raw data from all stages of sample processing, from PCR to genotyping, and presents them for data analysis and QC in a standardized interface. This allows the user to easily identify problems in sample processing by pre-qualifying data. The creation of an abstract generic genotyping data model by Sherpa allows the Centre to use a single informatic pipeline that analyzes data across multiple genomic platforms. In addition, Sherpa provides a number of unique data visualizations that are currently not available in commercial software packages; it is designed to go beyond current limitations in genotyping QC and will streamline the genotyping data feed into the Centre's data warehouse, the access point for data mining and analysis.

Sherpa guides technicians through multiple phases of data analysis and several levels of QC that enable the clinical accuracy required for pharmacogenomic testing. It uses a multi-algorithmic approach to give users a variety of different ways to look at the data in order to make the best decision and will also record the history of all genotyping calls. Sherpa reflects 21 CFR part 11 requirements by tracking changes and providing audit trails. The last phase of Sherpa development is the assignment of a proposed phenotype based on molecular profiles derived from pharmacogenomic research which includes literature text-mining in the data warehouse. This software's interface successfully addresses the current disconnect between genotyping results and the processes that generated them.

1035/T

Whole-genome association study identifies polymorphisms in the NPAS3 gene associated with super-response to iloperidone treatment in patients with schizophrenia. *C. Lavedan, S. Volpi, K. Mack, C. Heaton, R. Lannan, J. Hamilton, L. Licamele, C. Wolfgang, M. Polymeropoulos.* Vanda Pharmaceuticals, Inc, Rockville, MD.

Schizophrenia, a psychotic disorder affecting approximately 1% of the US population, is characterized by the presence of positive symptoms (eg, hallucinations), negative symptoms (eg, social withdrawal), and impaired cognitive functions. Treatment response to typical and atypical antipsychotics is highly variable. No specific, reliable markers predictive of response have been identified. Through a whole-genome association study conducted in a randomized, double-blind, placebo- and ziprasidone-controlled trial of iloperidone for treatment of schizophrenia, we identified several SNPs strongly associated with iloperidone efficacy, measured by change in PANSS total score. Two of these SNPs are located in 14q12-q13 in intron 3 of the *NPAS3* gene. Following 4-weeks iloperidone treatment, patients carrying the non-GG genotype for one SNP (approximately 30%) were 3 times more likely to experience approximately 20% improvement compared with patients with a different genotype. The association of non-GG genotype with better response was observed in both sexes and in all races. A study has described a mother and a daughter with schizophrenia who carried a t(9;14)(q34;q13) chromosome with a breakpoint in intron 3 of the *NPAS3* gene. This breakpoint disrupted the bHLH and PAS domains involved in DNA-binding and dimerization functions of the protein. Studies of mice with disruptions in *NPAS3* and *NPAS1* genes have shown that NPAS3 and NPAS1 transcription factors may control regulatory pathways relevant to schizophrenia. Our data suggest that *NPAS3* may affect response to antipsychotic treatment. More experiments are needed to better understand the function of *NPAS3*, its role in schizophrenia, and patient response to antipsychotic treatment. Additional studies specifically designed to confirm and fully appreciate the clinical value of this finding should provide new insight into response to iloperidone and ultimately may guide the clinician in directing iloperidone therapy to those patients most likely to respond.

1036/T

Pharmacogenomic study of iloperidone treatment in patients with schizophrenia identifies markers associated with efficacy. *L. Licamele, S. Volpi, C. Heaton, K. Mack, R. Lannan, J. Hamilton, I. Holt, C. Wolfgang, M. Polymeropoulos, C. Lavedan.* Vanda Pharmaceuticals, Inc., Rockville, MD.

In the practice of medicine, it is widely appreciated that the same dose of a medication given to patients with the same disease will result in many different outcomes relative to efficacy, tolerability, and safety. Practitioners frequently use a trial-and-error approach to identify the best treatment for each patient. Unfortunately, patients may experience weeks or months of suboptimal management of their illness. This inefficient approach is particularly evident in the treatment of schizophrenia. No single antipsychotic agent offers optimal efficacy and tolerability for every patient with schizophrenia. Pharmacogenomics (PG) provides the opportunity to discover genetic markers predictive of response. Knowing how a patient with schizophrenia will respond to a particular therapy based on his or her genetic makeup will allow clinicians to select the most optimal drug and dosage with less trial and error. We report the results of a whole-genome association study conducted to discover genetic markers of efficacy response to a novel atypical antipsychotic, iloperidone, in patients with schizophrenia. Three analyses of the change in the PANSS total score (PANSS-T) between baseline and Day 28 were performed: (1) 2-stage approach by which DNA samples were separated into 2 groups50% of the samples for a discovery phase in which only the top and bottom 30% of the change in PANSS-T were used and the other 50% as a hold-out group for a confirmatory phase; (2) ANOVA of last-observation-carried-forward data of all iloperidone-treated patients; (3) mixed-effects model repeated-measures analysis of all patients using the parsimonious genetic model of each SNP. We identified 6 SNPs associated with iloperidone efficacy, including 3 located in genes or regions previously associated with schizophrenia. Odds ratio, sensitivity, specificity, and predictive values were calculated. Results of this PG study provide new insight into markers of response to the novel antipsychotic iloperidone, developed with the ultimate goal of directing iloperidone therapy to those patients most likely to respond.

1037/T

Pharmacogenomic analysis shows differences between markers associated with responses of two atypical antipsychotics, iloperidone and ziprasidone, in the treatment of patients with schizophrenia. *S. Volpi, C. Heaton, K. Mack, L. Licamele, I. Holt, J. Hamilton, R. Lannan, C. Wolfgang, M. Polymeropoulos, C. Lavedan.* Vanda Pharmaceuticals, Inc., Rockville, MD.

Schizophrenia is a chronic, severe, and disabling disorder that affects about 1% of the US population. Symptoms include hallucinations, delusions, social withdrawal, and cognitive deficits. There is much evidence that schizophrenia is not caused by a single gene but, rather, by several interacting susceptibility loci and enviromental risk factors. Perhaps because of the heterogeneity of the underlying disease process, the etiology of schizophrenia has not yet been identified. Genetic factors are also expected to play a role in drug response, which is highly variable between individuals. Through a whole-genome association study conducted in a randomized, double-blind, placebo- and ziprasidone-controlled trial of iloperidone for the treatment of patients with schizophrenia, we identified several SNPs strongly associated with iloperidone efficacy, measured by change in the PANSS total score after 4 weeks of treatment. We report here on the analysis of 6 of these SNPs in 98 ziprasidone-treated patients. Allele and genotype frequencies were not statistically different between the iloperidone- and ziprasidone-treated patients. However, we showed that none of these SNPs were statistically significantly associated with ziprasidone efficacy response. Indeed, the genotype of an SNP associated with iloperidone best responders was more common among ziprasidone worse responders. Similarly, we observed that SNPs in the *CERKL* gene, associated with QT prolongation in iloperidone-treated patients, did not correlate with QT prolongation seen in ziprasidone-treated patients. Although some SNPs show a similar trend in association with response to each drug, our results suggest that the genetic signature of response is not identical for all drugs of the same class (here, atypical antipsychotics) but that it reflects the specificity of each drug, which may be mediated by its unique complex-binding profile, its individual interactions with other molecules, and its particular metabolism.

1038/T

Development of a Hypertension Candidate Gene Panel for use in Clinical Pharmacogenomics Studies. *N. Gaudreault[1], S. de Denus[2], M. White[2], M.S. Phillips[1].* 1) Genome Québec & Montreal Heart Institute Pharmacogenomics Centre, Montreal, Quebec, Canada; 2) Montreal Heart Institute, Montreal, Quebec, Canada.

Heart failure (HF) has reached epidemic proportions in the US with more than 5 million patients affected by the disease. The treatment of HF is complex, and although many treatments have been shown to be effective in selected populations, marked variability exists in the response of individuals. Currently, little data are available to identify which patients are most likely to benefit from specific treatments. Therefore, there is an urgent need to conduct studies to help identify which patients can benefit from specific therapeutic options. Pharmacogenomic studies have the opportunity to identify some of the variability contributing to a patient's specific response. Although thousands of polymorphisms have been identified, little is known about their biological and/or clinical significance. In this study, we propose to develop a focused panel (~300 SNPs) for genes involved in RAAS; hypertension and HF. Our comprehensive approach will evaluate an important number of candidate genes by genotyping both functional and haplotype tag SNPs in order to evaluate genotype-phenotype interactions. Some of the candidate genes incorporated into the panel include: REN, AGT, ACE, ACE2, ATR1, CYP11B2, NOS3, ADD1 and TGFB1. The panel will initially be used to support studies within the NIH HF Network. For this purpose, we have selected genes that might play a role in HF development, prognosis and modulation of its pathophysiology. Genes related to the biomarkers measured as part of the NIH HF Network were also included in the panel designs. Each assay has been optimized and validated for PCR genotyping reaction conditions against samples of known genotype (where available), as well as on multiple populations of diverse ethnic backgrounds. We are currently using this panel in a number of studies and believe that the combined use of rare variants and common haplotype SNPs within a target set of pathway-specific genes will expedite our ability to identify genes that are predictive for specific therapeutic responses observed in standard HF treatment.

1039/T

Whole-genome association study identifies polymorphisms in the CERKL gene associated with QT prolongation during iloperidone treatment of patients with schizophrenia. *C. Heaton, K. Mack, S. Volpi, J. Hamilton, R. Lannan, C. Wolfgang, L. Licamele, M. Polymeropoulos, C. Lavedan.* Vanda Pharmaceuticals, Inc., Rockville, MD.

Mutations in several genes can predispose to hereditary forms of long QT syndrome and to drug-induced prolongation of the QT interval of the electrocardiogram. Involvement of the *KCNH2* gene, which encodes the HERG potassium channel, has been well documented. Many drugs, including antipsychotics, have the potential to prolong the QT interval. We conducted a pharmacogenomic study to identify, among others, markers of QT prolongation during iloperidone treatment of schizophrenia. In a randomized, double-blind, placebo- and ziprasidone-controlled trial of iloperidone, an investigational atypical antipsychotic, patients were genotyped for approximately 500,000 SNPs. The change in QT interval from baseline to Day 14 was calculated based on Fridericia correction (QTcF), and a generalized linear model statistical analysis was performed. Two SNPs with highly significant associations are located on 2q31.3 in the *CERKL* gene, which encodes a ceramide kinase-like protein. Ceramide kinases convert the sphingolipid metabolite ceramide to ceramide-1-phosphate. Ceramide can decrease HERG currents and inhibit the basal turnover of HERG protein. The action of ceramide on ion channels is thought to be mediated mainly by kinase activity. Iloperidone-treated patients homozygous for an SNP were 6 times more likely than heterozygous patients to experience increased QTcF (>30 msec) by Day 14. The genotype of SNPs in the *CERKL* gene did not correlate with QT prolongation seen in ziprasidone-treated patients, even though ziprasidone showed a degree of QT prolongation similar to iloperidone. QT prolongation has been observed at therapeutic doses and at higher doses of other antipsychotic drugs, including risperidone, paliperidone, olanzapine, quetiapine, and aripripazole. Our results suggest that studying the involvement of *CERKL* and, more broadly, the ceramide pathway may lead to better understanding of the mechanism of QT prolongation induced by antipsychotic medications and other drugs known to affect the QT interval.

1040/T

Single-nucleotide polymorphisms in the *CYP2D6* gene are correlated with iloperidone drug exposure levels, impacting the degree of QTc prolongation associated with iloperidone treatment. *C. Wolfgang, M. Polymeropoulous.* Vanda Pharmaceuticals, Inc. Rockville, MD.

QT interval prolongation has been associated with numerous drugs, including antipsychotics. Genetic variants altering drug metabolism can result in higher than normal drug concentrations that may result in QT interval prolongation. Iloperidone, an investigational mixed D_2/5-HT_2 antagonist antipsychotic developed for the treatment of schizophrenia, is primarily metabolized by CYP2D6. The objective of this study was to identify and confirm genetic polymorphisms in *CYP2D6* that are associated with QT interval prolongation after iloperidone treatment. Seventy-four patients receiving iloperidone 16-24 mg/d in an open-label safety study (S1) and 300 patients receiving iloperidone 24 mg/d and 147 patients receiving placebo in a double-blind, efficacy/safety study (S2) were genotyped for two specific *CYP2D6* polymorphisms. Genotype/phenotype associations were characterized using iloperidone and metabolite concentration ratios in S1 to classify patients as either extensive metabolizers (EMs), intermediate metabolizers (IMs), or poor metabolizers (PMs). ECG was performed at protocol-specified intervals in each study. Maximum and mean QT interval changes from baseline (QTc) were calculated for each polymorphism. Mean and maximum QTc were significantly greater in IMs and PMs than in EMs among iloperidone-treated patients. Patients receiving placebo showed no change in mean QTc interval and a slight decrease in mean maximum change in QTc interval. *CYP2D6* polymorphisms were associated with higher levels of iloperidone exposure, resulting in larger QTc interval prolongations. These associations were characterized and then prospectively confirmed. Identifying patients genetically at risk for QT prolongation when treated with iloperidone before treatment initiation provides an opportunity to individualize therapy, balancing optimal tolerability and symptom relief.

1041/T

Development and evaluation of genome-wide strategies to identify pharmacogenetic contributions to adverse drug reactions in real-time. *M.R. Nelson, S.A. Bacanu, C.E. Bowman, S.L. Chissoe, M. Mosteller, A.D. Roses, E.H. Lai, M.G. Ehm.* Pharmacogenetics, GlaxoSmithKline, RTP, NC.

Adverse drug reactions (ADRs) can have a major impact on patients, doctors, regulatory agencies, and pharmaceutical companies. Risk factors known to contribute to ADRs include drug dose, environmental history and exposures, smoking, concomitant medications, as well as genetic variations. Identifying the genetic factors that contribute to ADR risk may lead to a better understanding of the underlying mechanism, identify patients at risk, or lead to more informed treatment decisions, all of which can provide better patient care and lower health costs. A review of the literature shows that several ADRs are strongly influenced by large pharmacogenetic effects. This suggests that proactive genome-wide genotyping of ADR cases coupled with pre-genotyped population controls could permit the rapid determination of whether there is a substantial common genetic component contributing to ADR risk. We explore the power of this approach through simulation and illustrate its application in a genome-wide search for markers associated with hypersensitivity to abacavir, an ADR which has a known major genetic risk factor (*HLA-B*5701*). Aggressive monitoring and genome-wide analysis of ADR cases as they are identified in clinical practice after drug approval can ensure that critical genetic biomarkers are discovered and have the opportunity for maximum impact on drug safety and patient care.

1042/T

Association of GIRK channel gene polymorphism *GIRK2* A1032G with postoperative analgesia. *D. Nishizawa[1], M. Hayashida[2], Y. Ogai[1], S. Kasai[1], J. Hasegawa[1], M. Tagami[3], M. Nagashima[4], K. Ikeda[1].* 1) Molecular Psychiatry, Tokyo Institute of Psychiatry, Tokyo, Japan; 2) Anesthesiology, Saitama Medical University International Medical Center, Saitama, Japan; 3) Anesthesiology, Toho University Sakura Medical Center, Chiba, Japan; 4) Surgery, Toho University Sakura Medical Center, Chiba, Japan.

Objectives: Opiates are commonly used as analgesics for the treatment of acute and chronic pain in the clinical scene today. However, it has been increasingly well recognized that there is considerable inter-subject difference in the sensitivity to opiate analgesics, which hampers satisfactory pain control. To explore polymorphisms responsible for the inter-subject difference in the opiate sensitivity, we performed an association analysis focusing on the SNPs in *GIRK2*, which is the gene encoding a subtype of G protein-activated inwardly rectifying potassium (GIRK) channel, known to play a key role in transmitting the analgesic effects of opioids. **Materials and Methods**: In the initial screening for polymorphisms in *GIRK2* gene and putative promoter regions, nine SNPs were identified. Among them, two SNPs of G-1250A and A1032G were selected for the following association study examining the relationships between the SNPs and requirement of postoperative analgesics. The subjects in the association study were a total of 129 patients who underwent surgical operation in hospitals and were treated with analgesics including opioids after surgery. **Results and Discussion**: During the first 24 postoperative hours, patients with the genotype A/A in the A1032G required analgesics more frequently than those with A/G and G/G in the SNP (p=0.011 and p=0.018, respectively), while no such associations were detected for the G-1250A. Although the molecular mechanism underlying the difference in the analgesic requirements remains to be elucidated, our data indicate that the SNP *GIRK2* A1032G could serve as a marker for predicting the analgesic requirements in patients. Our findings will lead to satisfactory pain control for patients suffering intolerable pain and open a path for personalized pain treatment in the future.

1043/T

Competitive allele-specific short oligonucleotide hybridization (CASSOH) method: Applications to genotyping of clinically important SNPs in pharmacogenetics. *S. Kure[1], M. Hiratsuka[2], A. Ebisawa[2], F. Kamada[1], S. Komatsuzaki[1], J. Kanno[1], A. Narisawa[1], Y. Aoki[1], M. Mizugaki[2], Y. Matsubara[1].* 1) Department of Medical Genetics, Tohoku University School of Medicine, Tohoku University Scholl of Medicine, Sendai, Japan; 2) Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University, Sendai, Japan.

Pharmacogenetics involves determining the genetic polymorphisms influencing drug exposure levels. We have developed a simple genotyping method of single nucleotide polymorphisms (SNPs) using immunochromatographic strip, CASSOH, which detects a SNP within 10 min after the competition of PCR by forming a visible gold-particle line on a chromatographic test strip (Matsubara Y and Kure S, Hum Mutat, 2003;22:166-172, Hiratsuka et al, Drug Metab Pharmacokin., 2004;19:303-307). The CASSOH method dose not demand either technical expertise or expensive instruments, and is readily performed in local clinical laboratories. We have applied this method for detection of ten SNPs that are clinically important in drug metabolism, ALDH2*2, CYP2C19*2, CYP2C19*3, NAT2*5, NAT2*6, NAT2*7, TPMT*3C, UGTT1A1*6, UGT1A1*27, and mitochondrial DNA 1555A>G. All the SNPs were successfully detected by the CASSOH method. The system developed here would facilitate poin-of-care genetic testing in local hospitals and out-patient clinics, promising potentially diverse clinical applications.

1044/T

Detecting unusual genotypic patterns in a single subject. *S-A. Bacanu, M.R. Nelson, E. Foot, M. Ehm, A. Slater.* GlaxoSmithKline, Res Triangle Park, NC.

Many adverse drug reactions (ADRs) are rare, and may only be observed in a small number of cases. Consequently, the limited number of cases for pharmacogenetic studies presents a challenge for analysis and inference using traditional methods. Under certain circumstances, we may even wish to make inferences about possible genetic causes within a single case. Even for larger case sample sizes, if we suspect the ADRs are genetically heterogeneous, it may be more appropriate to attempt case-specific genetic inferences. In such instances, instead of aggregating the information at each marker (usually single nucleotide polymorphisms, or SNPs) among cases, we choose to aggregate the genotypic information among different adjacent markers for each case and obtain a statistic capturing the likelihood of those patterns against a reference control set. The distribution of this statistic under the null hypothesis is estimated by computing the same statistic for each control relative to the pattern found in the remaining controls. An appropriate p-value comparing the case statistics relative to controls statistics is obtained using a newly developed method for bounding tail probabilities for a large class of distributions. Using this method we estimate the power to detect genetic aberrations such as deletions, loss-of-heterozygosity (LOH), translocations and inversions as a function of the number of SNPs spanning the aberrations and the linkage disequilibrium among SNPs. We apply this method to investigate if any such unusual genotypic patterns occur in a study involving one ADR case suspected of having LOH in a drug-modifying gene.

1045/T

Five genes are associated with colonic ischaemia and serious complications of constipation in a sample of diarrhoea predominant Irritable Bowel Syndrome patients treated with alosetron hydrochloride. *L.C. McCarthy[1], K.J. Davies[1], L.R. Budde[1], C.M. Vignal[1], S.W. Stinnett[1], C.J. Cox[1], A.J. Nelsen[1], D.P. Yarnall[1], H.C. Hollyfield[1], A.A. Flynn[2], I.E. Johnson[3], S.H. Gordon[3], V.Z. Ameen[4], J.S. Almenoff[5], S.S. Sundseth[1], E.H. Lai[1], M.G. Ehm[1].* 1) Pharmacogenetics, GlaxoSmithKline; 2) Drug Discovery Sciences, GlaxoSmithKline; 3) Clinical Development, GlaxoSmithKline; 4) CPDM, GlaxoSmithKline; 5) MIGU MDC, GlaxoSmithKline.

Objective: Determine whether there are any genetic biomarkers associated with the occurrence of ischaemic colitis (IC) or serious complications of constipation (SC) in diarrhoea predominant IBS (d-IBS) patients treated with alosetron. Patients & Methods: A 611,000 SNP whole genome screen and extensive sets of candidate genes were analysed for association with IC and/or SC, in 10 IC cases, 8 SC cases and 305 controls. All cases and controls were d-IBS patients and received alosetron treatment. All cases reported IC or SC following marketing of alosetron. Results: Five genetic biomarkers associated with IC and/or SC have been identified in this case sample, all with sensitivity and/or specificity >80%. HTR7 shows significant association with susceptibility to IC and SC; CAV2 is associated with susceptibility to IC; NR1I3, CSMD1 and COMMD10 are associated with susceptibility to SC. Six significant associations with polymorphisms in genomic loci which do not currently contain known genes were also identified for IC and SC phenotypes. HTR7, CAV2, NR1I3, CSMD1 and COMMD10 gene products have been previously implicated in the mechanisms of inflammation, ischaemia and/or Inflammatory Bowel Disease (IBD). Conclusion: We provide evidence that is consistent with the presence of genetic factors associated with the occurrence of IC or SC in d-IBS patients treated with alosetron. Due to the small case sample sizes in this study, all association results should be considered exploratory and require validation by independent replication and/or additional supporting evidence. If replicated in an independent sample, any validated genetic associations could contribute to a prognostic test to identify patients at increased risk of developing IC and/or SC.

1046/T

A genome-wide approach to identify pharmacogenomic candidate genes that contribute to variation in cytosine arabinoside (Ara-C) cytotoxicity. *L. Li[1], B.L. Fridley[2], K.R. Kalari[1,3], G. Jenkins[2], A. Batzler[2], M.A. Hildebrandt[1], D.J. Schaid[2], R.M. Weinshilboum[1], L. Wang[1].* 1) Division of Clinical pharmacology,Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN 55901; 2) Division of Biostatistics, Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55901; 3) Division of Biomedical Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55901.

Background: Ara-C is used in both induction and maintenance therapy for virtually all patients suffering from acute myelogenous leukemia. However, relatively few studies have addressed the possible contribution of inheritance to individual variation in response to Ara-C therapy. We set out to take both a "biased" pathway-based approach and a complementary "unbiased" genome-wide approach to study Ara-C pharmacogenomics. **Methods:** We utilized a data-rich cell-based model system consisting of 197 Coriell Institute "Human Variation Panel" lymphoblastoid cell lines from 3 ethnic groups. We generated indepth resequencing data for genes encoding proteins involved in the "Ara-C Pathway". In addition, we obtained basal expression array data for all of these cell lines and performed MTS-based cytotoxic assays. Genome-wide association studies and pathway-based analysis with basal expression array data and Ara-C cytotoxicity were then performed to identify genes associated with Ara-C response. **Results and Discussion:** Ara-C cytotoxicity was assayed for all cell lines, and IC50 (GI50) and LC50 values were calculated using a four parametric logistic model. These phenotypes were correlated with expression array data using Pearson's product moment correlation coefficients. This association study identified 21 genes - both within and outside of the Ara-C Pathway--with p-values < = 10-5. Pathway analysis of these genes showed that toll-like receptor (TLR) signaling, death receptor, IL-10 and apoptosis signaling pathways might be involved. Functional studies of these candidate genes are being performed to verify the correlation results. These results represent a step toward a global understanding of Ara-C pharmacogenomics.

1047/T

The efficacies of clozapine and haloperidol on refractory schizophrenia are related to DTNBP1 variation. *L. Zuo[1,2], X. Luo[1,2], R.A. Rosenheck[1,2], J. Krystal[1,2], J. Cramer[1,2], D.S. Charney[3], J. Gelernter[1,2,4,5].* 1) Dept Psychiatry, Yale Univ Sch Medicine, West Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) NIMH (DSC), Bethesda, MD, USA; 4) Dept Genetics, Yale Univ Sch Medicine, West Haven, CT; 5) Dept Neurobiology, Yale Univ Sch Medicine, West Haven, CT.

The prototypical atypical antipsychotic agent clozapine is more efficacious for refractory schizophrenia than the "typical" antipsychotics, but the mechanism is still under investigation. Since 2002, at least 18 association studies have demonstrated that the DTNBP1 is involved in the cause of schizophrenia. Thus, the DTNBP1 product is hypothesized to be the potential target of antipsychotics. The present study aimed to investigate the relationship between the DTNBP1 and the effects of clozapine and haloperidol on refractory schizophrenia. One thirty-nine patients with refractory schizophrenia were assigned to clozapine (n=62) or haloperidol (n=77) and followed for 3 months. Symptom improvement was evaluated by total PANSS score. Six markers at DTNBP1 and 38 ancestry informative markers (AIMs) were genotyped in all subjects. The relationships between the effects of antipsychotics and the diplotypes, haplotypes, genotypes, and alleles of DTNBP1 were tested by ANCOVA, ANOVA, and t-test. The results show that the patients with diplotype ACCCTC/GTTGCC, genotypes T/T+T/C, or allele T of marker P1333 have better response to clozapine (0.005≤p≤0.029), but the patients with diplotype ACCCTC/GCCGCC, genotype A/G, or allele A of marker P1583 have better response to haloperidol (0.007≤p≤0.080), in European-Americans, African-Americans, or the combined sample. The present study demonstrated that the DTNBP1 gene modulates the effects of both the atypical antipsychotic clozapine, and the typical antipsychotic haloperidol. Subjects with different DTNBP1 diplotypes, haplotypes, genotypes, or alleles may have different responses to these antipsychotics. Clozapine and haloperidol may have different pathways or efficacy although both are related to DTNBP1.

1048/T

Sex-specific differences in blood pressure response to metoprolol by ADRB1 Polymorphisms. *V. Bhatnagar[1,2], D.T. O'Connor[1], N.J. Schork[1,3], M.S. Lipkowitz[4].* 1) University of California San Diego, La Jolla, CA; 2) VA San Diego San Diego, CA; 3) Scripps Institute for Genomic Medicine, La Jolla, CA; 4) Mount Sinai School of Medicine New York, NY.

Purpose: This study explores the relationship between ADRB1 polymorphisms SER49GLY and GLY389ARG and blood pressure response to metoprolol among African American men and women with early hypertensive nephrosclerosis. Methods: 197 men and 131 women randomized to treatment with metoprolol from the African American Study of Kidney Disease and Hypertension were genotyped at SER49GLY and GLY389ARG. Of these, 161 were randomized to an aggressive treatment group and 167 to a usual treatment group. A Cox Proportional Hazards Model was used to determine the hazard rate of reaching a target a mean arterial pressure (MAP) of 107 mmHg, adjusting for baseline MAP, medications and potential co-variates, by genotypes and ADRB1 haplotypes; analyses were stratified by sex and treatment group. Results: Men and women were of similar ages (55 years) and had similar baseline MAPs (113 mmHg). Men had higher baseline glomerlular filtration rates, 48 (standard deviation 12) versus 45 (13) ml/min (p=.02); men had a lower body mass index, 31 (6) versus 32 (7) kg/m2 (p=.05). Genotype distributions were in Hardy-Weinberg Equilibrium. Among women randomized to aggressive treatment only, those with a GLY/GLY genotype at SER49GLY responded significantly faster than those with a SER/SER or SER/GLY genotype; the adjusted hazard rate with 95 percent confidence interval was 3.02 (1.20 to 7.60; p=.02 for genotype coefficient). Though of borderline significance, women with an ARG/ARG genotype at GLY389ARG responded faster that those with an ARG/GLY or GLY/GLY genotype: 2.01 (.95 to 4.23; p=.07). Women without a SER49/GLY389 ADRB1 haplotype responded significantly faster compared to those with this haplotype: 2.52 (1.40 to 4.52; p=.002). Conclusions: African American women (but not men) randomized to aggressive management with a SER49/GLY389 haplotype were less responsive to metoprolol. Genotyping at these sites may help identify women are less responsive to aggressive treatment with metoprolol.

1049/T

Association between GSTP1, MDR1, and MTHFR polymorphisms and the outcome of patients with breast cancer treated with FEC (Fluorouracil, Epirubicin, and Cyclophosphamide) adjuvant chemotherapy. *F.Y. Chung[1], M.Y. Huang[2], S.R. Lin[3], J.Y Wang[4].* 1) Graduate Institute of Medicine, College of Medicine; 2) Department of Radiation Oncology,; 3) Graduate Institute of Medical Genetics and BioMedi Innovation Incubation Center; 4) Department of Surgery, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Purpose: Chemotherapy is an integral part of multi-modality treatment of locally advanced breast cancer. Glutathione S-transferase P1 (GSTP1), multidrug resistance 1 (MDR1), 5, 10-methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthetase (TS) play important roles in chemotherapeutic drug transport, metabolism and target. In the present study, multiple genetic polymorphisms, including GSTP1 A313G, MDR1 C3435T, MTHFR C677T, and TS tandem repeats, were analyzed in breast cancer patients and used combining of gene polymorphism to predict the clinical outcome of breast cancer patients receiving FEC adjuvant chemotherapy. Methods: Genomic DNA was isolated from the blood samples of 192 postoperative breast cancer patients. The genotypes were determined by PCR-RFLP. Patients' prognoses of postoperative relapse and analyzed with GSTP1, MDR1, MTHFR and TS phenotype groups. Results: GSTP1 A313G, MDR1 C3435T, MTHFR C677T, and TS tandem repeats showed no significant association with clinicopathological factors and postoperative relapse (all P > 0.05). There were no differences in prognosis between each genotype when considered separately. However, we found the association between postoperative recurrence and patients with both MDR1 3435CC and MTHFR 677CC (OR: 2.97, P=0.026), and patients with additional GSTP1 313AA (OR: 3.48, P=0.024) in consideration. Conclusion: The GSTP1, MDR1, and MTHFR genotypes can be prognostic factors in breast cancer patients receiving FEC adjuvant chemotherapy, where gene-gene interactions between the genotypes may occur. Long-term follow-up breast cancer patients in a larger population may be prerequisite for further validate of the actual roles of these gene polymorphisms.

1050/T

Differential allele frequencies in drug-related SNPs in the Mexican population. *A. Contreras, I. Silva-Zolezzi, LA. Alfaro, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Genetic variation influence how humans respond to commonly used drugs. There is growing evidence showing that ethnic origin contributes to drug response. Most of the Mexican population results from admixture of any of 65 ethnic groups with Spanish, and in a lesser extent, Africans. To better understand drug-related SNPs distribution in the Mexican population, we genotyped seven SNPs known to influence drug response: *ADCY9* 2316A>G (Albuterol and Budesonide), *CRHR1* 30864G>T (Budesonide), *MDR1* 3435C>T (Carbamazepine), *MDR1* 2677G>T (Paclitaxel), *5-HT2C* -759C>T (Risperidone, Olanzepine), *5-HT2A* 4472G>A (Citalopram) and *PDE6A* 22620A>T (Fluoxetine) in 300 Mexican Mestizos from six different states: Guerrero, Guanajuato, Sonora, Veracruz, Yucatan and Zacatecas. Our results showed the following allele frequencies: *ADCY9* 2316A>G, G 0.226 (0.170-0.322) and GG 0.054 (0.034-0.071); *CRHR1* 30864G>T, T 0.221 (0.157-0.371) and TT 0.050 (0.000-0.168); *MDR1* 3435C>T, T 0.459 (0.368-0.553) and TT 0.195 (0.000-0.332); *MDR1* 2677G>T, T 0.459 (0.370-0.555) and TT 0.211 (0.137-0.349); *5-HT2C* -759C>T, T 0.185 (0.102-0.281) and TT 0.104 (0.067-0.237); *5-HT2A* 4472G>A, A 0.269 (0.204-0.366) and AA 0.067 (0.000-0.189); *PDE6A* 22620A>T, T 0.183 (0.134-0.366) and TT 0.003 (0.000-0.157). Allele distribution in Mexico showed significant differences between some states (p<0.05). Moreover, comparative analysis with other world populations evidenced different allele frequencies, examples include *MDR1* 2677G>T (MEX 0.46 vs YRI 0.0), *ADCY9* 2316A>G (MEX 0.23 vs CEU 0.40), and *PDE6A* 22620A>T (MEX 0.18 vs CHB 0.02). Our results indicate that these analyses will be of relevance to better design pharmacogenomic studies in Mexico, contributing to a more rational use of drugs in Mexican populations.

1051/T

GSTT1: Clinical Biomarker Assay Development for Copy Number Variation. *A.B. Freeman, C. Taylor, L. Gautier, Y. Xiang, C. Lopez-Correa.* Integrative Biology and Lilly Systems Biology, Eli Lilly and Company, Indianapolis, IN.

Glutathione S-transferase (GSTT1) is a Phase II metabolizing enzyme responsible for the conjugation of environmental carcinogens, pollutants, drugs and other xenobiotics. GSTT1 contains a deletion that reduces enzyme activity, is highly variable in the human population and therefore results in a Copy Number Variant (CNV). The GSTT1 copy number genotype correlates with conjugation phenotype; individuals with two gene copies have a "high conjugator" phenotype whereas individuals with no copies of GSTT1 (null genotype) have a "nonconjugator" phenotype. Therefore, a GSTT1 CNV assay could serve as a key clinical biomarker for tailored therapies and patient stratification.

We assessed the ability of PCR and Quantitative PCR (qPCR) technologies to detect the GSTT1 deletion status in 263 HapMap and 23 cancer cell lines. The two PCR assays used to genotype the GSTT1 CNV were optimized from previously described methods while the qPCR assay was similar to that described by McCarroll et al (2006). In an independent effort, we also utilized array Comparative Genomic Hybridization (aCGH) to detect the GSTT1 copy number in the cancer samples.

Using the qPCR assay in the HapMap cell lines, our GSTT1 CNV genotypes were in 100% agreement with McCarroll et al (2006) as compared to 90% agreement with the PCR assay. In the cancer samples, however, there was only 70% consistency between the qPCR and PCR assay results, while the aCGH genotypes had even less consistency with all of the above.

We conclude the qPCR assay is the most accurate and reliable for the detection of GSTT1 copy number in clinical patients. We further hypothesize that the high rate of discrepant GSTT1 CNV genotypes observed with all assays in the cancer samples is attributed to increased genomic rearrangements and instability within these cell lines. Cell heterogeneity within the cancer samples would directly affect the analysis of CNV genotypes with one or two copies. This is substantiated by the consistency among all four assays to identify the GSTT1 null genotype in both normal and cancer samples.

1052/T

Interaction of Genotype, Homocysteine and Vitamin Levels on Migraine. *L. Griffiths, R. Lea, N. Colson, S. Quinlan.* Genomics Res Ctr, Sch Med Sci, Griffith Univ Gold Coast, Southport, Australia.

Studies in our laboratory have investigated a number of vascular related genes to determine their involvement in migraine. These studies have identified a role for the methylene tetrahydrofolate reductase (MTHFR) gene in migraine, with our studies and others implicating the C677T variant in migraine, in particular migraine with aura (MA) susceptibility. The TT genotype of this variant which is associated with MA, is also associated with decreased MTHFR enzyme and elevated plasma homocysteine levels. High homocysteine levels have been associated with various vascular related disorders, but an increase in dietary vitamin B and folate levels can have an important effect reducing these levels. To determine the effects of vitamin supplementation on homocysteine levels and disease symptoms in migraine we are conducting a pilot trial. 53 MA patients were randomised to receive vitamin (folic acid, B6 and B12) supplementation over a 3 month period, with assessment at baseline for plasma homocysteine levels, folate and vitamin status and C677T genotype. The vitamin treatment was within maximum daily dosages, was well tolerated and no adverse events were recorded. For the total patient group, baseline homocysteine levels were negatively correlated with folate ($P = 0.001$), vitamin B12 ($P=0.006$) and vitamin B6 ($P =0.034$) with only minor variation between the treatment and placebo groups observed ($P>0.1$). Mean homocysteine was also similar in the two groups at baseline ($P = 0.95$) and baseline levels of vitamins between C677T genotype groups were not statistically significant ($P>0.05$). The mean homocysteine level was ~16% higher in the TT genotype group compared to CT/CC ($P=0.09$). After 3 months of vitamin supplementation the treatment group showed a mean reduction in homocysteine of 39% ($P < 0.0001$), whilst the placebo group showed no change ($P > 0.05$). The results indicate that vitamin supplementation has a marked effect on homocysteine levels in MA patients and suggest that this response is modified by MTHFR C677T genotype. Also subjective information received from patients has indicated a noticeable reduction in migraine severity, frequency and duration.

1053/T

Race-Marketing: BiDil and the Race Debate in Popular Media. *S. Harry, T. Caulfield.* Health Law Institute, Edmonton, Alberta, Canada.

The Food and Drug Administration's approval of the "race-specific" drug BiDil, marketed as a heart failure medication specifically for African-Americans or individuals who "self-identify" as black, has triggered the re-emergence of the race debate in genetic research literature and in the popular media. Supporters of the drug including the National Association for the Advancement of Colored People (NAACP) and the Association of Black Cardiologists have lauded the medication as the first step in decreasing health disparities within ethnic minority groups (Lerner, 2004). Critics have been very vocal in questioning the methodology of the drug trials and interpretation of the results, arguing that the trials were unduly influenced by commercial considerations (Gellene, 2005). Given the important role the media plays as a source of information and as a means of shaping public perceptions, exploring popular representations of "race" in this context seems essential.

This study examines popular representations of BiDil including major newspapers, magazines, and periodicals using a coding frame successfully implemented in other media studies. Preliminary observations show that while the popular press portrays both sides of the race controversy, a significant portion uncritically accept the existence of genetic differences between races despite extensive scientific evidence to the contrary. Only a few articles discuss socio-economic factors as contributors to heart failure in African Americans, but emphasize the significance of genetic predisposition. This data helps to inform the debate regarding the representations of race in the context of genetic research. While some have suggested that the development of BiDil is the first constructive step toward the pharmacogenomic goal of personalized medication (Roylance, 2005), others commentators have countered that race does not accurately reflect individual variation or variation within a sub-population. Future research should explore the degree to which popular representations accurately reflect this debate and the degree to which they result in the "racialization" of medicine (Stein, 2005).

1054/T

Pharmacogenomic Testing of CYP450 2C9, VKORC1 and Thrombophilic Factor II and Factor V using the Warfarin Sensitivity-Resistance Panel the INFINITI Analyzer. *P. Hujsak, Y. Fu.* R&D, AutoGenomics, Inc, Carlsbad, CA.

Warfarin targets the vitamin K epoxide reductase complex 1 (VKORC1) enzyme and affects the down stream proteins in Vitamin K metabolic cascade. Warfarin is metabolized mainly in humans by Cytochrome P450 2C9 (CYP450 2C9). Effective warfarin dosing has been correlated with genetic variants of both enzymes. Thrombophilic Factor II and Factor V mutations could affect Warfarin dosing of patients because of increase in blood clotting. Therefore, identifying an individual's genotype of these four enzymes may help determine initial Warfarin therapy levels and long term International Normalized Ratio (INR), thus ensuring maximum safety. We have developed a multiplex Warfarin-Sensitivity-Resistance (WSRP) pharmacogenetic assay for genotyping VKORC1, CYP450 2C9, Factor II, and Factor V using the BioFilmChip and the INFINITI Analyzer. Multiple alleles in each DNA sample are analyzed in a single tube by PCR amplification (off line), followed directly by Detection Primer Extension (DPE) using the INFINITI Analyzer. The INFINITI Analyzer performs SNP analysis by hybridizing the DPE product to universal molecular OligoZip immobilized on the BioFilmChip. For the VKORC1 enzyme, we are testing non-coding SNPs: C381T, C861A, G3673A, T5808G, G6853C and G9041A, and coding SNPs: G5396C, G5417T, T5445C, A5483G, G6642A and T8798G. For CYP450 2C9, the following SNPs are being tested: C430T (*2), A1075C (*3), C1080G (*5), and 818delA (*6). Our comprehensive panel also includes testing for Factor II G20210A and Factor V G1691A (Leiden) are also tested. Results obtained from 150 different samples using the WSR-P test correlated well with the standard known methods such as sequencing. This robust assay for genotyping VKORC1 coding and non-coding regions, and detecting mutations in the CYP450 2C9, Factor II and Factor V genes in individuals could be potentially useful in managing Warfarin therapy safely and effectively, and may account for differences in ethnic populations.

.

1055/T

FCGR3A genotypes influence response to tumor necrosis factor alpha inhibitors in patients with rheumatoid arthritis. *K. Ikari, S. Tsukahara, E. Sato, M. Shinozaki, T. Tomatsu, M. Hara, H. Yamanaka, S. Momohara, N. Kamatani.* Inst Rheumatology, Tokyo Women's Med Univ, Tokyo, Japan.

Tumor necrosis factor (TNF)-alpha inhibitors improve symptoms and physical function in patients with rheumatoid arthritis (RA). They have emerged as standard therapy for those whom traditional disease-modifying anti-rheumatic drugs have failed to control disease activity. However, a lower response to TNF-alpha inhibitor was observed in some patients in the clinical trials. Prediction of treatment outcome of patients with RA may allow better targeting of aggressive treatment. Recently, a polymorphism in the FCGR3A gene (encoding the Fc gamma IIIa receptor, which influences the binding of the Fc portion of the immunoglobulin) that results in an amino acid-changing polymorphism (phenylalanine /valine, rs396991) has been shown to be associated with increased likelihood of response to TNF-alpha inhibitors in the treatment of RA. The aim of the present study was to determine whether this FCGR3A polymorphism is associated with clinical response to TNF-alpha inhibitors in Japanese RA patients.

Today, 2 structurally different TNF-alpha inhibitor are commercially available in Japan: infliximab and etanercept. DNA samples were obtained from 33 patients treated with infliximab and 4 patients with etanercept. Genotyping of rs396991 was performed using TaqMan SNP genotyping assay. Responses to the treatment were assessed using the DAS28 and the EULAR response criteria. Each patient was categorized as being a good or moderate responder (responder), or a nonresponder according to the therapeutic response at 22 weeks. Genotype comparisons were performed with 2X3 tables and calculation of Chi-square test.

Thirty-one of the patients were considered to be responders and 2 were nonresponders. Four patients dropped out due to side effects. The distribution of genotypes was found to be significantly different between the patients with a differential response to TNF-alpha inhibitors ($P = 0.00017$). We conclude that the FCGR3A polymorphism is associated with clinical response to TNF-alpha inhibitors in Japanese RA patients.

1056/T

Analytical Validation of TaqMan® Allelic Discrimination and Multiplex MALDI-TOF Assays for CYP2C9, CYP2D6 and CYP3A5 Genotyping. *J.A. Isler[1], A.M. Slager[1], W. Zhong[2], O.E. Vesterqvist[1], M.E. Burczynski[1].* 1) Biomarker Laboratory, Clinical Translational Medicine, Wyeth, Collegeville, PA; 2) Biological Technologies, Wyeth, Collegeville, PA.

Genotyping assays for the detection of a core set of functionally relevant polymorphisms in three major ethnic populations (Caucasian, African-American, and Asian) in the CYP2C9, CYP2D6 and CYP3A5 genes were analytically validated using the TaqMan® allelic discrimination and MALDI-TOF platforms. For CYP2D6, to avoid false genotyping results by non-specific co-amplification of the highly homologous pseudogenes CYP2D7P and CYP2D8P, a two round PCR strategy was designed to amplify a 6.4 kb region of the CYP2D6 gene prior to amplification of smaller regions containing the targeted polymorphism, and the strategy was coupled with multiplex primer extension MALDI-TOF assays. MALDI-TOF assays were also designed and validated for several CYP2C9 alleles, and TaqMan allelic discrimination assays were validated for detection of additional CYP2C9 and CYP3A5 alleles. Analytical validation of all assays demonstrated that the various methods were of suitable specificity, efficiency, reproducibility and accuracy for conducting genotyping assessments in clinical trials. Storage stability experiments demonstrated that the assays were efficacious when performed on genomic DNA isolated from whole blood stored frozen for up to at least 6 months. The assays were applied to commercially available DNA samples of various ethnicities to establish a sample database of known CYP2C9, CYP2D6 and CYP3A5 genotypes. Finally, to demonstrate the clinical utility of the validated assays, CYP2C9 and CYP2D6 assays were applied in an early phase clinical studies to classify the metabolizer status of subjects to illustrate the dependence of pharmacokinetic characteristics of administered CYP2C9-metabolized drug substrates on CYP2C9, but not CYP2D6, genotypes.

1057/T

Whole genome scan revealed responsible loci for responses to short term analgesics in humans. *H. Kim[1], E. Ramsay[1], S. Wahl[2], R.A. Dionne[1].* 1) NINR/NIH, Bethesda, MD; 2) NIDCR/NIH, Bethesda, MD.

Because of the complicated mesh of contributing factors and the thousands of molecules involved in different pain phenotypes, it is challenging to detect responsible genetic variations for an individual's unique susceptibility to pain. Most of genetic associations reported with pain phenotypes so far are weak and debatable. Response to analgesics is one of the most feasible pain phenotypes for the genetic association studies due to its relative simplicity and clinical relevance. To find pharmacogenomic responsible loci for analgesic response on a genome wide scale, we have genotyped 500,768 single nucleotide polymorphisms (SNPs) with comparative genomic hybridization in humans who underwent standardized surgical removal of impacted third molars. Data collected from a total of 112 European American patients were analyzed with Helix Tree and Copy Number Analysis Tool (CNAT). We found 8 loci that showed significant associations with analgesic onset time (AOT) at the level of $p < 4.2 \times 10^{-7}$. Further analysis revealed a genomic region spanning 6 SNPs along with approximately 60 kbs from rs2562456 to rs2562507 that separated by approximately two-order of magnitude ($p < 10^{-9}$) from the next highest associated SNP ($p = 3.5 \times 10^{-8}$). CNAT suggests copy number variations of 7 loci in longer AOT (> 30 min) subjects compared to shorter AOT (< 5 min) subjects. Further characterization of these regions with dense genotyping may identify genetic loci that contribute to interindividual variability in analgesic responses of humans in pain due to tissue injury and acute inflammation following minor surgery.

1058/T

Mu-opioid receptor A118G polymorphism is associated with susceptibility to nausea and vomiting in tramadol-treated patients of osteoarthritis. *E. Kim[1], CB. Choi[2], JS. Song[3], YM. Kang[4], CH. Suh[5], J. Lee[6], JY. Choe[7], CK. Lee[8], WT. Chung[9], HA. Kim[10], SC. Bae[2], C. Kang[1].* 1) Dept Biological Sci, KAIST, Daejon, Korea; 2) Dept Int Medicine, Hosp Rheumatic Diseases, Hanyang Univ Coll Medicine, Seoul, Korea; 3) Dept Rheumatology, Chung-Ang Univ Coll Medicine, Seoul, Korea; 4) Dept Int Medicine, Kyungpook National Univ Sch Medicine, Daegu, Korea; 5) Dept Int Medicine, Ajou Univ Sch Medicine, Suwon, Korea; 6) Dept Int Medicine, Ewha Womans Univ Coll Medicine, Seoul, Korea; 7) Dept Int Medicine, Catholic University of Daegu School of Medicine, Daegu, Korea; 8) Dept Int Medicine, Yeungnam Univ Coll Medicine, Daegu, Korea; 9) Dept Int Medicine, Dong-A Univ Coll Medicine, Busan, Korea; 10) Dept Int Medicine, Hallym Univ Coll Medicine, Chuncheon, Korea.

Tramadol used for the treatment of moderate to severe pains is also effective in treatment of osteoarthritis but can cause various adverse events. Clinically active metabolites of tramadol act as agonists against mu-opioid receptor distributed in the chemoreceptor trigger zone, which then activates the vomiting center possibly leading to emetic responses. Mu-opioid receptor plays a key role in pain control as a primary target of opioid drugs. The A118G polymorphism in mu-opioid receptor gene (*OPRM1*), although encoding for an Asn40Asp substitution, has been shown to drastically alter translation efficiency of mRNA and hence the protein level. In this study, 193 patients with osteoarthritis who had been treated with tramadol were recruited. Among them 106 patients did not show any side effects and the remaining 87 patients showed one or more side effects including nausea, dizziness, and vomiting. The A118G polymorphism was significantly associated with susceptibility to nausea and vomiting in tramadol-treated patients of osteoarthritis ($P = 0.025$, OR = 0.579, 95% CI = 0.358-0.937) in comparison between the patients ($n = 54$) having suffered from nausea or vomiting and those ($n = 139$) not having suffered from either. The results suggest that the minor-allele G118 carriers are better protected from the tramadol-caused emetic responses possibly by reducing the protein level.

1059/T

Relative Warfarin Resistance in Two Patients with VKORC1 g5417t (D36Y). *C. King[1], C. Eby[1,2], R. Porche-Sorbet[2], P. Ridker[3], V. Luzzi[1,2], B. Gage[1].* 1) Department of Internal Medicine, Washington University School of Medicine, Saint Louis, Missouri; 2) Department of Pathology & Immunology, Washington University School of Medicine, Saint Louis Missouri; 3) Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

Common polymorphisms in genes for vitamin K epoxide reductase (*VKORC1*) and cytochrome P450 2C9 (*CYP2C9*) affect warfarin requirements. However, they do not explain warfarin resistance, which runs in some families. Recently, investigators identified a new *VKORC1* Asp36Tyr (g5417t) polymorphism that predisposed to relative warfarin resistance in 15 Israeli patients and 2 German patients taking warfarin. Our goal was to quantify the prevalence and relevance of this polymorphism in a diverse American population. We identified outliers whose ratio of therapeutic to predicted warfarin dose (based on *CYP2C9* & *VKORC1*-1639 genotype, body surface area, race, smoking, and medications) was either above the 90th (high-dose) or below the 10th (low-dose) percentiles. From a parent cohort of 900 patients taking warfarin, 39 outliers were African-American, 70 were Caucasian, and 3 identified as Hispanic. After primers were designed, PCR and Pyrosequencing were used to examine Asp36Tyr. None of the 54 low-dose outliers had this *VKORC1* variant. Two of 58 high-dose outliers carried the variant. The first was a homozygous (TT) patient with a therapeutic warfarin dose of 10 mg/day, 200% of what was predicted by the pharmacogenetic-based dosing algorithm at www.WarfarinDosing.org. The second patient was heterozygous (GT) with a therapeutic dose of 3.6, 170% of what was predicted in this elderly patient. Both patients were Caucasian. Larger studies of this polymorphism in patients taking warfarin are indicated.

1060/T

Genome-Wide Association study of Treatment Emergent Suicidal Ideation in the STAR*D Sample. *G. Laje[1], N. Akula[1], A.S. Allen[2], S. Paddock[3], H.K. Manji[4], A.J. Rush[5], D. Charney[6], F.J. McMahon[1].* 1) Genetic Basis of Mood and Anxiety Disorders, Mood and Anxiety Program, National Institute of Mental Health, National Institutes of Health, USDHHS, Bethesda, MD; 2) Dept of Biostatistics and Bioinformatics at Duke University, Durham, NC; 3) Karolinska Institutet, Stockholm, Sweden; 4) Laboratory of Molecular Pathophysiology, Mood & Anxiety Program, National Institute of Mental Health, National Institutes of Health, Dept. of Health & Human Services, Bethesda, MD; 5) Depts. of Clinical Sciences and Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX; 6) Mount Sinai School of Medicine, New York, NY.

Background: Suicidal ideation is an uncommon but potentially dangerous symptom than can emerge during antidepressant treatment. We have previously described association between treatment emergent suicidal ideation (TESI) and markers in GRIK2 and GRIA3. Now we have undertaken a genome-wide association study to search for additional genetic markers that may shed light on the causes of TESI and help identify individuals at high-risk who may benefit from closer monitoring, alternative treatments, and/or specialty care. Methods: A clinically-representative cohort of outpatients with nonpsychotic major depressive disorder who enrolled in the STAR*D trial were treated with citalopram under a standard protocol for up to 14 weeks. DNA samples from 90 white participants who developed TESI and equal number of treated participants who denied suicidal ideas were genotyped with 109,000 single nucleotide polymorphisms on the Illumina Infinium I chip. Findings: Two additional markers were significantly associated with TESI in this sample (marker rs10903034, allelic p =2.77 x 10-6, OR = 2.7; marker rs11628713, allelic p= 2.75 x 10-7, OR = 4.7). These markers reside within the genes IL28R and PAPLN, respectively. Conclusion: IL28R encodes an interleukin receptor and PAPLN encodes papilin, a proteoglycan-like sulfated glycoprotein. Together with our previous report, these findings may shed light on the biological basis of TESI and may help identify patients at increased risk of this potentially dangerous adverse event.

1061/T

μ-opioid receptor binding affinity to fentanyl is affected by sex but not by 304A/G polymorphism. *R. Landau[1], I. Charvet[1], J.-L. Blouin[2].* 1) Anesthesia, University Hospital of Geneva, Switzerland; 2) Genetic Medicine, University Hospital of Geneva, Switzerland.

Opioids are widely used for pain management even though large inter-individual variability in efficacy exists. A number of sequence variability in μ-opioid receptor gene (OPRM1) including non synonymous SNPs could explain some differences in analgesic response. The rs1799971 (c.304A/G, p.102Asn/Asp) is the most frequent non-synonymous variant since minor allele frequency is up to 49% (Hapmap-JPT). In vitro the G allele increases binding affinity of β-endorphin; women with the G allele have reduced spinal fentanyl requirements during labor analgesia. We tested whether Asn102Asp of OPRM1 increases binding affinity to fentanyl. In this IRB-approved study, 24 volunteers were genotyped for 102Asn/Asp of OPRM1. The ability of fentanyl to displace 3[H]-naloxone bound to freshly isolated lymphocytes was determined with radioligand assays (GraphPad software). A binding affinity constant (Ki) was also calculated in 4 selected subjects. While Asn102Asp of OPRM1 did not affect μOR binding affinity, we found a significant difference between ♂ and ♀.

Relative 'naloxone displacement ability' of fentanyl (%, *p<0.0001)

Men	%	Women	%
Total (n=12)	41 +/- 3	Total (n=12)	75 +/- 5 *
A/A (n=9)	40 +/-4	A/A (n=8)	73 +/- 4
A/G (n=3)	44 +/-2	A/G (n=3), G/G (n=1)	77 +/- 6

This is congruent with known sex differences in opioid analgesia and may explain why ♀ require less opioids for pain management. However other pathways such as expression, transduction or receptor trafficking rather than μOR binding should be explored to investigate mechanisms by which Asn102Asp of OPRM1 affects the clinical response to opioid therapy.

1062/T

BCHE genotype and clinical phenotype in patients following the muscle relaxant succinylcholine. *S. Levano, E. Schobinger, A. Matter, M. Singer, A. Urwyler, T. Girard.* Departments of Anesthesia and Research, University Hospital Basel, Switzerland.

Butyrylcholinesterase (BCHE) quickly hydrolyzes drugs containing ester bonds such as succinylcholine (SCh) and mivacurium widely used in anesthesia. However a delayed hydrolysis of these short acting neuromuscular blocking drugs is observed in patients with acquired or inherited reduced BCHE activity. The aim of this study was to compare the detected mutations in BCHE gene with the clinical phenotype. In those patients receiving a standard dose of SCh (1mg/kg) the time of recovery of neuromuscular function was recorded. In addition 20 patients with a normal duration of neuromuscular blockade (< 10 min) were included. The patient physical status was classified according to the American society of anesthesiology (ASA) grading system. The BCHE gene was analysed by dHPLC and sequencing. Prolonged muscle relaxation (≥10 min) was found in 221 out of 1480 patients (14.9%) with a median duration of 13.35 min (10.2 to 44 minutes). Most of the patients were classified as ASA II (n= 768) and ASA III (n=542). The average age of the patients was 64.3 years (18 to 97 years). Performing genetic analysis in 176 out of 221 patients the most frequently mutations in heterozygous forms were A209G (A-variant, 12.5%), G1615A (K-variant, 36.4%) and G1169T (F2-variant, 2.3%). The K-variant in homozygous form was detected in 18 cases. Double carriers of A- and K-variants were found in 19 patients. In addition single patients have shown recurrent and novel BCHE mutations. Multivariate linear regression analysis revealed a significant influence of ASA status ($p<0.001$) and genotype ($p<0.001$) but not age. The influence of the different genotypes compared to wild type was significant for the A-variant ($p<0.001$) and the F-variant ($p<0.05$) but not for the K-variant. In summary, prolonged neuromuscular blockade was measured in 15% of patients. About 50% of these patients were carriers of BCHE mutations. ASA physical status and BCHE genotype had a significant influence on the duration of action of SCh. The K-variant had no significant influence, neither in the heterozygous, nor in the homozygous state.

1063/T

Development of a cardiovascular risk panel For use in Clinical pharmacogenomics studies. *I. Mongrain[1], A.M.K. Brown[1], Y. Renaud[1], N. Gaudreault[1], J. Engert[2], M.S. Phillips[1].* 1) Genome Quebec & Montreal Heart Institute Pharmacogenomics Centre, Montreal, Quebec, Canada; 2) Victoria Hospital, McGill University, Montreal, Quebec, Canada &n.

Researchers are now working to identify genetic biomarkers that may be predictive of the pathogenesis of cardiovascular disease (CVD) and adverse drug reactions. Our group has developed clinical grade genotyping panels that may aid physicians evaluate a patient's risk of developing CVD, as well as directing physicians to more appropriate choices of drug therapies and dosing regimes. Our focused functional CVD panel consists of over 200 markers covering ~25 genes involved in lipid metabolism, notably APOE, LPL and CETP. Our panel consists of both functional and haplotype tag SNPs to evaluate genotype-phenotype interactions. This CVD risk panel has been developed using the following strategies: 1) Panel development is performed in parallel using two technologies (SNPstream, and Sequenom); 2) Assays are designed and developed for both DNA directions; and 3) Genotyping calls are validated against known genomic controls. In order to validate the CVD risk panel, the results of 2000 genotyping calls from 80 markers were compared to previous genotyping data with 99.9% concordance. This panel is now being used to screen a cohort composed of 284 patients with extreme HDL-cholesterol levels for an association study. We are currently developing a much larger broad-based statin-related gene panel (~4500 markers) using the iSelect chip on Illumina's Infinium platform. These panels will be used to support a large scale Genome Canada/Genome Quebec pharmacogenomics research project on statin myotoxicity at the Université de Montréal and the Montreal Heart Institute. As part of this project, the panel will be used to analyze 5000 patients presenting clear clinically relevant muscular intolerance phenotypes.

1064/T

Pilot survey on patient attitudes toward pharmacogenetic testing. *J. O'Daniel[1], P. Deverka[1], J. Lucas[2], G. Silvey[3], D.F. Lobach[3], S.B. Haga[1].* 1) Inst for Genome Sci & Policy, Duke Univ Med Ctr, Durham, NC; 2) Inst of Statistics & Decision Sciences, Duke Univ, Durham, NC; 3) Dept of Community and Family Medicine, Duke Univ Med Ctr, Durham, NC.

The successful integration of pharmacogenetic (PGx) testing into clinical care will require attention to patient perceptions and attitudes toward these tests. In this study, we aimed to identify the major reasons patients would or would not undergo PGx testing and whether these factors differed by socioeconomic and medical history variables. To achieve this goal, we developed a survey offered on hand-held computer tablets. The survey was evaluated by two focus groups for understandability and ease-of-use and piloted on the adult patient population of the Duke Family Medicine Center. Seventy-five completed surveys were collected. Seventy-two percent of respondents were female and 65% were African-American. Forty-nine percent were employed full-time, 42% had a high school education or less, and 57% had an annual income of $40,000 or less. About a third of respondents had a previous history of a side effect. The data were analyzed using linear-by-linear logistic regression after responses were clustered to achieve an ordinal relationship. Higher education was positively correlated with being more likely to consider PGx testing to reduce the risk of mild side effects only (p= .002), but was negatively correlated if respondents did not believe the test would help their doctor select which medication would work best or be safest (p=.004). Higher income was positively associated with being likely to consider PGx testing to determine which medicine is safest (p=. 001). Respondents with government insurance were more likely to request further information about PGx testing than those with no insurance or private insurance (p= .0012). Also, of the 23% of respondents who were unsure or unlikely to take a PGx test, only 18% of these respondents would change their mind if the test were not gene-based. These preliminary findings provide a glimpse into the attitudes of patients with diverse ethnic and economic backgrounds towards PGx testing and warrant further exploration.

1065/T

Evaluation of Commercial Platforms for Rapid Genotyping of Polymorphisms Affecting Therapeutic Warfarin Dose. *R. Porche-Sorbet[1], C. King[2], B. Gage[2], P. Ridker[3], A. Brown[4], Y. Renaud[4], M. Phillips[4], C. Eby[1,2].* 1) Department of Pathology & Immunology, Washington University School of Medicine, Saint Louis Missouri; 2) Department of Medicine, Washington University School of Medicine, Saint Louis Missouri; 3) Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; 4) Genome Quebec and Montreal Heart Institute Pharmacogenomics Center, Canada.

Warfarin initiation is challenging due to the risk of bleeding and the wide variability in therapeutic dose. A third of this variability is accounted for by patients' genotypes for 3 single nucleotide polymorphisms (SNPs): 2 in cytochrome P450 2C9 (CYP2C9) gene that slow warfarin metabolism (*2 and *3), and 1 in the promoter of vitamin K reductase (VKORC1) that affects warfarin sensitivity. Our goal was to compare the accuracy and efficiency of 3 commercial genotyping systems for these SNPs in 112 DNA archived samples. We evaluated 3 commercial genotyping platforms: INFINITI (Autogenomics, Carlsbad, CA) automated multiplex microarray platform; Invader® (Third Wave Technologies, Madison, WI) assay which employs Cleavase endonuclease enzymes; and Tag-it® Mutation Detection Assay (Tm Bioscience, Toronto, CA) which uses microspheres on a Luminex® 200 xMAP instrument (Austin, TX). The gold standard was Pyrosequencing® (Uppsala, Sweden) and direct sequencing. All three platforms were accurate, but they differ based on other characteristics.

Characteristics of Reagent-Instrument Platforms

Platform	Time (Hrs)	Complexity	DNA (ng)	CYP2C9 SNPs	VKORC1 SNPs
INFINITI	8	Low	50	*2,3,5,6,11	-1639(8 more)
Invader®	3	Moderate	250	*2,3	-1639
Tag-it®	8	High	15	*2,3,5,6	-1639(6 more)

.

1066/T

Sex-specific effects of GPRK haplotypes on metoprolol response. *E. Richard[1], M.S. Lipkowitz[2], D.T. O'Connor[1], N.J. Schork[1,3], R. Salem[1], V. Bhatnagar[1,4], AASK Study Committe.* 1) University of California San Diego, La Jolla, CA; 2) Mount Sinai School of Medicine, New York, NY; 3) Scirpps Institute for Genomic Medicine, La Jolla, CA; 4) VA San Diego, San Diego, CA.

Purpose: The objective of the study is to analyze sex-specific differences between genetic variants in the G protein-coupled receptor kinase 4 (GRK4) gene and blood pressure response to metoprolol among African Americans with early hypertensive nephrosclerosis. Methods: 197 men and 131 women randomized to treatment with metoprolol from the African American Study of Kidney Disease and Hypertension were genotyped at Ala142Val and Ala486Val. Of these, 161 were randomized to an aggressive treatment group and 167 to a usual treatment group. Mean arterial pressure (MAP) averages were determined over several time points within the first 200 days after randomization. MAP differences among GRK4 haplotypes were examined using repeated-measures ANOVA within a 45-day time frame and a 200-day time frame, adjusting for baseline MAP and other medications; analyses were carried out stratified by sex and treatment group. Results: Men and women were of similar ages, mean of 55 (standard deviation 10) years; men and women had similar baseline MAPs, mean of 113 (13) mmHg. Men had higher baseline glomerlular filtration rates, 48 (12) versus 45 (13) ml/min (p=.02); men had a lower body mass index, 31 (6) versus 32 (7) kg/m2 (p=.05). Genotype distributions were in Hardy-Weinberg Equilibrium. Among women only, those with Val142/Ala486 homozygous haplotypes responded significantly faster than Val142/Ala486 heterozygous haplotypes (p=0.045 for haplotype by time interaction). This effect was more significant among women randomized to usual treatment (p= 0.012). Although women randomized to the aggressive treatment group with homozygous Val142/Ala486 haplotypes had significantly lower MAP values (p=0.033), the interaction between time and haplotype was not significant. Conclusions: African-American women randomized to usual treatment goals with homozygous Val142/Ala486 haplotypes responded faster to metoprolol. Genotyping may help identify women may need to be treated more aggressively with beta-blockers.

1067/T

Warfarin Pharmacogenetics: VKORC1 Allele and CYP2C9/VKORC1 Genotype Frequencies in the Ashkenazi and Sephardi Jewish Populations. *S.A. Scott, L. Edelmann, R. Kornreich, R.J. Desnick.* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York, 10029.

Warfarin is a widely used anticoagulant with a narrow therapeutic range due to both environmental (e.g., vitamin K intake, co-medications, body surface area, etc.) and genetic factors. CYP2C9*2 (p.R144C) and *3 (p.I359L) and the VKORC1 promoter polymorphism (g.-1639G>A) frequently occur in patients who require a lower average warfarin dose, while patients with VKORC1 missense mutations require higher warfarin doses. To determine and compare the CYP2C9 and VKORC1 allele and genotype frequencies among Ashkenazi (AJ) and Sephardi Jewish (SJ) individuals, genotyping was performed on 260 AJ and 80 SJ individuals from the greater New York metropolitan area. Genotyping of 14 different CYP2C9 and VKORC1 alleles was performed using the Tag-It Mutation Detection Kit (Luminex Molecular Diagnostics); the recently identified VKORC1 p.D36Y mutation associated with warfarin resistance was analyzed using a PCR-RFLP assay. The AJ CYP2C9*1, *2, *3, and *5 (p.D360E) allele frequencies were 0.790, 0.127, 0.081 and 0.001, and the SJ frequencies were 0.663, 0.194, 0.144 and 0.000, respectively. Of note, the SJ CYP2C9 frequencies were determined for the first time and were significantly different from those observed in the AJ ($p \leq 0.025$). The VKORC1 g.-1639A allele was found at a high frequency in both AJ (0.467) and SJ (0.500) individuals whereas the warfarin resistant p.D36Y mutation had significantly different ($p \leq 0.025$) allele frequencies in the AJ (0.043) and SJ (0.006). All the CYP2C9 and VKORC1 allele frequencies were in Hardy-Weinberg equilibrium and CYP2C9*4 and *6, and the remaining VKORC1 coding region mutations analyzed by the Tag-It system were not detected in the AJ and SJ cohorts. Based on the recently described warfarin dosing algorithms that incorporate CYP2C9*2, *3 and VKORC1 g.-1639A, and given that in our cohorts 220 AJ and 72 SJ had a deficient CYP2C9 and/or VKORC1 allele, our results indicate that 84.6% of AJ and 90.0% of SJ individuals would benefit from genotyping prior to warfarin administration.

1068/T

IMPACT OF POLYMORPHISMS IN CANDIDATE GENES FOR PHARMACORESISTANCE IN MESIAL TEMPORAL LOBE EPILEPSY. *M.S. Silva[1], K.M. Siqueira[1], N.C. Ianni[1], E. Bilevicius[2], F. Cendes[2], I. Lopes-Cendes[1].* 1) Medical Genetics, FCM - Unicamp, Campinas, Sao Paulo, Brazil; 2) Neurology, FCM - Unicamp, Campinas, São Paulo, Brazil.

Mesial temporal lobe epilepsy (MTLE) is associated with the highest proportion of the drug-resistant patients. One hypothesis to explain differences in drug response in epilepsy treatment is the association with pharmacogenetic differences present in genes related to drug-metabolism and ion channels. Therefore, allelic variations in these genes could be responsible for degreased efficiency of antiepileptic drugs and failure to control seizures. The purpose of this study was to investigate whether single nucleotide polymorphisms (SNPs) on drug-transporter genes (ATP-binding cassette family: ABCB1, ABCC2, ABCC4; and RLIP76-ralA-binding-protein1) and ion channels (SCN11A-Na+channel α subunit; CACNA1B-Ca+2channel α1B subunit) could be associated with pharmacoresistance in a large group of MTLE patients. We chose 11 validated SNPs in dbSNPs database: rs12680, rs 12454987, rs8092935 (RALBP1); rs2235039, rs282564, rs2229109, rs3213619 (ABCB1); 2273697(ABCC2); 2274407(ABCC4); 2298771(SCN11A); 4422842(CACNA1B). Genotyping was carried out using the TaqMan system(Applied Biosystems). We included 90 drug-resistant MTLE patients and compared with 60 drug-responsive MTLE patients. Genotypic frequencies were in Hard-Weinberg equilibrium in both groups and no significant allelic differences were observed, for any of the SNPs tested, between the two groups ($p>0.01$). In addition, no differences were found between the allelic frequencies in both groups and the NCBI SNPs database. In conclusion, we found a lack of correlation between SNPs in candidate genes associated with drug-metabolism and ion channels and pharmacoresistance in MTLE.

1069/T

Association between the genetic polymorphisms in *DRD2* and the risk of tardive dyskinesia: a meta-analysis study. *H-T. Tsai.* Epidemiology Department, University of North Carolina-Chapel Hill, Chapel Hill, NC.

Background Tardive dyskinesia (TD), an involuntary movement disorder, is a serious and potentially irreversible adverse effect from antipsychotic therapy. Current understanding about TD is very limited. Genetic variants in dopamine receptor 2 (*DRD2*) have been studied for their associations with TD. However, study findings are very controversial.

Objectives To understand association between genetic variants in *DRD2* and TD among patients with schizophrenia, a meta-analysis study was conducted.

Methods A systematic search of literature was performed through a cross-search of several databases. Three genetic variants in DRD2 were studied: *TaqI*, rs1801028(*Ser311Cys*), and *-141C Ins/ Del*. Genotypic effects were compared in general, dominant and recessive models. Publication bias and heterogeneity test across studies was examined. Meta-regression analyses were also implemented using STATA 8.1.

Results Seven studies were identified for *-141C Ins/ Del*, with a total of 1724 schizophrenics (582 TD, 1142 non-TD) from Asia (4 studies), Europe (2 studies) and a mix of Europeans and African Americans (1 studies). Five studies about rs1801028 (462 TD, 964 non-TD), and *TaqI* A (390 TD, 549 non-TD) were collected. Schizophrenics with A2/A1 or A2/A2 genotype in *TaqI* A showed a lower risk of TD than those with A2/A2 genotype: OR $_{A1/A1+\ A2/A1\ vs.\ A2/A2}$ = 0.63 (95% C.I.= 0.47-0.85). No association was found in the analysis of TD with *-141C Ins/ Del*: OR $_{Del/Del+\ Ins/Del\ vs.\ Ins/Ins}$= 0.96 (95% C.I.= 0.65-1.43); and with rs1801028: OR$_{Cys/Cys\ vs.\ Ser/Ser}$ = 1.43 (95% C.I.= 0.77-2.62), OR $_{Ser/Cys\ vs.\ Ser/Ser}$ = 0.82 (95% C.I.= 0.49- 1.34). Heterogeneity or publication bias was not detected in this study.

Conclusions A systematic analysis of literature supported the association between *DRD2/ TaqI* A and TD among schizophrenics, but did not support associations between TD and *-141C Ins/ Del* or rs1801028 in *DRD2*.

1070/T

A polymorphism of the μ-opioid receptor influences analgesic response in labor. *J.-L. Blouin[1], C. Kern[2], M.O. Columb[3], R.M. Smiley[4], R. Landau[2].* 1) Genetic Medicine , Univ Hosps Geneva, Geneva, Switzerland; 2) Anesthesiology, Univ Hops Geneva, Geneva, Switzerland; 3) Dept. Anesthesia, University Hospital of South Manchester Wythenshawe, UK; 4) Dept. Anesthesiology, Columbia University College of Physicians and Surgeons, New York, NY.

Opioids are increasingly used to provide analgesia to women in labor. However a substantial inter-individual variability in the analgesic response is observed and could be explained by sequence variants in the μ-opioid receptor gene (OPRM1). It has been shown in vitro that minor allele (G) of the non-synonymous SNP rs1799971 (c.304A/G, p.102Asn/Asp), increases the binding affinity and potency of β-endorphin (3-fold). The aim was to determine whether the 304A>G variant could modify the analgesic (fentanyl) dose requirement during combined spinal-epidural analgesia in labor. Nulliparous women (n=224) were genotyped at 33 to 37 weeks gestation and clustered in 2 groups (Group A =genotype A/A, Group G =A/G, G/G). To determine the median effective dose of fentanyl (ED50), women were allocated to either up-down sequential (SA) or random dose (RA) allocation approaches. For the SA, initial dose was 18μg (interval of 2μg); for the RA, doses ranged 2.5-35μg (log increments). ED50 was estimated with the Dixon-Massey method and probit regression for SA and probit and logistic regression for RA with P<0.05 as significant. Prevalence of the G allele was 33%. In the SA part, ED50 was 26.8μg (95%CI, 22.7-30.9) in group A (n=26) and 17.7μg (95%CI, 13.4-21.9) in group G (n=24) (P<0.001); the G allele increased sensitivity to fentanyl by factor of 1.51 (95% CI 1.18-2.01). In the RA part, the ED50 was 27.4μg (95% CI 22.5-32.2) in group A (n= 80), and 12.8μg (95% CI 5.5-20.0) in group G (n=24), P<0.002. The G allele (RA study) increased the sensitivity to fentanyl by a factor of 2.14 (95% CI 1.30-5.17). We have demonstrated a 1.5 fold highly significant potentiation of spinal fentanyl effect by 304G allele of OPRM1 using the up-down sequential allocation method and replicated these results using random dose allocation. These findings suggest that OPRM1 polymorphism plays a significant role in inter-individual spinal opioid requirements.

1071/T

Whole Genome Association Study of Response to Citalopram in the STAR*D Sample. *H.A. Garriock[1], J.B. Kraft[1], E.J. Peters[1], G.D. Jenkins[2], M.S. Reinalda[2], P.J. McGrath[3], S.L. Slager[2], S.P. Hamilton[1].* 1) Department of Psychiatry and Institute for Human Genetics, University of California, San Francisco, CA; 2) Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 3) New York State Psychiatric Institute and Columbia University, New York City, NY.

Background: Inter-individual variability in response to antidepressants is thought to be influenced at least in part by DNA variation. To date, candidate gene approaches to antidepressant response have led to results of marginal impact. We have thus undertaken a genome-wide association study to look for novel genetic determinants of antidepressant response in a large clinical sample. Methods: We used a subset of subjects enrolled in the antidepressant trial Sequenced Treatment Alternatives to Relieve Depression (STAR*D). The number of subjects giving DNA was 1,953 and thus represents our sample in its entirety. Our citalopram response phenotypes included response (≥50% reduction in QIDS-SR), remission, and drug tolerance. In a two-stage design, we genotyped part of the sample for 590,000 SNPs (N= 831), and carried out a replication analysis in the remaining sample (N=835). Results: Markers were tested for association using Armitage trend test and results were ordered on the basis of the p-value. In our most highly powered discovery sample (white, non-hispanic) and combined across the three phenotypes, we observed about 185 markers with p-values less than 0.0001, with associated dominant odds ratios for SNP with MAF >0.05 ranging from 1.58-13.4. Assessment of our replication sample is underway. Conclusions: Results from the first stage of the study must still be confirmed in the validation stage. Data from the first stage of our study have implicated a large number of previously unconsidered loci involved in antidepressant response.

1072/F

Mice lacking the kinase domain of the Src protein develop osteopetrosis and lack incisors. *E. Ivakine[1], R. Zirngibl[2], C. Jung[1, 2], J. Aubin[2], R. McInnes[1, 2].* 1) Program in Developmental and Stem Cell Biology, Hospital for Sick Children Research Institute, Toronto, ON, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

The protein tyrosine kinase Src participates in diverse biological processes ranging from cell growth and differentiation to signaling and adhesion. It is expressed in a large variety of tissues with highest levels in brain, platelets and osteoclasts. Mice with a targeted disruption of the *Src* gene develop severe osteopetrosis due to impaired osteoclast function. The Src 60 kDa protein has a modular structure with a unique domain, and SH2, SH3 and kinase domains. However, whether the kinase domain is essential for osteoclast function has been controversial (Genes & Dev. 11:2835, 1997 vs JBC 279: 17660, 2004). Here we describe the positional cloning and characterization of a novel spontaneous *Src* gene allele (Src^{thl}). An insertion of a C nucleotide into *Src* exon 12 leads to a frameshift and a premature stop codon. The mutation predicts a deletion of 1/2 of the kinase domain, and immunoblots of $Src^{thl/thl}$ brain lysates identify a truncated 37kDa Src protein. At birth, $Src^{thl/thl}$ mice appear normal, but by 12 days of age can be easily identified by their small size, lack of incisors and variable numbers of molar teeth (usually 1-2 are absent). 65% of $Src^{thl/thl}$ mice maintained on a mashed food diet survive up to 6 months of age; the remainder die from as yet unidentified causes. Importantly, all the $Src^{thl/thl}$ mice have increased bone mineral density by 8 weeks of age and develop severe osteopetrosis indistinguishable from *Src*-null mice. We conclude, therefore, that the kinase function of *Src* is indeed essential for bone remodeling and that the lack of the kinase activity causes osteopetrosis in mice. Given the pleiotropic roles of the Src protein, this novel *Src* allele will facilitate studies that distingish between kinase-dependent and kinase-independent functions of the Src protein in a range of biological processes.

1073/F

Identification of a novel mutation for Cleidocranial dysplasia (CCD). *M.T.M. Lee[1], C.H. Chou[1], F.M. Sun[1], J.Y. Wu[1], F.J. Tsai[2], Y.T. Chen[1].* 1) National Genotoyping Ctr, Academia Sinica, Taipei, Taiwan; 2) China Medical University, Taichung, Taiwan.

Cleidocranial dysplasia (CCD; MIM 119600) is a rare autosomal dominant human skeletal disorder. The clinical features of CCD are facial and dental malformations characterized by delayed closure fontanelles, frontal bossing, absent clavicles, short stature, late eruption and supernumerary permanent teeth and other skeletal anomalies. Mutations causing CCD has been mapped to the RUNX2 (also known as CBFA1, PEBP2αA and AML3) gene located on chromosome 6p21. It is one of the three mammalian homologs of the Drosophila runt gene, which is a transcription factor required for osteoblast differentiation. RUNX2 spans a region over 220-kb in 6p21 and is composed of 8 exons. Heterozygous loss of function of CBFA1 appeared to be sufficient to cause CCD. A number of patients with CCD phenotypes also exist without any identifiable mutations in the RUNX2 gene. We recruited a family with CCD phenotypes from the China Medical University. The affected members of this family had the rarer CCD phenotype such as hypoplasisa in the distal phalanges and middle phalanges had cone-shaped epiphyses in addition to the phenotypes described above. Direct sequencing analysis revealed no mutations in the promoter, coding regions and the splice sites of RUNX2. Deletions in the RUNX2 gene were rare, nonetheless, the possibility of RUNX2 deletion in this family could not excluded by sequencing. Real Time quantitative PCR was performed to determine the copy number of each of the exons and 3'UTR. qPCR results and revealed that RUNX2 for the CCD patients in this family was deleted from exon 1 to exon 6 as indicated by the copy number of 1 while the normal individuals had normal copy number of 2. This was then confirmed by Southern blot analysis and the exact breakage point was mapped by sequencing the circular DNA generated from inverse PCR around the cleavage site. The deletion mutation presented in this study was the first intragenic deletion described for CCD. Use of qPCR was more sensitive than FISH analysis and could explain some of the CCD patients without any identifiable mutations in RUNX2.

1074/F

FBN2, FBN1, TGFBR1, and TGFBR2 analyses in congenital contractural arachnodactyly. *N.M. Matsumoto[1,2], A.N. Nishimura[1], H.S. Sakai[1], H.S. Saitsu[1], T.M. Mizuguchi[1].* 1) Dept Human Genetics, Yokohama City Univ Grad Sch Med, Yokohama, Japan; 2) SORST, JST, Kawaguchi, Japan.

FBN2, FBN1, TGFBR1, and TGFBR2 were analyzed by direct sequencing in 15 probands with suspected congenital contractural arachnodactyly (CCA). A total of four novel FBN2 mutations were found in four probands (27%, 4/15), but remaining 11 patients did not show any abnormality in either of the genes. This study indicated that FBN2 mutations were major abnormality in CCA, and TGFBR and FBN1 defects may not be responsible for the disorder. FBN2 mutations were only found at introns 30, 31, and 35 in this study. Thus analysis of a mutational hotspot from exons 22-36 (a middle part) of FBN2 should be prioritized in CCA as previously suggested. Collaborating doctors are acknowledged: Drs. Shiro Ikegawa, Hiroshi Kitoh, Nobuyuki Haga, Satoshi Ishikiriyama, Toshiro Nagai, Fumio Takada, Takako Ohata, Fumihiko Tanaka, and Hotaka Kamasaki.

1075/F

A novel missense mutation in the cartilage-derived morphogenetic protein 1 (CDMP1) gene of Grebe Type Chondrodysplsia patients in a Saudi Arabian family. *M. Ul Haque[1,3], E.A. Faqeih[2], H. Al-Zaidan[3], A. Al-Shammary[1], S.H.E. Zaidi[4].* 1) Molecular Genetics Lab, DPLM, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) Department of Pediatric Medicine, Children's Hospital, King Fahad Medical City, Riyadh, Saudi Arabia; 3) Department of Medical Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 4) Department of Medicine, University Health Network & University of Toronto, Toronto, Ontario, Canada.

Grebe type chondrodysplasia is a rare autosomal recessive skeletal disorder in which affected subjects exhibit markedly shortened limbs and tiny digits without abnormalities of the axial skeleton. This defect results from loss of function mutations in the cartilage-derived morphogenetic protein 1 (CDMP1) gene. Here we report a consanguineous family from Saudi Arabia, in which three children in two sib-ships display this rare disorder. All affected subjects exhibit typical features of Grebe type chondrodysplasia, which include severely shortened hands and legs, and appendage like fingers. In addition, these patients exhibit occipital prominence, bi-temporal narrowing and dental anomalies. Sequencing of the CDMP1 gene of the affected children identified a novel c.1285T>C change in the gene. The unaffected parents, who had no apparent skeletal abnormalities, were heterozygous for this mutation. In the amplified CDMP1 gene, the mutation creates a BstU1 cleavage site. The expected digestion pattern co-segregated in the heterozygous carriers and the affected individuals of this family. No BstU1 digestion was observed in the amplified CDMP1 genes of 100 control subjects. This mutation is predicted to result in a p.Cys429Arg substitution in the 2nd of the seven cysteines, which are highly conserved among various members of the transforming growth factor beta (TGF-beta) super family. It is possible that the replacement of Cys429 with an arginine has caused incorrect disulphide bond formations among the remaining six cysteines, resulting in an altered structure/activity of the mutant CDMP1 protein. It is likely that this has manifested in the children as Grebe-type chondrodysplasia with additional abnormalities that are unique to these patients.

1076/F

Genetic analysis of sporadic and familial ataxia in Wales. *E. Majounie[1], M. Wardle[1], M. Muzaimi[1], W. Cross[1], H.R. Morris[1], N.M Williams[2], N.P. Robertson[1].* 1) Department of Neurology, Cardiff University, Cardiff, United Kingdom; 2) Department of Psychological Medecine, Cardiff University, Cardiff, United Kingdom.

Autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders. To date, 13 autosomal dominant ataxia genes have been identified from familial studies. However, the majority of cases of adult onset ataxia are sporadic. Our aim was to determine whether non-pathogenic repeat length size influences the risk of ataxia. We used a fluorescent PCR approach using both flanking and intra-repeat primers, to analyze 10 candidate genes (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, DRPLA) in 112 sporadic ataxia patients, 26 unrelated patients with a family history of ataxia and 306 Welsh Caucasian blood donor controls. Patients with pathogenic SCA or DRPLA expansions were excluded, and we identified SCA6 and DRPLA as the two commonest causes of ADCA in Wales. We observed that the distribution of the larger normal allele (top 10%) in the Welsh population was similar to that previously reported in other Caucasian populations. Generally, the alleles most commonly represented in other Caucasian groups were also most common in the Welsh population. For each of the repeat expansion genes, there was no difference between repeat lengths in cases and controls, for either familial or sporadic ataxia cases. Testing for an additive effect of CAG repeats from the different genes also showed no difference between the cases and controls. Of note however, the proportion of DRPLA alleles over 19 repeats in the Welsh control samples reached 4.2% (13/306). While not as high as that reported in the Asian population (7.4%), this proportion considerably differs from that found in North American Caucasian populations (0%) and corresponds to a high prevalence of DRPLA amongst Welsh ADCA families. In conclusion, large normal repeat expansion size in known ataxia genes is not a risk factor for ataxia in the Welsh population.

1077/F

A novel duplication confirms the involvement of chromosome 5q23.2 in autosomal dominant leukodystrophy. *I.A. Meijer[1], A. Simoes Lopes[1], S. Laurent[1], T. Katz[1], D.J. Verlaan[1], S. Verreault[2], J-P. Bouchard[2], G.A. Rouleau[1].* 1) Centre de Recherche du CHUM, Hôpital Notre Dame, Université de Montréal, Montréal, PQ, Canada; 2) Hôpital de l'Enfant-Jésus, Québec City, Québec, Canada.

Leukodystrophies are a group of neurogenetic diseases characterized by demyelinisation of the central and peripheral nervous system. Previously, a locus on chromosome 5q23 was identified in five families with adult-onset autosomal dominant leukodystrophy. The reported critical genetic interval is flanked by markers D5S1495 and CTT/CCT15, which span 0.96 cM or 1.47 Mb (L. Marklund et al 2006). This region contains 13 known and putative candidate genes.

We have identified a large multigenerational French Canadian family with autosomal dominant adult-onset leukodystrophy. Six living affected family members were available for collection and presented with autonomic dysfunction as well as upper motorneuron signs affecting gait. Peripheral blood samples were obtained for DNA extraction and for the generation of lymphoblastoid cell lines. Two point linkage analysis confirmed linkage of this family to chromosome 5q23. In addition, a candidate gene screen of all the 13 genes was completed and no mutation was found.

Therefore a whole chromosome Comparative Genomic Hybridization (Nimblegen) for chromosome 5 was performed. The CGH analysis identified a 280 kb duplication within the chromosomal band 5q23.2 in the two tested affected individuals. The two samples were compared to a reference sample consisting of pooled DNA of 6 healthy individuals. The 280 kb duplication contains 3 genes, namely *LMNB1*, *FLJ36242* and *MARCH3*.

Our study supports the findings of Padiath et al. 2006 implicating duplicated *LMNB1* as the disease causing mutation. Further studies are necessary to elucidate the pathophysiology of lamin B1 in myelination and degenerative disorders such as ADLD and multiple sclerosis.

1078/F

Two distinctive dysfunctions of mutated FGD1 proteins found in patients with Aarskog-Scott syndrome. *K. Yanagi[1], T. Kaname[1, 5], H. Maehara[1, 2], Y. Chinen[3], N. Okamoto[4], K. Naritomi[1, 5].* 1) Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan; 2) Dept Orthopedics, Univ Ryukyus, Nishihara, Japan; 3) Dept Pediatrics, Univ Ryukyus, Nishihara, Japan; 4) Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; 5) SORST, Japan Science and Technology agency (JST), Kawaguchi, Japan.

Faciogenital dysplasia 1 (FGD1) gene was identified as a responsible gene for Aarskog-Scott syndrome (AAS), which is characterized by short stature, dysmorphic facial appearance, brachydactyly, and shawl scrotum. In some patients, neurobehavioral abnormalities have been also described in addition to the faciogenital dysplasia. FGD1 protein acts as a guanine nucleotide exchange factor (GEF) for CDC42, and possess Dbl homology domain (DH domain), pleckstrin homology domain (PH domain) that will be essential for the GEF activity. However, molecular pathology of FGD1 abnormalities and genetype/phenotype correlation of AAS are unknown.

We investigated cell biological (dys-)function for two types of mutated FGD1 protein found in two patients with faciogenital dysplasia only or the dysplasia plus neurobehavioral abnormality. Each mutation is substitution of an amino acid in the PH domain or in the DH domain, respectively. Expression vector for the mutants or wild type of FGD1 were constructed and transfected into HT1080, human fibrosarcoma cells. By comparison of each stable transformatns on protein localization, formation of membrane ruffles, cell migration, and cell proliferation, each mutant proteins display distinct dysfunction in vitro that might be able to explain differences of symptoms in AAS.

1079/F

Family based association analysis of TGFB1 as modifier gene in Cystic Fibrosis. *M.D. Bettin[1], C. Bombieri[1], G. Malerba[1], L. Xumerle[1], F. Belpinati[1], C. Castellani[2], B.M. Assael[2], P.F. Pignatti[1].* 1) Sec. Biology and Genetics, Dpt. Mother Child & Biology-Genetics, University of Verona, Verona, Italy; 2) Cystic Fibrosis Veneto Regional Centre, Hospital of Verona, Italy.

Cystic fibrosis (CF) is a lethal, multi-system autosomal recessive genetic disorder primarily affecting Caucasian populations, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Severity of clinical presentation in CF, particularly the pulmonary manifestation, are highly variable, even among CF patients presenting the same genotype. This variability is only partially explained by allelic heterogeneity at the CFTR gene. Literature data suggest that the severity in CF may be correlated with other genetic factors. Two polymorphisms (-509C/T and Leu10Pro) of the TGFB1 gene, which encodes for a cytokine involved in inflammation and tissue repair and expressed by several cells, have recently been associated to a more severe CF pulmonary manifestation in the American population (Drumm et al, NEJM 353:1443; 2005). We here report the results of TDT and haploscore association analyses of three TGFB1 functional polymorphisms (-509C/T, Leu10Pro e Arg25Pro) in Italian CF patients. Eightytwo family trios with a CF child and 52 unrelated CF patients were collected through the Veneto Regional CF Centre of Verona. All the patients were clinically evaluated for respiratory parameters, gastrointestinal and nutritional status parameters, and other clinical variables related to the common CF complicances (diabetes, DIOS, etc). TDT test result showed evidence of association between Pro25 and FEV1 (p=0.018). No association was found among other polymorphisms and studied clinical parameters. Single locus and haplotype analyses performed in all the unrelated CF patients confirm the association of TGFB1 gene polymorphisms with FEV1%. These results compared to literature data indicate that further studies are necessary to characterized the involvement of TGFB1 gene as modifier of disease severity in cystic fibrosis.

1080/F

A mutation in *APOA4* causes an autosomal dominant form of inflammatory bowel disease (IBD). *B.A. Johannes[1], B.G. Elyas[1], M. Hicks[1], S.M. Haase[1], J.S. Bamforth[1,2], H.F. Pabst[2], M.A. Walter[1], K.A. Sprysak[1], L.M. Vicen[1], M.J. Somerville[1,2].* 1) Dept Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Dept Pediatrics, University of Alberta, Edmonton, AB, Canada.

The causes of inflammatory bowel disease (IBD), characterized by inflammation of the large and/or small bowel, remain largely unknown. We report on the cause of a severe autosomal dominant form of IBD (Familial Enteropathy with Villous Edema [FEVE; OMIM 600351]) that typically manifests in childhood as a recurrent acute, life-threatening secretory diarrhea associated with distinctive jejunal histologic changes and IgG2 subclass deficiency. Genome-wide linkage analysis on a Mennonite kindred linked FEVE to D11S908 on chromosome 11q23 with a LOD score of 6.2 at theta = 0. A higher density microsatellite marker array refined the critical region to a 2 Mb interval between D11S4142 and D11S1364. We sequenced the open reading frames of nine genes from this critical region before identifying a 198 bp (66 aa) in-frame duplication in the *apolipoprotein (apo) A-IV* gene (APOA4) that segregates with disease in this family. This mutation was not detected in 200 unrelated controls (400 chromosomes). Apolipoprotein A-IV is a 46-kDa protein that is synthesized in the small intestine, upregulated in response to high lipid uptake, and secreted into the mesenteric lymphatic system on chylomicrons. In addition to apoA-IV's involvement in lipid transport, lipoprotein metabolism, and reverse cholesterol transport, recent evidence suggests that it acts as an endogenous anti-inflammatory protein. This has been demonstrated through dextran sulfate sodium-induced experimental colitis in Apoa4 knockout mice. ApoA-IV plasma levels have also been found to be inversely associated with disease activity in Crohn's disease patients. Our results, combined with those from previous reports, implicate APOA4 as a susceptibility gene for IBD. Identification of this APOA4 mutation in FEVE warrants further investigation into its role in IBD as well as the anti-inflammatory function of apoA-IV in the gastrointestinal system.

1081/F

Demonstration of Presumed Linkage Disequilibrium in the Posterior Polymorphous Corneal Dystrophy 1 Candidate Gene Region. *V.S. Yellore, M.C. Chen, S.A. Rayner, A.J. Aldave.* Cornea Service, Jules Stein Eye Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose: To identify the genetic basis of posterior polymorphous corneal dystrophy (PPCD1), an autosomal dominant disorder of the corneal endothelium associated with visually significant corneal edema and glaucoma. Linkage analysis in four families with PPCD1 has demonstrated linkage to a 2.4 cM common support interval bordered by the markers D20S182 and D20S139. We sought to identify the genetic basis of PPCD1 thorough screening of the 20 positional candidate genes between these markers in the fourth PPCD1 family mapped to this interval. **Methods**: DNA was obtained from 11 affected and 11 unaffected individuals from a family previously liked to the PPCD1 locus. The coding regions of all 20 positional candidate genes were amplified and sequenced in affected and unaffected individuals. **Results**: Four DNA sequence variants in three of the positional candidate genes demonstrated complete segregation with the affected phenotype: Thr109Thr (rs6111803) in *OVOL2*, Arg56Gln in *RPS19P1*, and Thr85Thr (rs1053834) and Pro99Ser (rs1053839) in *C20orf79*. While three of the identified sequence variants are known SNPs, Arg56Gln is a novel variant, although it has been identified in unaffected control individuals. While a number of other previously described and novel SNPs were identified in the 20 positional candidate genes, none segregated with the affected phenotype in the family. **Conclusions**: We have identified several sequence variants in genes mapped to the common PPCD1 region that are presumed to be in linkage disequilibrium with the as-of-yet unidentified pathogenic mutation. Screening of the non-coding regions of the 20 positional candidate genes is currently underway to identify the genetic basis of PPCD1.

1082/F

Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies, and specific neural tumors, associated with CM-AVM and RASA1 mutations. *N. Revencu[1], L.M. Boon[1], J.B. Mulliken[2], O. Enjolras[3], M.R. Cordisco[4], D. Chitayat[5], M. Vikkula[1].* 1) Lab Human Molec Genetics, Christian de Duve Inst, Brussels, Belgium; 2) Vascular Anomalies Center, Children's Hospital, Boston, USA; 3) Centre Multidisciplinaire des Angiomes de l'enfant, Hôpital d'enfants Armand-Trousseau, Paris, France; 4) Department of Pediatric Dermatology, Hospital de Pediatria Dr. J.P. Garrahan, Buenos Aires, Argentina; 5) Medical Genetics Program Mount Sinai Hospital, Toronto, Canada.

Background: Mutations in RASA1 were documented in 6 families (39 individuals) with autosomal dominant multifocal capillary malformations (CMs). Nine individuals had an associated arteriovenous malformation/fistula (AVM/AVF). One patient had Parkes Weber syndrome (PKWS), a disorder considered to be sporadic and non-genetic. Methods: We collected clinical information and DNA samples for 61 probands (from 21 centers) and their families with a phenotype similar to that observed in the original study: 56 had multifocal CMs, and 35 also a fast-flow vascular anomaly: 19 AVM/AVF and 16 PKWS; 5 had PKWS without multifocal CMs. RASA1 was screened by DHPLC followed by sequencing. Results: We identified 42 distinct mutations in 44/61 probands: 16/19 with AVM/AVF, 13/16 with PKWS, 15/21 with multifocal CMs only, and 0/5 with PKWS without multifocal CMs. RASA1 mutation was also found in 57 relatives. Overall, 17 individuals with a RASA1 mutation had an AVM/AVF: 8 were intracranial, 2 of which were vein of Galen aneurysmal malformations. Moreover, 7 patients had either a benign or a malignant tumor, 3 of which are known to occur in neurofibromatosis type I and/or type II. Penetrance of RASA1 mutations was 98% and de novo occurrence was 32%. Conclusions: Multifocal CM is the hallmark of RASA1 mutation. These patients often have extra- or intracranial AVM/AVF. This study confirms the original association designated capillary malformation-arteriovenous malformation (CM-AVM). In addition, PKWS, as well as vein of Galen aneurysmal malformation are genetic diseases, part of the CM-AVM spectrum. Specific neural tumors also may be linked to RASA1. (miikka.vikkula@uclouvain.be).

1083/F

The spectrum of the Factor VIII defects in Taiwanese patients with Hemophilia A. *G.C. Ma[1], S.P. Chang[1], M. Chen[1,2,3], M.C. Shen[1,4].* 1) Center for Medical Genetics and Department of Medical Research, Changhua Christian Hospital, Changhua, Taiwan; 2) Department of Obstetrics and Gynecology, College of Medicine, National Taiwan University, Taipei, Taiwan; 3) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua 500, Taiwan; 4) Department of Internal Medicine, Colleague of Medicine, National Taiwan University, Taipei, Taiwan.

Hemophilia A (HA) is an X-linked recessive bleeding disorder caused by various types of pathological defects in the FVIII gene. With the exception of two intron inversions (IVS22 and IVS1), no mutation hotspots in the FVIII gene were identified in the previous literature. To date, several studies on the spectrum of FVIII defects have been performed in Western populations, but similar studies in Asian races are scarce. Here, we report the distribution of the mutations within the FVIII gene in 31 Taiwanese unrelated patients with HA (19 severe and 10 moderate/mild males, and 2 severe females). Of these, 12 (38.7%) and one (3.2%) severely affected males were genotyped with the recurrent IVS22 and IVS1 inversion, respectively. These frequencies were similar to that in general populations (IVS22: 40-50%; IVS1: 2-5%) reported elsewhere. The FVIII defects in the remaining 18 inversion-negative patients cover a wide spectrum, in which 17 different mutations were identified, including ten missense and three nonsense mutations as well as two small and two large deletions. Twelve of these mutations are novel: seven caused nonsense substitutions and five resulted in truncated proteins. To assess the putative pathogenetic impacts of the newly amino acid substitutions, computer analyses were performed based on the molecular 3D modeling. The degree of conservation in cross-species FVIIIs and the position in known functional FVIII regions were studied. The novel missense mutations found in our series all occurred at evolutionary conserved residues that may carry a functional importance in our analyses. The results of this study add the short list of Taiwanese/Chinese FVIII mutations to the existing literature, and will enhance our understanding of the molecular basis of FVIII protein function and the mechanism underlying HA.

1084/F

The homolog of the intraflagellar transport protein, IFT80, is mutated in Jeune Asphyxiating Thoracic Dystrophy (JATD). *P.L. Beales[1], E. Bland[1], J.L. Tobin[1], C. Bacchelli[1], B. Tuysuz[2], J. Hill[1], S. Rix[1], C.G. Pearson[3], M. Kal[4], J. Hartley[5], C. Johnson[5], M. Irving[1], N. Elcioglu[6], M. Winey[3], M. Tada[4], P.J. Scambler[1].* 1) Molecular Medicine Unit, UCL Institute of Child Health, London, United Kingdom; 2) Department of Pediatrics and Genetics, Cerrahpasa Medical School, University of Istanbul, Turkey; 3) MCDB, University of Colorado, Boulder, Boulder CO 80309-0347 USA; 4) Dept of Anatomy and Developmental Biology, University College London, London, WC1E 6BT UK; 5) Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham Medical School, Birmingham, UK; 6) Department of Pediatric Genetics, Marmara University Hospital, Istanbul, Turkey.

JATD is an autosomal recessive chondrodysplasia that often leads to death in infancy because of a severely constricted thoracic cage and respiratory insufficiency; retinal degeneration, cystic renal disease and polydactyly may be complicating features. A bioinformatic approach combining clinical and proteomic searches supported the idea that JATD could be a ciliopathy and enabled prioritization of genes within a 3q24-3q26 linkage interval. Mutations of IFT80 were detected, the first association of a defective intraflagellar transport protein in human disease. Knockdown of IFT80 in zebrafish resulted in abnormal hedgehog signalling and cystic kidneys, and knockdown in Tetrahymena thermophila resulted in shortened or absent cilia. An *Ift80*$^{-/-}$ mouse model was lethal around E9.5 as might be predicted.

1085/F

Aggregation of lamin A/C or progerin in fibroblasts derived from Hutchinson-Gilford progeria syndrome (HGPS) is not resulted from interaction between progerin and the promoter of LMNA gene. *Y. Huang[1,2], H. Guo[1,2], N. Zhong[1,2,3].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Dept. Medical Genetics of Peking University Health Science Center; 3) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by dramatic premature aging. Classic HGPS is caused by a de novo point mutation in exon 11 of the LMNA gene that encodes lamin A protein, activating a cryptic splice donor and resulting in a mutant lamin A protein, the progerin, that lacks the normal cleavage site to remove a C-terminal farnesyl group. Our previous study has shown that there is an aggregation in HGPS cells. We hypothesized that this aggregation is resulted from a pathological accumulation of uncleaved progerin. We proposed that the accumulated progerin might interact with the promoter of LMNA gene and interfere with the gene expression. To test our hypothesis, we have employed a dual-luciferase reporter assay to study the progerin, compared to a normal lamin A, effects on the promoter of LMNA gene. The dual-luciferase reporter system includes firefly Luciferase and Renilla Luciferase. The construct was transfected into 293T cells. Our pilot results present no evidence that progerin or lamin A would have a obvious interaction with the LMNA promoter, although alternative approaches are being under conducted to characterize the effects of progerin. Our studies have preliminarily excluded that mutant progerin may have pathological interaction on the gene expression of either normal lamin A/C or mutant progerin in HGPS.

1086/F

Utility of microarrays in characterizing two patients with 11q deletions and clinical features of Jacobsen syndrome. *C. Haldeman-Englert[1], D.M. McDonald-McGinn[1], E. Geiger[1], L. Medne[1,2], C. Bonnemann[1,2], K. Brigatti[1], N.B. Spinner[1], E.H. Zackai[1], T.H. Shaikh[1].* 1) Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Neurology, Children's Hospital of Philadelphia, Philadelphia, PA.

Terminal deletions of 11q have previously been associated with Jacobsen syndrome (11q23-qter), which is characterized by FTT, thrombocytopenia, MR and dysmorphia. Here we report two patients with overlapping deletions of 11q identified cytogenetically, which we have further characterized using high-density oligonucleotide arrays. Patient 1, a 5 month-old male, had a perimembranous VSD, ASD, umbilical hernia, undescended testis, hypotonia with resultant motor delay, FTT and dysmorphia. Patient 2, a 3 year-old male, had unilateral cerebellar and vermis hypoplasia, coarctation of the aorta, thrombocytopenia, truncal ataxia, hyperreflexia, global developmental delay with autistic behaviors and dysmorphia. Patient 1 was found to have an 11q terminal deletion using standard cytogenetic techniques [46,XY,del(11)(q24)], whereas, Patient 2 was initially reported to have an interstitial 11q deletion [(46,XY,del(11)(q24.2q25)]. However, using high-density oligonucleotide array we more precisely mapped the proximal breakpoint in Patient 1's ≈11 Mb deletion to 11q24.1 and Patient 2's ≈5.7 Mb deletion to be nested within the deletion of Patient 1. Of note, Patient 2 appears to have the smallest 11q interstitial deletion reported to date which contains ≈20 genes. Importantly, one of the genes, *FLI-1*, is thought to be associated with hematopoiesis, and haploinsufficiency of this gene may effect platelet production causing thrombocytopenia, as seen only in Patient 2. In summary, the cases presented here do in fact have some overlapping features of Jacobsen syndrome, but they do not match entirely and have smaller deletions. Thus, using high-density oligonucleotide microarrays to analyze identified patients with 11q deletions will certainly help to further characterize the nuance of this diagnosis and may provide important genotype-phenotype correlations which will aide in providing appropriate management and counseling.

1087/F

Molecular analysis of the CHD7 gene in CHARGE syndrome: identification of 22 novel mutations and evidence for a low contribution of large CHD7 deletions. *P. Vuorela[1], S. Ala-Mello[2], C. Saloranta[2], M. Penttinen[3], M. Pöyhönen[4], K. Huoponen[1], W. Borozdin[5], B. Bausch[6], E.M. Botzenhart[6], C. Wilhelm[5], H. Kääriäinen[1,7], J. Kohlhase[5,6].* 1) Department of Medical Genetics, University of Turku, Turku, Finland; 2) Department of Clinical Genetics, Helsinki University Hospital, Helsinki, Finland; 3) Clinical Genetics Unit, Department of Pediatrics, Turku University Central Hospital, Turku, Finland; 4) Department of Medical Genetics, University of Helsinki, Finland; 5) Praxis für Humangenetik Freiburg, Germany; 6) Institut für Humangenetik und Anthropologie, Universität Freiburg, Freiburg, Germany; 7) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland.

Autosomal dominant CHARGE syndrome (OMIM #214800) is characterized by choanal atresia and/or cleft lip/palate, ocular colobomas, cardiovascular malformations, retardation of growth, ear anomalies and deafness, and caused by mutations in the CHD7 gene. Here we describe the outcome of a molecular genetic analysis in 18 Finnish and 56 German patients referred for molecular confirmation of the clinical diagnosis of suspected CHARGE syndrome. In this group of 74 patients, we found mutations in 30 cases. 22 mutations were novel, including 11 frameshift, 5 nonsense, 3 splice site and 3 missense mutations. One de novo frameshift mutation was found in the last exon and is expected to result in a minimally shortened CHD7 polypeptide. Since the mutation is associated with a typical CHARGE syndrome phenotype, it may indicate the presence of an as yet unknown functional domain in the very carboxyterminal end of CHD7. qPCR or MLPA assays did not reveal deletions in mutation negative cases, suggesting that larger CHD7 deletions are not a major cause of CHARGE syndrome. Our mutation detection rate of 40.5% is relatively high in an unselected cohort of patients, who were referred to DNA analysis because of suspected diagnosis of CHARGE syndrome, and one can expect it to be markedly higher in a cohort of patients who meet strict criteria of the disorder.

1088/F

A Genome Wide Study to Identify a Disease Specific Expression Profile and Downstream Targets of Cohesin Regulation in Cornelia de Lange Syndrome. *J. Liu[1], Z. Zhang[2], E. Rappaport[1], S. Tandy[1], I.D. Krantz[1].* 1) Division of Human Genetics, Abramson Research Institute; 2) Bioinformatics Core, Center for Biomedical Informatics, The Children's Hospital of Philadelphia, PA 19104.

Cornelia de Lange Syndrome (CdLS) (OMIM 122470) is a dominant disorder of multiple congenital anomalies including characteristic facial, physical, and developmental features. Our laboratory has identified mutations in the *Nipped B-like* (*NIPBL*) gene, a regulator of the cohesin complex, as a cause of CdLS. Gene screening has identified *NIPBL* mutations in approximately 50% of probands who met clinical criteria for CdLS. Recently mutations in the genes that encode the two structural arms of the cohesin complex, *SMC1A* and *SMC3*, were found to contribute to approximately 5% of CdLS cases. This finding confirms genetic heterogeneity in CdLS and emphasizes the possibility that there may yet be additional genes that contribute, either individually or in combination, to the CdLS phenotype. We hypothesized that genome-wide expression array analysis in CdLS would provide insights into the underlying molecular mechanisms contributing to the phenotype as well as identifying a specific profile of the differentially expressed genes. We performed array based expression profiling from lymphoblastoid cell lines (LCLs) from 16 severely affected CdLS patients (with confirmed mutations in *NIPBL*) as controlled by 17 matched healthy subjects and further validated with 6 additional samples with various diagnoses. The unsupervised Principle Component Analysis (PCA) clearly separated CdLS samples from the controls, and leave-one-out cross validation successfully categorized the testing samples, suggesting a distinctive molecular profile for CdLS. We then identified a unique list of 420 probe sets, with *NIPBL* ranked as the most significantly changed gene, that are differentially expressed in CdLS. We further defined a 7-gene CdLS-specific expression signature. Further work is in process to delineate the identified genes with altered expression to investigate their relevance to the phenotypic differences in CdLS as well as to see if expression profiling can be used as a diagnostic tool for other developmental disorders.

1089/F

Mice Carrying Novel Mutations in *Frem1* Have Phenotype Similar to that Seen in Fraser Syndrome. *O. Shchelochkov[1], D.A. Scott[1], X. Lin[1], M. Justice[1], D.W. Stockton[2], B. Lee[3].* 1) Dept of Mol and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Wayne State University School of Medicine, Detroit, MI; 3) Howard Hughes Medical Institute.

Fraser syndrome is a complex developmental malformation syndrome with autosomal recessive inheritance. In humans it is characterized by cryptophthalmos, limb and genital anomalies, and internal organ abnormalities, which may include renal agenesis, and gastrointestinal malformations. "Blebbed" mouse mutants are considered the murine model of Fraser syndrome with mutations described in *Fras1*, *Grip1*, *Frem1* and *Frem2* genes. Here we describe two mouse strains generated by ENU mutagenesis, EYE2 and CRF11. Phenotypic analysis of these non-complementing strains revealed a spectrum of eye defects ranging from unilateral microphthalmia to bilateral cryptophthalmos, renal defects and hemorrhagic blisters *in utero* similar to previously described 'bleb' strains. Linkage analysis was used to define the location of the mutations in these mice within a ~3 Mb interval on mouse chromosome 4 that contained the *Frem1* gene. Sequencing of *Frem1* in these stains revealed a missense mutation in EYE2 in exon 8 (c.1687A>T, 563I>F) and a nonsense mutation in CRF11 in exon 13 (c.2477T>A, 826L>X). Additional phenotypic findings not previously identified in *Frem1* mice included lung segmentation defects, a propensity to the development of rectal prolapse and, in one EYE2 mouse, a retrosternal diaphragmatic hernia. Similar defects have been reported in a small portion of patients with Fraser syndrome. In summary, we have described two novel *Frem1* mutations in mouse strains, which share both common and rare phenotypic findings with patients with Fraser syndrome. These mutations include the first missense mutation to be described with this phenotype. Although to date no *FREM1* mutations have been identified in human cases of Fraser syndrome, this gene remains an attractive candidate gene for this syndrome.

1090/F

Identification of HNF1alpha mutations in MODY3 patients. *W. Wuyts[1,2], I. Callebaut[2], L. Rooms[1], L. Vits[2], K. Storm[2].* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Dept Medical Genetics, University Hospital Antwerp, Antwerp, Belgium.

Maturity Onset Diabetes of the Young (MODY) is a monogenic form of diabetes mellitus accounting for approximately 1 to 2% of non-insulin dependent diabetes. MODY is characterised by early onset pancreatic β-cell dysfunction and autosomal dominant inheritance. It is a genetic heterogeneous condition with already several causal genes identified. In Europe, MODY type 2, caused by mutations in the glucokinase gene and MODY type 3 caused by mutations in the HNF1alpha transcription factor gene are the most prevalent forms.

We have performed genetic testing for MODY type 3 in 286 probands referred to our center. All probands full filled at least two of the following criteria: early-onset hyperglycaemia (age of onset < 40 years), the absence of beta cell auto-antibodies and a positive familial history for diabetes with at least two successive generations affected.

Molecular screening of the HNF1alpha gene was performed by PCR amplification and sequencing of all coding exons (exon 1-10). In 47 probands (16,4%) a potential pathogenic variant was detected. Fourty-one different mutations were identified while six mutations were detected in more than 1 patient. Approximately 50% of the mutations were missense mutations, 30% truncating mutations and 20% variants (likely) affecting splicing. Although mutations were present in every exon, exon 4 appeared most affected with a mutation in this exon in 20% of the patients.

These results show that mutations in the HNF1alpha gene are a significant cause of MODY in our patient population.

1091/F

Mutational analysis of Japanese families with childhood-onset dominantly inherited diabetes mellitus. *T. Yorifuji, S. Nagai, M. Kawai, T. Momoi, T. Nakahata.* Pediatrics, Kyoto University Hospital, Kyoto, Japan.

(Background) Dominantly-inherited diabetes mellitus (DM) comprises approximately 5% of all type 2 DM. Typical forms with adolescence-onset have been called MODY. So far 6 causative genes have been identified and termed MODY1-6. Mutational analyses of Caucasian families have shown that most MODY cases could be explained by MODY1-6. However, in east Asian populations, mutations in these genes can be identified in only 10-20%. (Aims) To understand the mutational spectrum of Japanese patients with dominantly inherited DM.(Methods) Twenty-five Japanese families with dominantly inherited DM were analyzed. At least one member of each family had the onset of DM during childhood. Then, all exons, exon-intron boundaries, and the promoter region of the known MODY genes, KCNJ11, and ABCC8 were amplified from genomic DNA and directly sequenced.(Results) Mutations were identified in 11 out of 25 families (Table), much more frequently than previously reported for Japanese patients. GCK mutations were identified more frequently than TCF1 mutations. Notably, KCNJ11 mutations were identified in two families, more frequently than other MODY genes.

Gene	Mutation
GCK	P59S, E40K, G299R, P417Q
TCF1	R131W, L348P, p291fsdelC
KCNJ11	C42R, D323G
TCF2	S148W
HNF4A	-82G>C (exon 1D)

.

1092/F

Hereditary pancreatitis caused by a double 'gain' mechanism. *C. Le Marechal[1,2,3,4], E. Masson[1,2], J. Chen[1,3], C. Férec[1,2,3,4].* 1) INSERM, U613, 29220 Brest, France; 2) Faculté de Médecine de Brest et des Sciences de la Santé, Université de Bretagne Occidentale, 29238 Brest, France; 3) Etablissement Français du Sang - Bretagne, 29220 Brest, France; 4) Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Universitaire de Brest, Hôpital Morvan, 29220 Brest, France.

Hereditary pancreatitis was often reported to be caused by 'gain-of-function' missense mutations in the cationic trypsinogen gene (PRSS1). Recently, we have reported that triplication of a ~605-kb segment containing the PRSS1 gene on chromosome 7 was responsible for the disease in five French Caucasian families (Le Marechal et al. Nat Genet 2006;38:1372-4). This triplication, which seems to result in a 'gain' of trypsin through a gene dosage effect, represents a previously unknown molecular mechanism causing hereditary pancreatitis. Here, we further identified a hybrid trypsinogen gene due to unequal homologous recombination in a new French family. This mutational event was initially revealed by quantitative fluorescent multiplex PCR (QFM-PCR) and then fully characterized by long-range PCR at the nucleotide level; it resulted in the formation of an additional copy of trypsinogen that fused the anionic trypsinogen gene (PRSS2) with the PRSS1 gene. The resulting hybrid gene seems to be apparently functional because its expression was detected from the peripheral blood cells by RT-PCR. Most importantly, the hybrid gene was predicted to encode a protein with a known missense mutation that has been previously reported to cause hereditary pancreatitis. In summary, this mutation appears to cause the disease through a new mechanism viz. a double 'gain' of trypsin through an increased trypsinogen copy that in turn encodes a clearly 'gain of function' mutant protein.

1093/F

A novel missense mutation p.R178Q in the SLC40A1 gene encoding ferroportin is present in 28% of a Belgian cohort of autosomal-dominant hemochromatosis families. *X. Pepermans[1], V. Lambot[1], M. Lambert[2], G. Matthijs[3], K. Dahan[1].* 1) Center for Human Genetics, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium; 2) Department of Internal Medicine, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium; 3) Department of Human Genetics, University of Leuven, Leuven, Belgium.

Inherited iron overload, a very common condition is characterized by a large genetic heterogeneity with at least 4 responsible genes, HFE1, HJV, HAMP and SLC40A1. Even if it is usually an autosomal-recessive pattern of transmission, 15 mutations (14 missenses and one in-frame deletion) in the SLC40A1 gene have been identified so far in patients with an autosomal-dominant hemochromatosis. In this study, we screened 7 probands with a family history of at least more than two individuals on two generations with either a precocious raised ferritin or an accumulation of iron in the liver by magnetic resonance imaging for mutation in the SLC40A1 gene. We identified in 2 of them a novel heterozygous misses change (c.533G>A) in exon 6 resulting in the arginine to glutamine substitution at amino acid 178 (p.R178Q).This mutation altered a highly conserved residue within the third extracellular domain of the ferroportin was not found in 370 control alleles making it unlikely that it represents a rare polymorphism. Subsequently a panel of 230 samples from individuals with iron overload unlinked to pathogenic mutations in HFE1 gene was tested for the p.R178Q allele. Analysis revealed one additional individual with the same change (1/230, 0.4%). This mutational study shows a mutation detection rate of 28% ; in a small cohort of autosomal-dominant hemochromatosis families, making it sense to propose the SLC40A1 gene screening in patients with a family history of raised ferritin. At contrary, its contribution in the pathogenesis of iron overload without family criteria appears to be low in this Belgian cohort of patients.

1094/F

Molecular determination of mutations in the *GLA* gene in Mexican patients with Fabry's disease. *B.A. Rodriguez-Espino[1], D. Olvera-Castillo[2], J. Morales[3], J. Granados-Arriola[4], A.E. Cataneo-Davila[2], R. Correa-Roter[2], M. Ramos-Kuri[1].* 1) Molecular Biology, Medical School, Universidad Panamericana, Mexico City, Mexico; 2) Nephrology and Mineral Metabolism; 3) Genetic; 4) Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran. Mexico City, Mexico.

Introduction. The Fabry disease is a recessive metabolic illness, caused by mutations in the *GLA* gene (locus Xq22.1), that codifies for the lysosomic enzyme α-galactosidase A (α-GAL). The affected individuals show incapacity for catabolizing lipids with α-galactosyl terminal residues, which are accumulated gradually in the vascular endothelium and the visceral tissues of the body. There are more than 400 known mutations for Fabry disease, but in most cases they are unique for each family and there are not registers in Mexican population. **Objective**. To determine the type of mutations in the *GLA* gene in three positive Mexican index cases for Fabry disease. **Methods**. Genomic DNA was isolated from male patients and a control group of healthy and ethnically related individuals. All the seven exons of the *GLA* gene were amplified by means of PCR using specific oligonuclotides. The amplified products were sequenced and compared against international data bases. **Results**. In one patient the loss of an A in position 260 of the codifying DNA was detected (*c.*260delA), this produces a framework shift in the polypeptide translation. In the second patient, the change of a G by C in the 243 codon replaces the amino acid L by F (*p.*L243F). In the third patient there were not mutations. **Conclusions**. It is the first molecular study of the *GLA* gene in Mexican population, in which a new mutation is reported (*c.*260delA). These studies altogether with the enzymatic analyses, would allow to improve the early detection, to define the status of carrier in asymptomatic individuals, to select probable familiar kidney donors, and to identify the molecular variables of the disease in Mexico.

1095/F

MLPA identification of whole exon and single nucleotide deletions in the CFTR gene of Hispanic individuals with cystic fibrosis. *I. Schrijver[1], K. Rappahahn[1], L.M. Pique[1], M. Kharazzi[2], L-J. Wong[3].* 1) Pathology, L235, Stanford Univ, Stanford, CA; 2) Genetic Disease Branch, California Department of Health Services 850 Marina Bay Parkway, Room F175, Richmond, CA 94804; 3) Molecular and Human Genetics, Baylor College of Medicine One Baylor Plaza, NAB 2015, Houston, TX 77030.

Identification of CFTR mutations in Hispanics with cystic fibrosis (CF) can facilitate diagnosis, improve management, and guide genetic counseling. In both carrier screening panels and molecular diagnostics, however, a disparity between Caucasian and Hispanic mutation detection continues to exist. In order to more fully characterize the Hispanic CFTR mutation spectrum, we aimed to identify exonic deletions in 39 self-identified Hispanic patients who had previously completed extensive mutation analysis. Using a commercial multiplex ligation-dependent probe amplification (MLPA) assay, exon deletions appeared present in 10/39 patients. Two recurrent pathogenic deletions (of exons 2 and 3 and of exons 22 and 23) were identified in 3 patients each (15.4%). Based on MLPA results, 3 apparently novel deletions were identified in 4 additional patients. However, during the process of confirmation, single nucleotide deletions at the probe binding sites were identified (exon 6b: 935delA, exon 19 : 3791delC and exon 20 : 3961delA). All resulted in false positive deletion signals with MLPA and all were close to the ligation site. Interestingly, none of the 3 deletions are common in Caucasians and the 935delA mutation is one of the most common mutations identified in U.S. Hispanics. A total of 76 mutations and 5 silent variants reported in the Cystic Fibrosis mutation database (http://www.genet.sickkids.on.ca/cftr/app) are located under the sequences that immediately surround the MLPA ligation sites in this assay. Twenty-three occur in non-Caucasians, including 9 Hispanic, 6 from the greater Middle-East, 4 Asian, and 4 African. These mutations are not all rare. Thus, apparent exonic deletions by MLPA may indicate both large deletions and point mutations, with important implications for pan-ethnic MLPA testing in CF and other genetic conditions.

1096/F

INCREASED SENSITIVITY TO IONISING RADIATION IN MADA LAMINOPATHY. *A. di Masi[1], R. Ricordy[2], M.R. D'Apice[3], F. Lombardi[3], F. Gullotta[3], C. Tanzarella[1], G. Novelli[3,4].* 1) Department of Biology, University "Roma Tre", Rome, Italy; 2) Centro Genetica Evoluzionistica, CNR, Rome (Italy); 3) Department of Biopathology and Diagnostic Imaging, Tor Vergata University of Rome, Italy; 4) Arkansas Medical School, Little Rock, USA.

Mandibuloacral dysplasia type A (MADA; OMIM # 248370) is a premature ageing disease caused by the homozygous R527H mutation in the LMNA gene. At cellular level, MADA is characterized by unprocessed prelamin A accumulation, nuclear architecture alterations, chromatin defects and increased incidence of apoptosis. These biochemical and morphological alterations involve genomic instability as demonstrated in other progeroid laminopathies (e.g. HGPS). We investigated the sensitivity of MADA cells to the ionising radiation-DNA-induced damage. MADA and age-matched normal fibroblasts were exposed to X-rays, with doses comprises between 1 and 4 Gy. We observed that the ability of MADA cells to repair the damage was significantly reduced compared to control fibroblasts, as demonstrated by the increased percentage of chromosome aberrations and the higher percentage of residual γ-H2AX foci, corresponding to unrepaired DNA-damage sites. MADA cells showed a markedly reduced phosphorylation of p53 at Ser15 and a lower induction of p53 and CDKN1A proteins after irradiation, compared to the control cell line. We also detected expression differences of some p53 downstream target genes in their response to DNA damage. In addition, MADA cells' showed defects in checkpoint response, particularly in G1/S activation. Our results indicate that accumulation of the lamin A precursor protein determines a defect in DNA damage response after X-ray exposure, supporting a crucial role of lamin A in regulating DNA repair process and cell cycle control.

1097/F

Novel SOX3 mutations in patients with various forms of syndromic pituitary defects. *I. Giurgea[1,3], K. Machinis[1], M.-P. Vié-Luton[1], G. Pinto[4], B. Mignot[5], A.-M. Bertrand[5], C. Naud-Saureau[6], S. Rose[1], F. Kurtz[7], M. Legendre[1,2], M.-L. Sobrier[1], J. Léger[8], P. Czernichow[8], S. Amselem[1,2].* 1) INSERM U654, Hôpital Armand Trousseau, Paris, France; 2) AP-HP, Hôpital Armand Trousseau, Service de Génétique et d'Embryologie médicales, Paris, France; 3) AP-HP, Groupe Henri Mondor-Albert Chenevier, Service de Biochimie et Génétique, Créteil, France; 4) AP-HP, Hôpital Necker Enfants Malades, Service d'Endocrinologie, Paris, France; 5) Hôpital Saint Jacques, Service d'Endocrinologie, Besançon, France; 6) Hôpital de Lorient, Service de Pédiatrie, Lorient, France; 7) Hôpital de Saint-Avold, Service de Pédiatrie, Saint-Avold, France; 8) AP-HP, Hôpital Robert Debré, Service d'Endocrinologie, Paris, France.

SOX3 is a transcription factor involved in the control of pituitary function and development. So far, few mutations have been identified in this X-linked gene. To assess SOX3 involvement in human pathology, we investigated 50 unrelated boys with pituitary dysfunction and midline central nervous system (CNS) defects. Mutations were identified in 8 patients from 6 unrelated families. Expansions of the polyalanine tract (+8 and +11 Ala) were found in 4 boys from 2 families with isolated growth hormone deficiency (IGHD) and strabismus. A contraction of the polyalanine tract (-9 Ala) was found in a patient with a combined pituitary hormone deficiency (CPHD) and callosal anomalies. Missense mutations were observed in 3 patients: p.Ala102Ser in a patient with blindness related to septo-optic dysplasia and severe midline CNS anomalies, p.Ala5Pro in one with IGHD and behaviour problems, p.Pro3Ser in a patient with CPHD. All patients had morphological pituitary anomalies: anterior pituitary hypoplasia (7/8), abnormal pituitary stalk (5/7), and ectopic posterior pituitary (4/8). This report broadens the clinical spectrum of SOX3 related disorders.

1098/F

Characterization of SOX10 deletions in Waardenburg-Hirschsprung disease. *N. Bondurand[1], F. Dastot-Le Moal[1,2], V. Baral[1], N. Collot[1,2], I. Giurgea[1,2], P. Sarda[3], A. Echaieb[4], R. Touraine[5], J. Amiel[6], M. Goossens[1,2], V. Pingault[1,2].* 1) Genetic department, INSERM U841, IMRB, creteil, France; 2) AP-HP, Groupe Henri Mondor-Albert Chenevier, biochimie et génétique, Créteil, France; 3) Department of Medical genetics, CHU Montpellier, France; 4) Chirurgie pediatrique, Hopital Pierre Zobda Quitman, Fort de France, France; 5) CHU-Hôpital Nord, Génétique, Saint Etienne, France; 6) INSERM U781 et département de Génétique, Hôpital Necker, Paris, France.

The SOX10 transcription factor is involved in development of neural crest derivatives including melanocytes, enteric nervous system and glial cells. Accordingly, SOX10 mutations have been found in patients presenting with intestinal aganglionosis, pigmentation anomalies and deafness, an association known as Waardenburg-Hirschsprung disease or WS4. Associated neurological signs have been reported in some cases leading to a syndrome called PCWH (Peripheral demyelinating neuropathy-Central dysmyelinating leukodystrophy-Waardenburg syndrome-Hirschsprung disease). The mutations identified so far include mostly truncating point mutations. Much evidence indicates that the more severe disease phenotype PCWH is realized only when the mutant mRNAs escape the nonsense mediated decay RNA surveillance pathway. Besides SOX10, mutations of EDN3 and EDNRB have been observed in WS4 patients. However, not all cases are explained at the molecular level, raising the possibility that other genes are involved or that some mutations within the known genes are not detected by commonly used genotyping methods. Here, we used semi-quantitative fluorescent multiplex PCR and Fluorescent in situ hybridization to search for SOX10 deletions. We identified the first 3 heterozygous deletions in patients presenting with WS4 or PCWH. Full characterization of the deletions revealed different events ranging from the deletion of a single exon to that of up to 220kb. Interestingly, one of the patients also carries a SOX10 Valine to Leucine substitution at the hemizygous state (V92L). Functional consequences of the V92L variation, and the influence of additional deleted genes on the phenotype severity will be discussed.

1099/F

***Cirh1a,* mutated in North American Indian Childhood Cirrhosis, plays important roles in the genesis of multiple organs during mouse embryonic development.** *B. Yu, G. Mitchell, A. Richter.* Medical Genetics, Hopital Sainte-Justine, Montreal, PQ, Canada.

Missense mutation R565W in human *CIRH1A* causes North American Indian childhood cirrhosis (NAIC), a hereditary cholestasis frequent in native children from Western Quebec. The gene product, cirhin is a nucleolar protein of unknown function that interacts with HIVEP1, a component of the Dpp/MBP/TGFβ signalling pathway. To elucidate gene function, we generated a *cirh1a* knockout mouse using exon targeted XH230 ES cells that have a β-gal-NEO insertion in intron 9 of the gene. Expression of the β-gal reporter is driven by the *cirh1a* promoter. Heterozygotes are fertile and show no physiologic or histological signs of liver dysfunction. In contrast, we obtained no liveborn *cirh1a* (-/-) animals. To determine the timing of embryonic lethality, we performed X-Gal staining of whole mount embryos and cryostat sections. Our results show that the homozygous knockout state is embryonic lethal before mid-gestation: as early as E8.5 we found no (-/-) embryos. To understand this embryonic lethality, we examined gene expression patterns in (+/-) embryos. No expression was detected in early (<E5.5)or late (>E12.5) embryos. Between E6.0 and E12.0, we observed changes in spatial and temporal expression patterns: initial expression of *cirh1a* in liver coincides with the appearance of the hepatic diverticulum (E9.5), followed by expression in the liver buds and the cystic (gall bladder) primordium. Between E10.0 and E12.0 *cirh1a* expression becomes progressively restricted. By E12.0, only liver and stomach show high level expression.

The expression pattern of cirhin during embryogenesis is similar to that of proteins in the Dpp/MBP/TGFβ signalling pathway. We hypothesize that *cirh1a* may have a role in early embryonic patterning, morphogenesis, and in the genesis of multiple organs. This is compatible with a role for cirhin in development, although a physiological function can not be excluded. Study of its direct role in liver and biliary tract development will be undertaken in R565W *cirh1a* knock-in animals. Supported by the CIHR.

1100/F

Survival in Machado-Joseph Disease (SCA3). *C. Kieling[1], P.R. Prestes[2], R. Giugliani[1,2], M.L.S. Pereira[1,3], L.B. Jardim[1,4].* 1) Medical Genetics Service, Hospital de Clinicas, Porto Alegre, RS, Brazil; 2) Department of Genetics, UFRGS, Porto Alegre, RS, Brazil; 3) Department of Biochemistry, UFRGS, Porto Alegre, RS, Brazil; 4) Departmento of Internal Medicine, UFRGS, Porto Alegre, RS, Brazil.

Machado-Joseph disease, one of the most prevalent autosomal dominant cerebellar ataxias, is a neurodegenerative disease that starts during adulthood, with patients presenting difficulties in gait, and later becoming bedridden. There is scarce data quantifying disease impact on patient survival. We then investigated the overall survival of a large series of MJD patients and compared it with the survival of their asymptomatic relatives. 412 affected and 413 unaffected individuals were ascertained from a consecutive sample of 82 families with a molecular diagnosis of MJD. Estimated mean survival time was 63.96 (95% CI, 62.09-65.83) and 78.61 years (95% CI, 74.75-82.47) for the affected and unaffected group, respectively (p<.001). Each additional year of birth increased the HR by 1.03. Mean age at onset was 36.37 years (95% CI, 35.21-37.53). Mean survival after disease onset was 27 years. Early onset and large CAG length predicted a shorter survival time. Therefore, MJD reduced survival, and this phenomenon was related to CAG length, age at onset, and year of birth.

1101/F

Atypical Manifestation of Macrothrombocytopenia with Leukocyte Inclusions. *J.R. Choi, J. Song, J.W. Choi.* Dept Laboratory Medicine, Yonsei Univ Col Medicine, Seoul, Korea.

Macrothrombocytopenia with Leukocyte Inclusions is a group of rare disorders including May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome and Epstein syndrome which share common features of giant platelets, thrombocytopenia and Döhle body-like cytoplasmic inclusions on neutrophils. They are all thought to be caused by mutations of MYH9 gene and categorized together as MYH9 disorders. We have experienced two cases with not all of aforementioned cardinal features together with some atypical features not described in literature. First patient was a female aged 30 who was referred to our institution for the evaluation of thrombocytopenia revealed by health screening program. Platelet count was 25,000 /μL but actual count estimated by peripheral blood smear was 60,000 - 80,000 /μL and giant platelets were frequently seen. Cytoplasmic inclusion was not observed in neutrophils. Besides these findings, moderate to severe elliptocytosis was also present. Bone marrow finding was not remarkable other than abundant megakaryocytes and frequently seen sea-blue histiocytes. Macrothrombocytopenia and elliptocytosis were also found in peripheral blood of her father and sister. Second patient was recently referred for evaluation of incidentally found leukopenia (2,610 /μL). Basophilic inclusions with somewhat floppy appearance were observed in nearly all neutrophils without any recognizable toxic finding in peripheral blood smear. Platelet count was absolutely normal (271,000 /μL) and there was not any notable aberration in platelet size. Sea-blue histiocytes were also frequently observed as the first case. Screening for mutation targeting exons of MYH9 gene implicated in MYH9 disorders of previously defined cases was performed for the first case and missense mutation corresponding to K373N was found. The same mutation was reported before in one German family of May-Hegglin anomaly. Increased sea-blue histiocytes may imply increased cellular turn over in these patients and cautiously be generalized for other MYH9 disorder cases warranting careful follow up for possible risk of hematologic malignancy.

1102/F

A new paradigm for the inheritance of familial Mediterranean fever (FMF). *I. Aksentijevich, M.G. Booty, E.F. Remmers, D.L. Kastner.* Genetics & Genomics Branch, NIH/NIAMS, Bethesda, MD.

FMF is a recessively inherited autoinflammatory disease caused by mutations in MEFV, which encodes pyrin. To date, substantial numbers of patients have been observed with only one demonstrable MEFV mutation, and rare cases of dominant inheritance have been documented. Here, we report 10 patients with typical FMF clinical histories and good responses to colchicine treatment. Eight patients were carriers of M694V, a mutation associated with the most severe FMF phenotype, one patient was a carrier of R653H and the other of the complex allele E148Q /V726A. To identify the second disease-associated mutations, all patients were sequenced using two methods. Standard sequencing using the ABI 3100 was performed on all 10 exons of MEFV, and the entire 15 kb genomic region encompassing MEFV was sequenced using a chip-based resequencing system (Callida). A second FMF mutation was not identified, but numerous heterozygous nucleotide changes were found throughout the gene, downplaying a potential role for genomic deletions. To investigate the presence of both transcripts, we evaluated allelic expression in 6 patients by cDNA sequencing. We considered a digenic model of inheritance by looking for mutations in pycard and siva, proteins known to interact with pyrin, and in POP1 and POP2, proteins known to have a similar function to pyrin in the regulation of IL-1β pathway. We identified two novel nucleotide changes and one of them proved to be a polymorphism after evaluating panels of Caucasians and ethnically matched controls. The second missense mutation is still under investigation. We compared the relative levels of MEFV transcripts between FMF patients with one mutation, to FMF patients with two mutations and healthy controls in an attempt to examine possible copy number variation. We did not observe a significant difference in MEFV expression between FMF patients with one mutation compared to patients with two mutations after using two different Taq-Man probes. Our data indicate that the existence of one MEFV mutation may be sufficient in some patients in the presence of some other modifying genes to cause the inflammatory phenotype in FMF patients. *MEFV.*

1103/F

Myotonic dystrophy type 2 in Japan: distinct ancestral origin from Caucasian families. *Y. Amakusa[1], T. Matsuura[1], T. Saito[2,3], T. Kimura[2], O. Yahara[2], H. Aizawa[3], K. Ohno[1].* 1) Div Neurogen & Bioinfo, Nagoya Univ Grad Sch Med, Nagoya, Japan; 2) Dept Neurol, National Dohoku Hosp, Asahikawa, Japan; 3) Div Neurol, Dept Internal Medicine, Asahikawa Med Univ, Asahikawa, Japan.

Myotonic dystrophy type 2 (DM2) is an autosomal dominant, adult-onset muscular dystrophy characterized by myotonia and multisystemic features, caused by expansion of the tetranucleotide CCTG repeats in intron 1 of the zinc finger protein 9 (ZNF9) gene on chromosome 3q21. The size of expansion is extremely large and variable, ranging from 75 to 11,000 repeats, with a mean of 5,000. This unprecedented size and somatic heterogeneity of the expansion make the molecular diagnosis of DM2 more complicated. Genetically confirmed DM2 patients are all Caucasians, and haplotype analysis suggests that the mutation arises from a single founder. No DM2 mutation has been identified to date in sub-Saharan or east-Asian population. Herein, we report a Japanese family with the DM2 mutation. No consanguinity or genetic admixture with other ethnicities is documented. The cardinal clinical feature is a combination of adult-onset proximal muscle weakness and myotonia, consistent with the typical DM2 phenotype. PCR-amplification of the DM2-repeat detected a single normal allele at 228 bp and subsequent Southern blot analysis showed an expanded DM2 allele of 18.1kb (corresponding to 3400 repeats) in the proband. To our knowledge, this is the first DM2 family identified in non-Caucasian population. Although DM2 mutations were reported in non-European populations including Morocco, Algeria, Lebanon, Afghanistan and Sri Lanka, all reported DM2 patients were white and likely arose from a single common founder of European descent because they shared an identical haplotype. To investigate the ancestral origin of our DM2 family, we performed a haplotype analysis using SNPs and short tandem repeat markers flanking the DM2 CCTG repeat. Our data suggest this family has an expansion-associated haplotype distinct from that found in the Caucasian DM2.

1104/F

KRAS gene mutation analysis in a cohort of 50 Brazilian patients with Noonan and Noonan-like syndromes. *D.R. Bertola[1], A.S. Brasil[1], A.C. Pereira[2], L.M.J. Albano[1], R.S. Honjo[1], C.A. Kim[1], J.E. Krieger[2].* 1) Pediatrics, Instituto da Criança, São Paulo, SP, Brazil; 2) Heart Institute (InCor), Cardiology, São Paulo, SP, Brazil.

Noonan and Noonan-like syndromes comprise a group of disorders caused by deregulated RAS-MAPK signaling. In Noonan syndrome, three genes (PTPN11, KRAS and SOS1) in this pathway were described as responsible for its phenotype. In the case of cardiofaciocutaneous (CFC) syndrome, the main genes are: BRAF, MEK1 and MEK2 and in Costello syndrome, the HRAS gene is responsible for more than 80% of the cases. Mutations in KRAS gene have been described in a small proportion of Noonan syndrome patients (3%), as well as in CFC and some Costello syndrome individuals. We performed KRAS gene sequencing in 38 Noonan syndrome individuals, negative for PTPN11 mutations (2 with Noonan-like/multiple giant cell lesion syndrome), and 12 patients with Noonan-like syndromes (10 patients with CFC syndrome and 2 with Costello syndrome). None gene alterations were found in 38 Noonan syndrome and 10 CFC syndrome patients. A K5E substitution was found in exon 2 of the KRAS gene in one of the Costello syndrome individual. This patient showed some atypical findings, such as lymphedema and prominent corneal nerves, characteristics of Noonan syndrome. The involvement of several genes in Noonan and Noonan-like syndromes pose a complex task in the delineation of the molecular defect of these disorders. KRAS gene mutations were reported in some cases with a clear phenotypic overlapping of Noonan syndrome, CFC and Costello syndromes. Further descriptions of atypical cases and their molecular basis could improve the establishment of a more precise genotype-phenotype correlation and give a better direction of which gene should be screened first in each case.

1105/F

Recessive non-syndromic prelingual hearing impairment, GJB2 mutation scope in cochlea implant patients. *R. Birkenhäger, R. Laszig, A. Aschendorff.* Department of Otorhinolaryngology , University Hospital Freiburg, Germany.

Congenital sensorineural hearing impairment affects approximately 1-2/1000 newborn. Especially mutations in the GJB2 gene connexin-26 are the reason for non-syndromic hearing impairment. In the present study 289 patients with severe to profound hearing impairment and no evidence of any additional syndrome and abnormality in CT images of the temporal bones and inner ear, were analyzed for genetic alterations in the GJB2 and GJB6 genes. Mutations in the GJB2 gene were found in 133/289 (46,02 %) patients. 24/289 patients were heterozygous for GJB2 alterations, no other mutations were detectable (8,31 %). In three cases the mutation delGJB6-D13S1830 was found in combination with the c.35delG mutation in the GJB2 gene. In 129/289 patients with severe to profound hearing impairment, no mutations or gene alterations were detectable in the GJB2 gene (44,63 %). Twenty-eight different mutations were detected, ten of these gene alterations are novel (Met1Ile, Trp24Leu, c.146delC. Trp134 Stop, Val167Met, Cys169Tyr, Ser183Phe, Ile196Val, Leu213Met, Lys221Asn). These mutations were found partially in combination with the most common c.35delG mutation. These results demonstrate that individuals with severe to profound hearing impairment or deafness should be investigated for GJB2 (connexin-26) mutations. In the case of identification of GJB2 mutations genetic consulting can be offered. Additional screening of newborns with suspected hearing impairment can provide early identification of patients who require intensive speech therapy, need hearing aid and might be candidates for cochlear implantation.

1106/F

genetic analysis in patients with a clinical picture of Myotonic Dystrophy. *I.C. Ciro Candiano[1], P. Tarantino[1], S. Carrideo[1], E.V. De Marco[1], D. Civitelli[1], F. Annesi[1], F.E. Rocca[1], V. Greco[1], M. Caracciolo[1], C. Rodolico[2], A. Toscano[2], G. Vita[2], G.* 1) 1Intitute of Neurological Sciences, National Research Council, Mangone (Cosenza), Italy; 2) 2Institute of Neurology, University of Messina.

Myotonic dystrophy (DM) is a dominantly inherited disorder; the classic form (DM1) is caused by an expanded CTG repeat in the 3'-UTR of the dystrophia myotonica-protein kinase gene (DMPK) on 19q13. Disease severity varies with the number of repeats: normal subjects have 5 to 37 repeats; individuals with premutation (38÷49 repeats) are asymptomatic but premutation alleles are unstable; mildly affected persons have 50 to 80 repeats (protomutation); severely affected patients have 2,000 or more repeats. DM can also be caused by a CCTG expansion (mean ~5,000 repeats) in intron 1 of the zinc finger protein 9 (ZNF9) gene on 3q21 (DM2). We performed a molecular study of DMPK gene in patients with clinical features of DM. We analyzed the probands of 10 families and 71 unrelated patients. All patients' samples were initially screened by PCR identify unaffected individuals, demonstrating two alleles in the normal range and small expansions often observed in minimally affected cases. The patients showing only one PCR allele within the normal range require a Southern confirmation that has two possible outcomes: A) the patient is ruled homozygous for the normal allele, B) an expanded allele is detected, thus confirming the diagnosis of DM. Thirty-three subjects are wild-type; among them one presents a premutation. Fourty-seven patients are genetically confirmed DM1. Molecular analysis of DMPK gene in 81 subjects revealed the expanded CTG repeat in 47 patients with varying clinical severity and various sizes of repeat amplification; one patient from a consanguineous family DM1-linked had an expansion of CTG repeat on both alleles (65 and 933 repeats) but in DM homozygotes do not differ phenotipically from heterozygotes. Two individuals carried a protomutation (50÷80 CTG repeats). For the 33 unaffected patients other neuromuscular diseases should be considered; alternatively, many milder phenotypes may be caused by DM2 mutations.

1107/F

Point mutations in Spanish Charcot-Marie-Tooth families: relevance to clinical genetic testing. *C. Concheiro-Alvarez[1], P. Blanco-Arias[1], B. Quintáns[3], M.T. Darnaude[5], J. Pardo[3], S. Gómara[4], A. Carracedo[1,2], M.J. Sobrido[2].* 1) Grupo de Medicina Xenómica, Universidad de Santiago de Compostela, Spain; 2) Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela; 3) Hospital Clínico de Santiago de Compostela, Santiago de Compostela; 4) Hospital Xeral Cíes, Vigo, Spain; 5) Hospital de Móstoles, Spain.

Genetic analysis and counseling of CMT families without duplication of the PMP22 region at 17p12 is hampered by the extensive genetic heterogeneity. Knowledge of the genetic epidemiology of CMT in our region is relevant to the rationalisation of a protocol for molecular analysis, diagnostic decision making and genotype-phenotype correlation. A group of 47 Spanish patients (most of them Galician) with a clinical diagnosis of probable CMT and negative for the PMP22 duplication was selected for this study. Systematic sequencing of the coding region and exon-intron junctions of PMP22, MPZ, GDAP1, GJB1, EGR2, NEFL, and LITAF was performed. We found six patients carrying previously described mutations (two in MPZ, three in GJB1, 1 in NEFL). Additionally, we identified three novel coding sequence alterations in PMP22 (L5F), GDAP1 (R224del, two independent patients) and GJB1 (P172H). Four synonimous nucleotide changes were identified in NEFL, EGR2 and GJB1, as well as five nucleotide substitutions in intronic regions not contained in dbSNP. All the new sequence variations were screened in 296 Caucasian controls, 200 of which are Galician individuals without any neurological disorder. In all, 17% of patients (8/47) negative for PMP22 duplication could be diagnosed by sequencing. In addition, 10/47 (21%) patients have changes not seen in controls and therefore with a putative pathogenic effect. The most frequently mutated gene was GJB1 (3 known, 1 probable, 2 possible mutations), followed by MPZ (2 known mutations) and NEFL (1 known, one possible). No definite mutations were identified in PMP22, GDAP1, LITAF and EGR2. In conclusion, mutation screening of GJB1, MPZ and NEFL is cost-efficient in our population and should be offered to patients with demyeliniting or mixed neuropathy who tested negative for PMP22 dosage alterations.

1108/F

Multiplex Ligation-dependent Probe Amplification analysis identifies ENG and ALK1 deletions in Hereditary Hemorrhagic Telangiectasia. *T.G.W Letteboer[1], M. Tjong-Pon-Fong[1], C.J.J Westermann[2], J.J. Mager[2], R.J. Snijder[2], J.K. Ploos van Amstel[1].* 1) Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; 2) St.Antonius Hospital, Nieuwegein, Netherlands.

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder characterized by vascular malformations in multiple organs, resulting in mucocutaneous telangiectases and arteriovenous malformations, predominantly in the lungs (PAVM), brain (CAVM) and liver (HAVM). Mutations in the ENG gene and ALK1 (ACVRL1) gene cause HHT1 and HHT2 respectively. In 2004, SMAD4 mutations have been found to cause the combination of HHT and Juvenile Polyposis and recently two possible other loci were associated with HHT. In our laboratory in a population of HHT probands with a diagnosis according to the Curacao criteria(telangiectases, epistaxis, AVM, first degree relative with HHT), mutations in ENG or ALK1 were detected in more than 90% of the HHT families. Here we report on the results of DNA analysis performed in all HHT patients since the start of the DNA analysis in HHT. Analysis was performed in 302, apparently unrelated, probands. Of 42% of these, the presence of HHT symptoms was not well described. Mutation analysis was performed using direct sequencing of both ENG (exons 1-14) and ALK1 (exons 1-10). In probands without a mutation, subsequent Multiplex Ligation-dependent Probe Amplification (MLPA) was performed. In the ENG gene 112 probands showed 69 different pathogenic mutations. Two probands showed deletions identified by MLPA: one deletion of exon 3 and one deletion consisting of exons 3-14. In the ALK1 gene 87 probands showed 50 different pathogenic mutations. One deletion was detected, encompassing exons 2-8. No pathogenic mutations were identified in the remaining probands. These results indicate that large rearrangements are rare in both ENG (2.9%) and ALK1 (2.0%) and do not add significantly to the mutation detection rate. Secondly, when DNA analysis is performed in probands with confirmed diagnosis according to the Curacao criteria, the detection rate is high, whereas, when uncertainty exists on the clinical diagnosis, the mutation detection rate decreases significantly.

1109/F

Identification of Mutations in *CLN5* Gene in Neuronal Ceroid Lipofuscinosis (NCL) Patients with Diverse Ethnic Backgrounds and Variant Clinical Presentations. *W. Xin[1,2], D. Sleat[3,4], R. Kiely[1], X. Feng[1], L. O'Malley[1], Y. Shen[1], H. Zheng[3], P. Lobel[3,4], K. Sims[1,2].* 1) Neurogenetics DNA Diag Lab, Mass Gen Hosp, Boston, MA; 2) Dept of Neurology, Mass Gen Hosp, Boston, MA; 3) Ctr for Adv Biotech & Med, Piscataway, NJ; 4) Dept of Pharm, Robert Wood Johnson Med Sch-UMDNJ, Piscataway, NJ.

Neuronal ceroid-lipofuscinosis (NCL) are a group of autosomal recessive neuro-degenerative disorders. Disease characteristics include progressive cognitive and motor deterioration, visual loss, seizures and early death. Clinical suspicion of specific NCL type is based on the age of onset, clinical symptoms, and pathologic nature of inclusions by EM. Eight subtypes have been described. The Finnish variant late-infantile form of NCL (fLINCL; *CLN5*) has been reported primarily in patients from Finland. To date, 7 mutations have been described in *CLN5* gene in patients with onset of clinical symptoms in late infancy.

We now report mutation identification in 6 patients out of 16 screened who had clinical NCL features but no defect in other CLN genes. These patients had late-infantile, juvenile or adult onset NCL and were not of Finnish extraction. DNA sequencing of *CLN5* gene identified 1 NS mutation (in 2 patients), 2 MS mutations, and 2 out-of-frame deletions. A large deletion of all 4 exons was suspected in 1 patient. We have also identified previously unreported intronic and 3' UTR nucleotide changes and a silent mutation. To assay the functional consequences of these DNA mutations and to confirm pathogenicity, relative quantitation using mass spectrometry has been done. Loss of *CLN5* product has been documented.

The results from our study suggest that mutations in *CLN5* gene are 1) more common in NCL patients than originally reported, 2) are found in NCL patients with diverse ethnic backgrounds, and 3) can be identified in NCL patients with clinical presentations outside of the late-infantile age range. *CLN5* genetic testing is warranted in a wider population with clinical features suggestive of a NCL disorder.

1110/F

Obstructive uropathy in cystinuria knockout mice. *A. Sahota[1], E. Cui[1], H.J. Vernon[2], M. Yang[1], S. Ridgely[3], D. Reimer[3], S. Bledsoe[4], A.P. Evan[4].* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Dept Pediatrics, Johns Hopkins Univ Sch Med, Baltimore, MD; 3) Laboratory Animal Services, Rutgers Univ, Piscataway, NJ; 4) Dept Anat and Cell Biol, Indiana Univ Sch Med, Indianapolis, IN.

Introduction: Obstructive uropathy is a major cause of renal failure in children. Unilateral ureteral ligation in animals has provided valuable insight into the pathophysiology of urinary tract obstruction, but this is an acute injury model. It provides little information on the development of chronic obstruction as may occur in patients with bladder or ureter stones. Cystinuria, an inherited disorder of dibasic amino acid transport, is the most common cause of urinary tract stones in children. Cystinuria is classified as type I or non-type I, and these are caused by mutations in *SLC3A1* and *SLC7A9* genes, respectively. We created *SLC3A1* knockout mice to investigate the molecular basis of stone disease in cystinuria. **Methods:** Gross pathology was carried at age 12 months, and perfused kidneys, ureters, and bladder was examined by micro computed tomography (micro CT). In previous studies, perfusion and gross pathology were done at ages 4 and 8 months. **Results:** Bladders of *SLC3A1* male knockout mice at age 12 months were distended and filled with numerous uroliths up 4 mm in diameter, and the mucosal lining was thickened. Kidneys from these animals were enlarged and showed regional thinning of the cortex and dilation of the medulla and pelvis. A nephrolith was present in the medulla in a few mice. Ureters were grossly enlarged, but there was no evidence for large stone obstruction. Micro CT demonstrated extensive stone deposition in the bladder, and one animal also showed stones in the kidney and ureter. Bladder stones were also present in 4- and 8-months-old male mice, and there was hydronephrosis. Knockout female mice at any age group showed no gross lesions. **Conclusions:** These studies indicate that the bladder is the major site of stone deposition in cystinuria mice. The progressive accumulation of cystine stones and the subsequent hydronephrosis and hydroureter suggest that these mice may be more relevant models for studying obstructive uropathy compared with ureteral ligation.

1111/F

Identification of rare mutations and polymorphisms in α globin genes using denaturing high performance liquid chromatography (dHPLC) followed by sequencing analysis. *H.Y. Law[1], R.S. Roch[1], E.S. Tan[1], A.H.M. Lai[1], K.S. Aung[1], I.S.L. Ng[1,2].* 1) Dept Pediatrics, KK Women's & Children's Hosp, Singapore, Singapore; 2) National Thalassaemia Registry, Singapore.

Molecular analysis of gene mutation provides accurate diagnosis of genetic disease. However, analysis of an entire gene for all mutations is often not possible or cost effective. Current DNA diagnosis of α-thalassaemia screens for 11 mutations, including 3 2-gene deletions, 2 single gene deletions and 5 point mutations in α1 globin gene and 1 point mutation in α2 globin gene, which account for 99% of α-thalassaemia alleles in Singapore. To find out if patients suspected to be α-thalassaemia but negative for the screening test carry other rare mutations, a strategy has been developed to 1) determine the region that may habour a mutation in the α1 and α2 gene using dHPLC, and 2) confirm the mutation by sequencing. DNA samples from 636 patients from National Thalassaemia Registry (NTR) and outpatient clinics were analysed. Patients have either family history, MCV<85dL, presence of HbH inclusion bodies or a combination of the above. All were tested for 11 common α-thalassaemia mutations. For dHPLC analysis, α1 and α2 globin genes were specifically amplified, followed by nested amplification to generate 4 overlapping amplicons in each gene for heteroduplex analysis on dHPLC (Transgenomic WAVE). Amplicons displaying heteroduplexes were sequenced using ABI Cycle Sequencing kit to identify mutations. Mutations were detected in 68 samples: 30 in α1 and 38 in α2 gene. Mutations in 18 led to known Hb variants and 2 novel variants (in Cd5 and Cd122). Eight thalassaemia mutations were found in poly A (4), Start Codon (3) and promotor (1). Interestingly the Cd122 (CAC/CTC) in α2 globin gene appeared to be associated with HbH phenotype in combination with Hb Bleuland (Cd108 (ACC/AAC))detected by dHPLC. As various mutations displayed characteristic chromatograms on dHPLC, prescreening using dHPLC followed by confirmation with sequencing is effective in detecting mutations, making screening rare mutation more affordable.

1112/F

Molecular genetics of Meckel syndrome. *J. Tallila[1], R. Salonen[2], L. Peltonen[1,3,4], M. Kestilä[1].* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, Väestöliitto, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) The Broad Institute, MIT Boston, MA, USA.

Meckel syndrome (MKS, MIM 249000) is a lethal developmental disorder characterized primarily by a combination of occipital meningoencephalocele, large polycystic kidneys, fibrotic changes of the liver and polydactyly. The inheritance mode is autosomal recessive. Although the frequency varies greatly among populations, MKS represents the most common form of syndromic neural tube defects (NTDs). In Finland the estimated frequency is 1:9000. The genetic heterogeneity of MKS is well established and the *MKS1* (17q23) and *MKS3/TMEM67* (8q21.13-q22.1) genes were characterized recently. In addition, the *MKS2* gene has been localized to chromosome 11q13. The founder mutation in the novel *MKS1* gene is a 29 bp intronic deletion that is mainly found in Finnish patients, but also in other Caucasian patients. This deletion interrupts the splicing, which causes a frame shift leading to a non-functional protein. We have additionally identified two novel mutations in the *MKS1* gene. In 70% of the Finnish MKS families the patients are homozygous for the founder mutation and the remaining 30% of the families represent mutation(s) in other gene(s). As we have several families without a mutation in the *MKS1* gene and with no linkage to the MKS2 or MKS3 loci, we are currently in search of new MKS locus/loci by using Illumina SNP arrays (317K) to map the disease loci. Because *MKS1* and *MKS3* are known to encode polypeptides having roles in cilia function, we will first analyze positional candidate genes that are known to be linked to cilia. Identification of the first MKS proteins moved the link between cilia and neural tube closure beyond animal models and into the area of human disease. Because the cellular role of cilia is poorly understood in human embryonic development, MKS serves as an excellent model for ciliary dysfunction. Studies of MKS will undoubtedly provide new insights into early embryonic development; information critical for better understanding of molecular details behind several human malformation syndromes.

1113/F

Evidence of genetic heterogeneity for Primary Ciliary Dyskinesia in an inbred Amish-Mennonite community. *M.A. Zariwala[1], M.W. Leigh[1], H. Lie[2], A. Lori[1], R. Herrin[2], A. Bowcock[2], T. Ferkol[2], M.R. Knowles[1].* 1) Department of Pathology, Medicine, and Pediatrics, University of North Carolina, Chapel Hill, NC; 2) Department of Pediatrics and Genetics, Washington University, St. Louis, MO.

Primary ciliary dyskinesia (PCD) is genetically heterogeneous, autosomal recessive trait with impaired mucociliary clearance leading to sino-pulmonary disease and situs inversus in half of the patients (termed Kartagener syndrome). Ciliary ultrastructural analysis in most patients reveals defective outer dynein arms (ODA). Mutations in *DNAI1* and *DNAH5* (encode intermediate and heavy chain dyneins of ODA respectively) have been identified in 38% of PCD patients. We discovered an increased frequency of PCD in an inbred Amish-Mennonite community in Missouri and suspect that this population is enriched with novel disease-causing mutations. We identified nine patients (with seven sub-families) with an unequivocal diagnosis of PCD based on clinical evaluation and defective ODA. Majority of patients from this community could be traced back to two couples from the initial founders. Mutation analysis was carried out for known mutations in *DNAI1* and *DNAH5*. Three affected patients from three sub-families were heterozygous for *DNAI1* (IVS1+2_3insT) mutation, but full coding gene sequencing did not reveal the second mutant allele. Haplotype analysis of *DNAI1* using intragenic SNP and microsatellite markers showed no evidence of concordance for the second allele in any of these patients, implicating another gene as causative. Another sub-family harbored a monoalleliec *DNAH5* (10815delT) mutation in two affected sibs, and haplotype sharing at DNAH5 locus in both affected sibs suggests second mutant allele; full sequencing of *DNAH5* is underway. Analyses of several intragenic SNP and/or microsatellite genotyping confirmed that *DNAI1* and *DNAH5* and seven other candidate genes (*DNAH11*, *DNAH7*, *DNAH9*, *DNAI2*, *DNAL4*, *DNAH3* and *TCTEL1*) were not linked to PCD. In conclusion, we detected mutations in two genes in inbred Amish-Mennonite cohort. Additionally, there is an evidence for another mutation in *DNAH5*, plus, a novel gene. Supported by GCRC00046, MO1 RR00046-42, RO1 HL071798 (NHLBI), 5U54 RR019480 NCRR) and R21 HL07024.

1114/F

Genetic defects in patients with X-linked Lymphoproliferative Syndrome in North American. *K. Zhang[1], JA. Johnson[1], J. Villanueva[2], B. Tinkle[1], J. Bleesing[2], AH. Filipovich[2].* 1) Division of Human Genetics, Cinncinnati Children's Hosp, Cincinnati, Ohio, USA; 2) Division of Hematology /Oncology, Children's Hospital Medical Center, Cincinnati, Ohio, USA.

X-linked Lymphoproliferative Syndrome (XLP) is an immunodeficiency disorder, characterized by fatal infectious mononucleosis, hypogammaglobulinemia, lymphohistiocytosis and B-cell lymphomas. Historically, mutations in the signaling lymphocyte activation molecule (SLAM)-associated protein SAP (SH2D1A gene) have been associated with XLP. More recently, a study of 18 XLP families from a French group reported mutations in the X-linked inhibitor-of-apoptosis XIAP (BIRC4) gene. The mutation spectrum of BIRC4 gene in XLP patients in the North American is not known. In the last three year, we tested more than 200 patients with suspected clinical diagnoses of XLP. We identified 41 patients who were hemizygous for mutations in the SH2D1A gene, of which 12 are previously unreported novel mutations. Interestingly, gross deletions involving one or multi exons, account for more than 30% of the mutations found in SH2D1A. SAP protein analysis by flow cytometry was also performed whenever the sample is available for testing. Very good correlation was observed between the type of mutation and the level of SAP expression. However, the correlation between the genotype of SH2D1A gene and the disease phenotype is much more complex. The type of mutation itself can not predict the course and severity of the disease. We also tested 20 XLP patients who have normal SH2D1A analysis for the presence of BIRC4 mutations. We found two patients who carried gross deletions in BIRC4 gene. Both patients have hepatosplenomegaly, one patient has had a liver transplant, and the other will undergo HCST. Our findings highlight the importance of molecular diagnosis in patients with XLP especially the great indication for their family members with atypical late onset XLP. Farther elucidation of the molecular basis for XLP will continue to provide an expanded understanding of the immune system's role in responding to EBV infection and the biological function of SAP and XIAP in adaptive immune responses and development.

1115/F

Osteofibrous Dysplasia: Description of Mendelian Inheritance and Identification of Positional Candidates. *X. Gao[1], D. Zhang[2], L.A Karol[1,2], E. Smith[1], C.A Wise[1,2].* 1) Seay Center, and Orthopaedics, Texas Scottish Rite Hospital, Dallas, TX; 2) Department of Orthopaedic Surgery, and McDermott Center, University of Texas Southwestern Medical Center at Dallas.

Osteofibrous dysplasia (OD) (MIM #607278) is an early-onset condition marked by isolated, tumor-like lytic lesions bone in skeletally immature patients. The site of involvement is typically the cortex of the tibia, although other bony involvement is described. Symptomatic children usually present at less than five years of age with anterolateral bowing or subsequent pathologic fracture of the affected bone. The differential diagnosis of OD includes congenital pseudoarthrosis of the tibia (CPT), a condition usually associated with neurofibromatosis type 1, and the more common fibrous dysplasias. Resemblance to adamantinoma of long bones, a low-grade malignant neoplasm affecting mostly the tibia of young adults, has led to the suggestion that OD may be related to this condition. Interestingly, cytogenetic studies of OD and adamantinoma bone specimens have revealed apparently overlapping results, with trisomies 7, 8, and 12 observed in each disease. Despite these observations, the pathogenesis of OD is unknown, and until recently was not generally considered heritable. We ascertained a three-generation family in which eight individuals were considered affected with OD and performed a genome-wide scan of polymorphic loci spaced at 10-15 cM density. LOD scores were calculated from the resulting genotypes under a model of dominant inheritance and varying penetrances. Importantly, evidence of linkage was not detected for loci in the region of the NF1 or TNFRSF11A genes responsible for neurofibromatosis type 1 and familial expansile osteolysis, respectively. We obtained strongest results for regions of chromosomes 3q13-21 and 8q23-24, where subsequent fine-mapping produced maximum multipoint LOD ~ 2.0 for the chromosome 8 linkage peak. This is of particular interest given the previous cytogenetic observations in OD. Genes encoded in linked regions, particularly those that function in pathways of early osteogenesis, are candidates for further investigation in both familial and sporadic cases of OD.

1116/F

Sporadic Non-Immune Hydrops Fetalis Can Be Caused by *VEGFR3* Mutations. *A. Ghalamkarpour[1], C. Debauche[2], N. Van Regemorter[3], N. Revencu[1], Y. Gillerot[4], Y. Sznajer[5], D. Thomas[6], L.M. Boon[1,7], M. Vikkula[1].* 1) Human Molecular Genetics, de Duve Institute, Brussels, Belgium; 2) Department of Neonatology, Cliniques Universitaires Saint-Luc, Belgium; 3) Center de Génétique ULB, Hôpital Erasme, Belgium; 4) Center for Human Genetics, Cliniques Universitaires Saint-Luc, Belgium; 5) Unité de Génétique Clinique Pédiatrique, ULB, Belgium; 6) Unité Diagnostic Anténatal, Hôpitaux Iris Sud, Belgium; 7) Centre for Vascular Anomalies, Cliniques Universitaires Saint-Luc, Belgium.

Mutations in the vascular endothelial growth factor receptor 3 gene, *VEGFR3/FLT4*, have been identified in a subset of families with hereditary lymphedema type I or Milroy disease (MIM 153100). The classical clinical phenotype of the patients with a *VEGFR3* mutation is congenital lower limb lymphedema, which is usually bilateral and below the knees. We have shown that an inherited *VEGFR3* mutation can cause hydrops fetalis, indicating for a systemic lymphatic dysfunction. We hypothesized that sporadic non-immune hydrops fetalis may also result from an altered VEGFR3 signaling. Here, we report two such cases. The first patient presented *in utero* with severe generalized skin edema. The edema limited to the lower limbs after birth, yet in the presence of chylous ascites. We identified a heterozygous *VEGFR3* mutation in this patient, which was not present in either of the non-affected parents, and thus constituted a dominant *de novo* mutation. The second case was diagnosed *in utero* with thickening of subcutaneous tissues and chylous ascites. Similar to the first patient, the edema spontaneously limited to the lower limbs after birth. A heterozygous *VEGFR3* mutation was identified in this patient, however, we were unable to collect samples from his healthy parents and thus it is either a *de novo* or a non-penetrant familial mutation. Our data indicate that *VEGFR3* mutations can be the pathophysiological cause of non-immune hydrops fetalis in the patients with no family history of edema. This has implications for genetic counseling and a *VEGFR3* screening can be suggested in such individuals. (http://www.icp.ucl.ac.be/vikkula) (Miikka.Vikkula@uclouvain.be).

1117/F

Hydrolethalus syndrome: From molecular genetics to functional studies. *H. Honkala[1], J. Lahtela[1], K. Wartiovaara[2], R. Salonen[3], L. Peltonen[1,4,5], M. Kestilä[1].* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Developmental Biology, Institute of Biomedicine, University of Helsinki, Helsinki, Finland; 3) Department of Medical Genetics, Väestöliitto, Helsinki, Finland; 4) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 5) The Broad Institute, MIT, Boston, MA, USA.

Hydrolethalus syndrome (HLS) is a severe malformation syndrome of the fetal stage belonging to the Finnish disease heritage which leads to stillbirth or death shortly after birth. HLS is characterized by multiple developmental defects including CNS malformations such as hydrocephaly and absent midline structures, polydactyly and defective lobation of the lungs. The disease-causing mutation in the *HYLS1* gene in chromosome 11 is an A to G transition that causes the D211G change in the 299 amino acid polypeptide with so far unidentified function. Mutation analysis has also been performed for several foreign cases with the phenotype resembling HLS, but no mutations have been found. Although the precise function of the protein is currently unknown, the severe effects of the mutation suggest an important role of HYLS1 in fetal development.

Present studies include *in silico* expression array and pathway analyses, initial results showing downregulation of several genes involved in lipid metabolism whereas upregulation of genes related to cell cycle events in patient cells. We have studied e.g. cell cycle regulation by using cultured neuronal progenitor cells obtained from an aborted HLS fetus and from normal fetuses from the pregnancies terminated for social reasons. The results indicate an increased cell proliferation rate of the patient cells. In addition, experiments with the existing transgenic knock-in *D. melanogaster* model expressing human *HYLS1* have been started recently, the purpose being to compare both the overall morphology as well as the central nervous system of *HYLS1* wt and mutant flies using HYLS1 specific antibody. These results will give us novel information about the function of HYLS1 and about the essential molecular events during the embryonic development in general.

1118/F

The unfolding clinical spectrum of POLG mutations. *M.J. Blok[1,2], B. van den Bosch[2], E. Jongen[1], C. van de Burg[2], A. Hendrickx[1], M. Pieters[1], J. Bierau[1], I. de Coo[3], H. Smeets[1,2].* 1) Clinical Genetics, academic hospital Maastricht, Maastricht, Netherlands; 2) Department of Genetics and Cell Biology, Maastricht University, Maastricht, Netherlands; 3) Department of Neurology, Erasmus Medical Center Rotterdam, Rotterdam, Netherlands.

Mutations in the nuclear encoded polymerase-gamma (POLG) gene turn out to be a major cause of a broad spectrum of mitochondrial diseases and of secondary mtDNA defects, like mtDNA depletion and multiple mtDNA deletions. We have screened 114 patients, based on clinical criteria and/or the presence of multiple deletions and mtDNA depletions in muscle, for POLG mutations. We have identified 39 mutations in 22 patients, the majority of which cause recessive disease and only a few display a dominant segregation pattern. The group of 39 consisted of 16 different mutations, in which the substitution p.A467T was most frequently observed (n=13). Four new mutations, p.A804T, p.S1095R, p.L966X and p.R275X were found, of which the mutations p.S1095R and p.L966X mutation were detected in severe childhood cases, together with the known mutations p.D1184N and p.A467T, respectively. We also detected for the first time the presence of the known mutation p.R943H in a family with dominant chronic progressive opthalmoplegia and premature ovarian failure. Furthermore, we question the pathogenicity and dominant nature of the previously reported p.G517V mutation, based on the detection of this mutation in multiple unaffected individuals from different families. The clinical presentation of POLG defects is extremely variable, but, in contrast to other reports, we do not observe a gender bias for the childhood cases with this mutation. In addition, the p.A467T mutation did not appear to be more frequent in childhood cases than in adult cases, since we found equal frequencies. Patients with POLG mutations are at risk of death from status epilepticus and liver failure, if exposed to sodium valproate. This requires that the result of genetic testing is available within a day and prior to treatment.

1119/F

New insights into the understanding of premature suture closure: increased plasticity of cranial periosteal cells harboring Apert p.Ser252Trp FGFR2 mutation. *R. Fanganiello[1], A.L. Sertié[1], E. Yeh[1], D.F. Bueno[1], M.T. Martins[2], I. Kerkis[3], M.R.S. Passos-Bueno[1].* 1) Dept Genetics & Evolution, Biosciences Inst, Sao Paulo, Brazil; 2) Department of Pathology, School of Dentistry, São Paulo, Brazil; 3) Instituto Butantã, Sao Paulo, Brazil.

Apert syndrome (AS), a severe form of craniosynostosis, is caused by dominant gain-of-function mutations in FGFR2. Accelerated suture fusion during development and after surgery has been attributed to an increased osteogenic potential of the osteoblasts. However, as we have shown in a previous report (Fanganiello et al, 2007, in press), it is possible that the FGFR2 mutant periosteal cells also contribute to the accelerated process of suture fusion. In order to better understand the function of this tissue in AS cranial pathophysiology we compared the proportion of mesenchymal surface markers and the differentiation potential of the coronal suture periosteum cells from 3 AS patients (p.Ser252Trp mutation) to wild type periosteal fibroblasts from 3 controls. In flow-cytometry experiments, we observed that both AS and control cells stained positively (>95%) for mesenchymal stem cell (MSC) markers (SH2, SH3, CD29, CD90) but negative for hematopoietic (CD45, CD117) or endothelial SC markers (CD31), indicating a highly homogeneous population (95-98%) of mesenchymal cells. Under in vitro differentiation conditions AS periosteal cells had a strikingly higher differentiation potential towards osteoblast and adipocytes when compared to non-mutated control cells. This was verified by cell/tissue specific staining techniques (von Kossa and Oil-Red) and confirmed by quantitative real-time PCR of tissue specific gene expression (Osteopontin and COL1A1 for osteoblasts and LPL for adipocytes, $p \leq 0.05$). We are currently evaluating the in vivo ability of these cells to reconstruct large-sized cranial bone defects in rats with a model previously established in our lab. Our findings suggest that the mutant periosteal cells might play an important role in the suture ossification of AS patients as well as in the recurrent suture closure after surgery due to its increased osteogenic potential. FAPESP, CNPq.

1120/F

Syndromic isolated growth hormone deficiency in a patient with a splice mutation in the GHRHR gene. *L. Hilal[1], Y. Hajaji[1], M-P. Vié-Luton[2], Z. Ajaltouni[2], B. Benazzouz[1], M. Chana[3], A. Chraibi[3], A. Kadiri[3], S. Amselem[2], M-L. Sobrier[2].* 1) Neuroendocrin Genetic and Physiology, Ibn Tofail University, Kenitra, Morocco; 2) Inserm U654, Hopital A Trousseau, Paris, France; 3) Endocinology Diabetology and Nutrition, CHU Ibn Sina, Rabat, Morocco.

Isolated growth hormone deficiency (IGHD) may be of genetic origin. One of the very few genes involved in that condition encodes the growth hormone releasing hormone receptor (GHRHR), which, through its ligand (GHRH), plays a pivotal role in the regulation of GH synthesis and secretion by the pituitary. We investigated two siblings born to a consanguineous union presenting with a marked growth retardation (> 5SD) associated with anterior pituitary hypoplasia and severe GH deficiency. In addition to these classical phenotypic features for IGHD, one of the patients had a Chiari I malformation, an arachnoid cyst and a dysmorphic anterior pituitary. No abnormality was found in the GH-N gene. However, sequencing of all GHRHR coding exons and their flanking intronic regions led to the identification, in both patients, of a homozygous sequence variation located in the consensus donor splice site of intron 1 (c.57+2T>G). Using in vitro transcription assays, we showed that this mutation results in abnormal splicing of GHRHR transcripts that, if translated, would lead to the synthesis of a severely truncated protein lacking all transmembrane and intracellular domains. Such developmental abnormalities, which were not already described for this type of IGHD, point to the possible role of the GHRHR in the proper development of extrapituitary structures, through a so far unknown mechanism that could be direct or secondary to severe GH deficiency.

1121/F

Gene expression profiling reveals complement mediated regeneration in skeletal muscle from Leigh syndrome patients with a SURF1 mutation. *H. Smeets[1], R. Van Eijsden[1], R. Mineri[2], P. Lindsey[1], L. Eijssen[1], C. van den Burg[1], E. de Wit[3], T. Ayoubi[1], C. di Blasi[2], J. Zeman[4], M. Zeviani[2], I. de Coo[3], W. Sluiter[3], V. Tiranti[2].* 1) Dept Genetics & Cell Biol, GROW, Univ Maastricht, Maastricht, NL; 2) Unit Molec Neurogenet & Neuromusc Dis, Neurol Inst C Besta, Milano, I; 3) Dept Biochem & Neurol, Mitoch Res Unit, ErasmusMC, Rotterdam, NL; 4) Dep Pediatr, 1st Fac Medic, Charles Univ, Prague, CZ.

Leigh syndrome is an early-onset, progressive and often fatal neurodegenerative disorder, characterized by necrotic lesions in the brain basal ganglia. Leigh syndrome with a decreased cytochrome c oxidase (COX) activity is frequently caused by mutations in the SURF1 gene, which disturb COX-assembly. To characterize molecular pathophysiological processes, gene expression profiling was performed in skeletal muscle biopsies from SURF1 Leigh syndrome patients and controls. No significant alterations in transcription levels of oxidative phosphorylation (OXPHOS) genes were observed. Altered processes were protein synthesis, DNA metabolism, cell cycle, skeletal muscle development, and intriguingly, the complement system. Genes of the classical complement pathway (C1R, C1S, and C3) - not only involved in immune response, but also in tissue regeneration - were significantly upregulated. This regenerative response is also observed in gene expression changes in other processes. Most likely, SURF1 mutations lead to increased production of reactive oxygen species (ROS), causing protein damage and increased turnover. This could trigger complement activation and induce a process of muscle regeneration as a rescue process. A causative relation has to be established, but our data provide new insight in the molecular processes occurring in muscle of SURF1 patients, which could be involved in other OXPHOS disorders as well.

1122/F

Allgrove Syndrome in a Mexican-American Family is Caused by an Ancestral Mutation Derived from North Africa. *A.J. Chang[1], M.M. Kline[1], Y. Currie[2, 3], H. Wijesuriya[2, 3], M. Perez-Ospina[2, 3], J. Hartiala[2, 3], T.B. Buchanan[1], R.M. Watanabe[2], H. Allayee[2, 3].* 1) Department of Medicine; 2) Department of Preventive Medicine; 3) Insitute for Genetic Medicine; USC Keck School of Medicine, Los Angeles, CA.

Allgrove syndrome is an autosomal recessive disorder characterized by alacrima, achalasia, and adrenocorticotropic hormone-resistant adrenal insufficiency. Nearly 30 different mutations have been described in the AAAS gene but it has been difficult to obtain consistent phenotype-genotype correlations due to the rarity of the disorder and phenotypic variability amongst patients. We describe the clinical and genetic profile of a Mexican-American family in which two siblings display classic Allgrove syndrome features, with the older sister becoming symptomatic in adolescence compared to her brother who began displaying abnormalities at approximately the age of seven. Genetic analysis revealed that the mother carries a previously described 1191insA mutation in exon 13. The father is a carrier of a G>A substitution at the first position of intron 14, which abolishes the splice donor site, and was originally identified as a founder mutation in inbred Allgrove patients from North Africa. As expected, both affected siblings are compound heterozygotes. Comparison of haplotypes in the family with those observed in several North African patients using ~10 microsatellite markers dispersed evenly in a 1Mb region flanking the AAAS gene revealed that the father's haplotype harboring the mutation matched the North African haplotype nearly perfectly. This North African haplotype was present in less than 5% of ~300 individuals from 132 Mexican-American families without Allgrove syndrome. We conclude that previously described mutations are the cause of Allgrove syndrome in this Mexican-American family and that the father's mutation is most likely derived from an ancestral North African chromosome. This latter notion is consistent with historically known migration patterns of North African individuals into Spain approximately 1200 years ago followed by the subsequent colonization of Central and South America by Spanish explorers.

1123/F

Intragenic deletions represent a significant disease causing mechanism: Evidence from a select group of rare genetic disorders. *E.V. Haverfield, A.J. Platteter, M.A. Dempsey, W.B. Dobyns, S. Das.* Department of Human Genetics, University of Chicago, Chicago, IL.

Genetic abnormalities associated with human diseases range from whole chromosome abnormalities to point mutations within genes. Chromosome gain or loss and structural rearrangements as well as most microdeletions or duplications are identified by chromosome analysis, FISH and array CGH. Point mutations and small insertions or deletions are detectable by DNA sequencing and mutation scanning methods. Intragenic deletions and duplications that affect single or multiple exons are an important disease-causing mechanism and are a category of mutations that remain undetected by these methods. We determined the frequency of intragenic deletions and duplications in a select group of rare genetic disorders: lissencephaly and subcortical band heterotopia (*LIS1* and *DCX* genes), Sotos syndrome (*NSD1* gene) and Cornelia de Lange syndrome (*NIPBL* gene). Analysis was performed with multiplex ligation probe amplification (MLPA) and real-time quantitative PCR. All patients were negative for mutations in their respective gene and large microdeletions were excluded in *LIS1* and *NSD1*. In 43 phenotypically well-characterized patients with lissencephaly, deletions or duplications in the *LIS1* gene were identified in 19 (44%). In 11 patients with subcortical band heterotopia, deletions in the *DCX* gene were identified in 3 (27%). *NSD1* deletions were found in 1 of 6 patients (17%) with suspected Sotos syndrome and *NIPBL* deletions were observed in 1 of 52 patients (2%) with suspected Cornelia de Lange syndrome. Partial deletions of the *PANK2* gene in patients with autosomal recessive pantothenate-kinase associated neurodegeneration were identified when apparent homozygosity for a rare mutation was due to compound heterozygosity for a mutation and an intragenic deletion. A systematic study of intragenic deletions in the *PANK2* gene is in progress. Our studies illustrate the presence of intragenic deletions and duplications in rare genetic disorders and indicate that they may contribute significantly to disease etiology. Our results have important implications for the diagnostics of genetic disease.

1124/F

Molecular Characteristics of X-linked retinoschisis(XLRS) in Koreans. *S.Y. Kim[1], H.S. Ko[1], Y.S. Yu[2], J.M. Hwang[3], J.Y. Kim[1], M.W. Seong[1], S.S. Park[1].* 1) Department of Laboratory Medicine, Seoul National University Hospital and Seoul National University Hospital Clinical Research Institute, Seoul, Korea; 2) Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea; 3) Department of Ophthalmology, Seoul National University Bundang Hospital, Sungnam, Korea.

X-linked retinoschisis(XLRS) is one of the most common causes of juvenile macular degeneration in young males. It is characterized by a mild to severe decrease in visual acuity, foveal schisis due to splitting of the retinal layers, progressive macular atrophy, and reduction in the ERG b-wave. The gene RS1 has been identified as a cause of XLRS. We investigated mutation spectrum of the RS1 gene in Korean patients diagnosed with XLRS. A Total of 13 unrelated probands were included in this study with their available family members. All six exons of the RS1 gene were amplified by polymerase chain reaction and directly sequenced. We identified 13 genetic variations. Nine missense mutations and one intronic polymorphism have previously been reported: c.214G>A(Glu72Lys), c.305G>A(Arg102Gln), c.426T>G(Cys142Trp), c.544C>T(Arg182Cys), c.589C>T(Arg197Cys), c.590G>A(Arg197His), c.625C>T(Arg209-Cys), c.638G>A(Arg213Gln), c.647T>C(Leu216Pro) and c.184+35T>C. Three sequence variations were novel: One missense mutation(c.227T>G, Val76Gly) and two splice-site variations(c.78+1G>T and c.78+5G>A). Ten patients had one or more mutations. No sequence variation in RS1 was detected in three patients. All the missense mutations were located within the discoidin domain of retinoschisin protein. The clinical diagnosis of XLRS can be challenging. Therefore, population genetic studies of XLRS and identification of the causative mutations in the RS1 gene will be helpful for confirmation of the diagnosis and in genetic counseling.

1125/F

Missense and non-sense mutations in the alternatively spliced exon 2 of COL2A1 cause the ocular variant of Stickler Syndrome. *M. Mannikko[1], A. McAlinden[2], M. Majava[1], P.N. Bishop[3, 4], R. Perveen[4], G.C.M. Black[4], M.E. Pierpont[5], L. Ala-Kokko[1, 6].* 1) Collagen Research Unit, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland; 2) Department of Orthopaedic Surgery, Washington University School of Medicine, St Louis, Missouri, USA; 3) Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences,University of Manchester, UK; 4) Medical Genetics Research Group, School of Medicine, University of Manchester, UK; 5) Children's Hospital of Minnesota, Department of Pediatrics and Ophthalmology, University of Minnesota, Minneapolis, Minnesota, USA; 6) Connective Tissue Gene Tests, Allentown, PA, USA.

Stickler syndrome type I (STL1) is a phenotypically heterogeneous disorder characterized by ocular and extraocular features. It is caused by null-allele mutations in the COL2A1 gene which codes for procollagen II. COL2A1 precursor mRNA undergoes alternative splicing, resulting in two isoforms, a long form including exon 2 (IIA) and a short form excluding exon 2 (IIB). The short form is predominantly expressed in adult cartilage, and the long form during early development and in the vitreous, the only adult tissue containing procollagen IIA. Recent evidence indicates that due to the tissue-specific expression of these two isoforms, premature termination codon mutations in exon 2 cause Stickler syndrome with minimal or no extraocular manifestations. We describe here two mutations in exon 2 of COL2A1 in three patients with predominantly ocular Stickler syndrome: Cys64Stop in two patients, and a novel structural mutation, Cys57Tyr, in one. RT-PCR of total lymphoblast RNA from one patient with the Cys64Stop mutation revealed that only the normal IIA allele was present, indicating that the mutation resulted either in complete loss of the allele by non-sense-mediated mRNA decay or by skipping of exon 2 via non-sense-mediated altered splicing, resulting in production of the type IIB isoform. The results of COL2A1 mini-gene expression studies suggest that both Cys64Stop and Cys57Tyr alter positive cis regulatory elements for splicing, resulting in a lower IIA:IIB ratio.

1126/F

Unusual association of combined pituitary hormone deficiency with deafness in a patient with two novel mutations in the LIM-homeodomain transcription factor LHX3. *M-L. Sobrier[1], C. Heinrichs[2], M-P. Luton[1], S. Rose[1], S. Amselem[1].* 1) Inserm U654, Hopital A Trousseau, Paris, France; 2) Pediatric Endocrinology, Reine Fabiola Children Hospital, Bruxelles, Belgium.

LHX3 encodes a LIM-homeodomain transcription factor that plays important roles in the proper development of the pituitary and motoneurons. Only seven LHX3 mutations have so far been reported in humans. All of them were found in the homozygous state in patients with combined pituitary hormone deficiency (CPHD) (involving GH, TSH, PRL, FSH/LH , and sometimes ACTH); all but one mutations were identified in patients with a rigid cervical spine, a phenotypic feature believed to result from a LHX3-dependent neurological defect that is not rescued by the closely related protein LHX4. Here, we report the identification of two new LHX3 gene defects found in a compound heterozygous state in a patient presenting not only with CPHD and a rigid cervical spine, but also with persistent motor delay and profound sensorineural hearing loss. One defect (c.252-3 C>G) affects the acceptor splice site preceding exon 3, while the other is a missense mutation (p.C123Y) involving a well-conserved amino acid of the LIM2 domain. To determine the consequences of the c.252-3C>G mutation on the splicing of LHX3 transcripts, HEK293 cells were transfected with expression vectors containing the normal or the mutant LHX3 minigene consisting of a genomic fragment spanning exon 1b to exon 5. RT-PCR amplification of LHX3 transcripts isolated from those cells revealed an abnormal splicing of transcripts that, if translated, would lead to a severely truncated protein. The missense mutation was found to be associated with a partial loss of transcriptional activity on several pituitary gene promoters. Taken together, these data demonstrate that these two novel sequence variations are not rare polymorphisms but represent disease-causing mutations. The unusual report of a hearing loss in this patient, who was born to a non-consanguineous union, now prompts us to investigate the possible role of LHX3 in the proper development of the auditory system in humans.

1127/F

Identification of the G1363S mutation of the lactase gene (LCT) in two siblings of Turkish origin. *S. Torniainen[1], C. Gijsbers[2], M. Potter[3], I. Jarvela[1, 4].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) The Juliana Children's Hospital, The Hague, The Netherlands; 3) Department of Pathology and Molecular Medicine, McMaster University, Ontario, Canada; 4) Helsinki University Hospital, Laboratory Services, Helsinki, Finland.

Congenital lactase deficiency (CLD) is a rare, severe gastrointestinal disorder where the activity of lactase is very low or absent in the intestinal wall since birth. The main symptoms are watery diarrhoea and severe dehydration after ingestion of lactose. CLD is inherited as an autosomal recessive trait and it is enriched in the Finnish population. Five mutations in the lactase gene (LCT) have recently been identified to underlie CLD in the Finnish population. Of them, the founder mutation (Y1390X) is present in 90% of the disease alleles. The other four mutations S1666fsX1722, S218fsX224, G1363S, Q268H are family specific. We have obtained DNA from three foreign origin patients with clinical features compatible with CLD. Two of the patients are siblings from the Netherlands whose parents are second cousins. Their ethnic origin is Turkish. The third patient is from Canada with Portuguese origin. In order to characterize the spectrum of mutations in non-Finnish CLD patients we have sequenced the LCT gene in these patients. We have identified the previously know mutation G1363S in exon 9 in homozygous form in Turkish siblings. Both parents were carriers of the same mutation. No mutation has so far been identified in the third patient. In conclusion, we report here the first non-Finnish CLD patients whose mutations have been identified. Our results further confirm the role of LCT in CLD. We hypothesize that the G1363S mutation could have arrived Finland from Asia.

1128/F

Novel de novo SOX2 mutations in patients with severe eye defects and associated developmental abnormalities. *M-P. Vié-Luton[1], M-L. Sobrier[1], C. de Barace[2], E. Feigerlova[3], S. Rose[1], N. Idres[2], S. Odent[4], M. Tauber[3], S. Amselem[1].* 1) Inserm U654, Hopital A Trousseau, Paris, France; 2) Paediatric Unit, Hospital Center, Saint-Brieuc, France; 3) Children's Hospital, Toulouse, France; 4) Medical Genetic's Unit, CHU Rennes, France.

SOX2 is a transcription factor involved in the regulation of embryonic development and cell fate determination. Although mice carrying a targeted disruption of Sox2 in the heterozygous state have abnormal anterior pituitary development with reduced levels of GH, LH and TSH, and no eye defect, the few heterozygous SOX2 mutations identified in humans can cause bilateral anophtalmia-microphtalmia and/or developmental delay, defects of the corpus callosum, esophageal atresia, sensorineural hearing loss. Here, we investigated two independent patients with severe eye defects; the first one had right anophtalmia and left optic nerve hypoplasia, while the second, who was born to a consanguineous union, had bilateral anophtalmia associated with hypogonadotropic hypogonadism and short stature. Brain magnetic resonance imaging revealed a thin corpus callosum in the first patient, and, in the second patient, a cyst of the septum pellucidum, whereas pituitary morphology was normal. The two patients were found to carry novel de novo SOX2 mutations in the heterozygous state: patient #1 bears a missense mutation (p.W51R) involving a residue located in the HMG domain of the protein and that is highly conserved throughout evolution. A single base deletion (c.540delC) was identified in patient #2; this latter defect would result in a frameshift that introduces 21 novel amino acids before a premature stop codon at position 202, thereby leading to a truncated protein. Additional studies are underway to assess the functional consequences of these two novel SOX2 defects.

1129/F

Molecular Analysis of Progressive Familial Intrahepatic Cholestasis In Israel. *T. Yardeni[1], Y. Anikster[2], R. Shapiro[3], Y. Bujanover[4], D. Bercovich[1,5].* 1) Genetics, migal, Kiryat-shmona, Israel; 2) Metabolic Disease Unit Sheba Medical Center Tel-Hashomer; 3) Institute of Gastroenterology,Schneider Children's Medical Center; 4) Paediatric Gastroenterology Unit Sheba Medical Centre; 5) Tel Hai Academic College, Israel.

Progressive familial intrahepatic cholestasis (PFIC) syndromes are a rare autosomal recessive disorder, characterized by defects in the mechanisms involved in bile formation. PFIC leads to death in the first decade of life if liver tranplant is not preformed. This disorder has multi-phenotypes which are divided in to subtypes PFIC:1,2,3. The correlation between genotype and phenotype are not always clear. There are three known suspected genes which could be involved in this disorder: The PFIC1 (ATP8B1) gene that codes the P-type ATPase protein, which is expressed in the liver parenchymal cells; the PFIC2 (BSEP) gene codes for ATP-binding cassette (ABC) transporter protein, which is expressed only in the liver canalicular membrane. The MDR3 is the third gene that codes a different ATP-binding cassette (ABC) transporters protein, which plays a key element in the availability of phospholipids to the canalicular bile. The aims of this study were to characterize the genetic molecular basis of patient with PFIC subtypes. Blood samples collection & DNA extraction were preformed on 14 different families, 10 of the children had PFIC1 or PFIC2 and 4 children with PFIC3 phenotypes. Mutations screening were preformed using DNA chromatography (DHPLC) of patient samples and controls, loci SNP's linkage study was preformed. Out of the 10 families with the PFIC1 or PFIC2 phenotypes, for two of them, a novel mutations were found in the BSEP gene, one (G877R) was homozygous in the patient from family number 2 and the two missense mutations (G19R & S226L) were compound heterozygous in the patient from family number 3. In family number 14 with the same phenotype, another heterozygous missense mutation was fond in the ATP8B1 gene (R600W), which is in the most conserve region of the protein. These findings have clinical and epidemiological aspects and may lead to a better understanding of the correlation between genotype and phenotype.

1130/F

How modifiers genes influence in pulmonary disease? An overview in a group of Mexican patients with Cystic Fibrosis. *M. CHAVEZ-SALDAÑA[1,2], E. YOKOYAMA[1], C. VILLARROEL[1], F. CUEVAS[3], A. CARNEVALE[4], J.L. LEZANA[5], S. FRIAS[1], B. MOLINA[1], L. OROZCO[6].* 1) Depto. de Investigación en Genética Humana, INP; 2) Universidad Autónoma Metropolitana; 3) Servicio Neumología, INP; 4) Coordinación Nal de Medicina Genómica, ISSSTE; 5) Depto. de Neumología, HIM; 6) Lab de Genómica de Enf Multifactoriales, INMEGEN.

Introduction. Cystic fibrosis (CF) is the most common autosomal recessive disorder in the Caucasian population. There are reports about the strong correlation between pancreatic function and the *CFTR* genotype, whereas others clinical signs may be different in patients with the same genotype. Because of this, it has been proposed that modifiers genes such as *TNF-α, MBL, α1-AT, α1-ACT,* β*-2AD, IL-10,* and others influence the severity of CF. **Objectives.** The aim of this study is to determine the frequency of allelic variants of the genes previously mentioned and their association with severity of pulmonary disease in Mexican patients with CF. **Methodology.** Sixty patients with CF were included. Thirteen single nucleotide polymorphisms (SNP's) of the genes already mentioned were analyzed. The typification was done with Taq-Man methodology. Pulmonary phenotype was measured by positive *Pseudomona* (Pae) cultures, health condition, age at diagnosis and death, age of onset of symptoms, first positive Pae culture, Brasfield's score and the best FEV1 in three years. The association was analyzed with the statistics program SPSS®, and a value of $p \leq 0.05$ was considered significant. **Results**. The frequency of allelic variants does not show differences with the literature reports. Significant differences were found in the variables MBL-550 with health condition, *IL10-819* and β*-2AD/16Gly→Arg* with age at diagnosis, *α1-ACT/[-15]Thr>Ala* with age at death and Brasfield's score, and *α1-AT/alleleZ* with best FEV1 in three years. **Conclusion.** This is the first report on the association of some SNPs in genes of specific response with the pulmonary phenotype among Mexican patients with CF. Our results suggest that genomic analysis of patients with CF may predict the clinical evolution of the disease and may be useful to establish an individualized treatment of patients.

1131/F

ELUCIDATION OF THE RMRP PATHOGENESIS IN CARTILAGE HAIR HYPOPLASIA. *P. Hermanns[1], K. Reicherter[1], A. Bertuch[2], B. Lee[3,4], B. Zabel[1].* 1) pediatric genetics section, Centre for Pediatric & Youth Medicine, Freiburg, Baden-Württembe, Germany; 2) Pediatrics-Hematology & Oncology, Texas Children's Feigin Center, Houston, TX, USA; 3) Baylor College of Medicine, Houston, TX, USA; 4) Howard Huges Medical Institute Houston, TX, USA.

CHH is an autosomal recessive disease chracterized by dwarfism, fine, sparse hair, deficient cellular immunity and predisposition to malignancy. CHH is caused by mutations in RMRP, which is the RNA component of a ribonucleoprotein complex. Yeast studies suggest its involvement in several cellular processes. Mutations include promoter insertions and duplications that are exclusively located between the TATA box and the transcription start site. Also point mutations and small insertions and deletions spread out through the entire RMRP transcript have been identified. In vitro studies show that promoter duplications found in CHH patients cause a hypomorphic allele affecting RMRP transcription. qRT-PCR analysis of patient lymphoblasts revealed a 7-fold decrease in RMRP RNA level. RMRP mutations introduced into the yeast ortholog NME1 neither altered mitochondrial function nor, affected mitochondrial content in a CHH patient fibroblast cell line. The 70A>G causes an alteration in rRNA processing and microarray studies performed with two patients suggest that RMRP mutation is associated with significant up-regulation of several cytokines and cell cycle regulatory genes. These data suggest that alteration of ribosomal processing leads to altered cytokine signaling and cell cycle progression in terminally differentiated cell types involved in CHH pathogenesis, i.e., lymphocytic and chondrocytic lineages. Additionally, preliminary studies suggest that RMRP might be regulated by miRNAs. To elucidate this further a miRNA microarray will be performed with total RNA isolated from whole blood of CHH patients, who have two identified mutations. This result will be compared to an expression profiling performed with sample of the same patients. This way we expect to identify differentially regulated miRNAs and their target genes in CHH patients compared to normal controls, thus gaining more insights in the pathogenic mechanisms in CHH.

1132/F

Mutations in Wnt5A in Patients with Autosomal Dominant Robinow Syndrome. *J.L. Lohr[1, 7], A.D. Person[2,8], C.M. Sieben[1,7], S. Hermanson[2], A.N. Neumann[1,7], M.E. Robu[2,8], J.R. Schleiffarth[1], H. van Bokhoven[4], J. Hoogeboom[5], J.F. Mazzeu[6], A. Petryk[1,2], H.G. Brunner[4], S.C. Ekker[2,7,8], S. Beiraghi[3].* 1) Department of Pediatrics, University of Minnesota, Minneapolis, MN; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 3) Department of Developmental/Surgical Sciences/Pediatric Dentistry, University of Minnesota Minneapolis, MN; 4) Department of Human Genetics, University Medical Center Nijmegen, Nijmegen, The Netherlands; 5) Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands; 6) Departments of Genetics and Evolutionary Biology, Institute of Biosciences, University of Sao Paulo, Brazil; 7) Institute of Human Genetics, University of Minnesota, Minneapolis, MN; 8) Minnesota Craniofacial Research Training Program (MinnCResT), Minneapolis, MN.

Robinow Syndrome is a heritable condition with both autosomal dominant and autosomal recessive transmission described. Both forms are characterized by short stature, mesomelic limb shortening, craniofacial abnormalities and genital hypoplasia. The recessive form has been associated with mutations in the tyrosine kinase receptor, *ROR2*, a putative mediator of Wnt signaling. We have shown, using a candidate gene approach, that dominant Robinow syndrome in all affected members of the original family described by Dr. Robinow, is associated with a heterozygous missense mutation in a highly conserved region of exon 4 of *WNT5A*. The single living unaffected family member has two wild type *WNT5A* alleles. A second *WNT5A* mutation has been found in exon 3 in an unrelated patient with sporadic Robinow Syndrome with a dominant phenotype. Both mutant proteins show reduced function in a zebrafish cell migration assay. This data suggests that a *WNT5A* signaling pathway dependent on *ROR2* for signal transduction is important in human craniofacial, skeletal and genital development, and that normal development of these structures is sensitive to variations in *WNT5A* function.

1133/F

Molecular Basis of Short Stature in Trichorhinophalangeal Syndrome. *D. Napierala[1], K. Sam[1], R. Morello[1], Q. Zheng[1], T. Bertin[1], T. Sibai[1], R. Shivdasani[3], B. Lee[1,2].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

Mutations in the human *TRPS1* gene cause a dominantly inherited craniofacial and skeletal dysplasia trichorhinophalangeal syndrome (TRPS). Patients with TRPS have short stature, hip abnormalities, cone-shaped epiphyses, and premature closure of growth plates reflecting defects in endochondral ossification. The *TRPS1* gene encodes for the transcription factor TRPS1 that has been demonstrated to repress transcription *in vitro*. To elucidate the role of Trps1 in endochondral bone formation, we analyzed *Trps1* mutant mice deleted for the GATA DNA-binding domain (TrpsΔGT mice). Histological analyses of long bones demonstrated delayed onset of chondrocyte hypertrophy in TrpsΔGT mice in comparison to WT littermates. Morphometric analyses of the growth plate and RNA in situ hybridizations demonstrated that zones of proliferating, prehypertrophic and hypertrophic chondrocytes are elongated in TrpsΔGT mice in comparison to WT littermates. Interestingly, the mineralization of perichondrium was more advanced in TrpsΔGT mice than in WT littermates. Since both mineralization and chondrocyte maturation are regulated by the Runx2 transcription factor, and because the expression of Runx2 and its target genes in chondrocytes is increased in TrpsΔGT mice, we tested whether Trps1 could repress *Runx2* gene expression and/or Runx2 protein function. Our in vitro studies demonstrated that Trps1 directly interacts with Runx2; moreover Trps1 strongly represses the Runx2-mediated transactivation of the target reporter construct. Taken together, abnormalities in the growth plate in the TrpsΔGT mice and *in vitro* functional studies strongly suggest that one of the roles of Trps1 during endochondral ossification is spatial and temporal repression of Runx2. Dysregulation of this function underlies the growth plate alterations in TRPS1 loss of function.

1134/F

***SOX9* micro- and macrodeletions are not uncommon in campomelic dysplasia.** *G. Scherer[1], W. Jin[1], M. Wessels[2], R. Hordijk[3], C. van Ravenswaaij[4], E. Obersztyn[5], E. Blair[6], E. McPherson[7], D. Sillence[8].* 1) Inst Hum. Genet., Univ. Freiburg, Germany; 2) Dept. Clin. Genet., Erasmus Univ. Rotterdam, Netherlands; 3) Dept. Clin. Genet., Academic Hospital Groningen, Netherlands; 4) Dept. Hum. Genet., UMC Nijmegen, Netherlands; 5) Dept. Med. Genet., Inst. Mother and Child, Warsaw, Poland; 6) Dept. Clin. Genet., Churchill Hospital, Headington, UK; 7) Marshfield Clinic, Marshfield, WI, USA; 8) Dept. Clin. Genet., New Children's Hospital, Parramatta, Australia.

Campomelic dysplasia (CD), an autosomal dominant skeletal malformation syndrome, results from mutations within *SOX9*, a 5.4 kb gene consisting of three exons, or from translocations interrupting the 1 Mb *cis*-regulatory domain upstream of *SOX9*. Only three cases with complete deletion of *SOX9* have been reported, all several Mb in size, and one case with a 1.5 Mb deletion located 380 kb upstream of *SOX9*. We have screened 60 cases with clinically and radiologically confirmed (15 cases, group 1) or suspected CD (45 cases, group 2) for *SOX9* deletions, using quantitative PCR. In these non-translocation CD cases, no *SOX9* coding region mutation had been detected by sequencing. We found deletions in four cases of group 1. Two are microdeletions that removed exons 1 and 2 (2225 bp deletion) or exon 2 plus part of exon 3 (2177 bp deletion), while two are macrodeletions of 2.2 Mb and 4.4 Mb. Sequencing of breakpoint-spanning PCR products showed 3 bp homologies at the deletion junctions in three of these deletion cases. Short 2-6 bp homologies occur frequently at deletion junctions in the human genome. In group 2, three deletions of approximate sizes of 150 kb, 850 kb and 1.4 Mb all encompassing the *SOX9* gene were detected, but due to sample limitation, their exact endpoints could no be delineated. Scanning the 1 Mb *SOX9* upstream control region with amplicons spaced 100 kb apart in 11 group 1 and 23 group 2 cases did not uncover another upstream deletion case. In conclusion, *SOX9* deletions are more frequent than previously supposed and can efficiently be detected by quantitative PCR, including deletions in the range of a few kb that go undetected by array CGH.

1135/F

Understanding the function of Ataxin1 and its paralog Ataxin1-like, and their role in SCA1 pathogenesis. *J. Crespo-Barreto[1], A.B. Bowman[2], H.T. Orr[3], H.Y. Zoghbi[1,2].* 1) Program in Cell and Mol. Biology; 2) Dept. Mol. and Hum. Genetics, Baylor College of Medicine, Houston, TX; 3) Laboratory Medicine and Pathology, U. Minnesota, Minneapolis, MN.

Spinocerebellar ataxia type 1 (SCA1) is a dominant neurodegenerative disease caused by an expanded polyglutamine tract in ataxin-1. Mice expressing polyQ-expanded *Atxn1* but not *Atxn1*$^{-/-}$ mice reproduce SCA1, implicating a gain-of-function mechanism. We identified a conserved Atxn1 paralog, Atxn1-like, which lacks a polyQ stretch but contains an AXH domain, a region involved in polyQ-mediated toxicity. Since overexpression of *Atxn1l* suppresses SCA1 phenotypes in *Atxn1*$^{154Q/+}$ mice, we propose that *Atxn1* and *Atxn1l* are functionally related and that their normal function is likely to modulate SCA1 pathogenesis. To test this, we set out to understand the *in vivo* function of *Atxn1* by studying *Atxn1*$^{-/-}$ mice. In Open Field Analysis, *Atxn1*$^{-/-}$ mice stay more at the center of the arena than wt littermates, as determined by the center/total distance ratio ($p<0.005$), and spend more time in the open arms of the elevated plus maze than wt littermates ($p<0.00001$). These data suggest that *Atxn1*$^{-/-}$ mice are less anxious than wt littermates. In the Conditioned Fear Test, *Atxn1*$^{-/-}$ mice have decreased fear responses ($p<0.05$), indicating impaired learning and memory. To determine if *Atxn1* and *Atxn1l* genetically interact, we tested the effect of *Atxn1l* overexpression on the behavior of *Atxn1*$^{-/-}$ mice. Our data show that a duplication of *Atxn1l* partially suppresses *Atxn1-/-* defects both in OFA and CF, suggesting that Atxn1l can compensate for Atxn1's loss-of-function. To determine if the endogenous function of Atxn1 plays a role in SCA1 pathogenesis, we compared the SCA1 phenotypes of *Atxn1*$^{154Q/+}$ mice to those of *Atxn1*$^{154Q/-}$ mice. Removing the wt copy of *Atxn1* worsened SCA1 phenotypes in the Rotating Rod and decreased *Atxn1*$^{154Q/-}$ mice survival by 13 weeks ($p<0.001$), indicating that Atxn1's endogenous function can modulate SCA1 pathogenesis. Taken together, our data show that *Atxn1l* can functionally substitute for *Atxn1*, and that the wild-type copies of *Atxn1* and *Atxn1l* are neuroprotective in a SCA1 knock-in mouse model.

1136/F

Partial maternal isodisomy of chromosome 17p in a case of infantile cystinosis. *A.S. Lebre[1], V. Morinière[1], O. Dunand[3], N. Morichon[1], C. Antignac[1,2].* 1) Département de Génétique, Hôp Necker-Enfants Malades, Paris, France; 2) Inserm U574, Hôp Necker-Enfants Malades, Paris, France; 3) Hôpital Trousseau, Paris, France.

Cystinosis is an autosomal recessive disorder characterized by an accumulation of intralysosomal cystine due to a defect in cystine transport across the lysosomal membrane. Three clinical forms of cystinosis (infantile, juvenile and ocular cystinosis) have been delineated based on severity of symptoms and age of onset. The causative gene, CTNS, maps to 17p13, spans 23 kb of genomic sequence, and encodes the lysosomal cystine transporter, cystinosin. CTNS mutations have been detected in individuals affected with all forms of cystinosis. The most common mutation is a large 57 kb deletion spanning exons 1 to 10, detected in ~60-70% of affected northern European individuals.

Here, we report a maternal uniparental disomy of chromosome 17 (mat UPD17) in a 2.5-year-old girl presenting with isolated infantile cystinosis. This patient was detected homozygous for a 57-kb deletion of the CTNS gene. The mother's proband was found heterozygous for the deletion but surprisingly the father did not bear the deletion. Haplotype analysis with polymorphic markers spanning the whole chromosome 17 demonstrated no paternal contribution for all the markers of chromosome 17 and only one maternal contribution for markers of chromosome 17p. As a deletion 17p would not be viable, these results suggest a maternal uniparental disomy 17 with an heterodisomy of 17q and a isodisomy of 17p. They are the first evidence of mat UPD of chromosome 17 and in cystinosis.

1137/F

Functional and biophysical characterisation of wild type fibulin 5 and mutants associated with age-related macular degeneration. *C.E. Ridley[1], R.P.O. Jones[1], K. Mellody[2], A.C. Lomas[2], T. Wang[1], M. Howard[2], T. Jowitt[2], C. Baldock[2], A. Lotery[3], P.N. Bishop[2], C.M. Kielty[2], D. Trump[1].* 1) Medical Genetics, University of Manchester, Manchester, UK; 2) Wellcome Trust Centre for Cell-Matrix Research, School of Biological Sciences, University of Manchester, UK; 3) Clinical Neuroscience, University of Southampton, UK.

Introduction Age-related macular degeneration (AMD) is the leading cause of visual loss in the Western world. The pathogenesis is complex, and missense mutations in *Fibulin 5 (FBLN5)* are implicated in up to 2% of patients. The pathogenicity of these missense mutations has been questioned. *FBLN5* mutations also cause cutis laxa. The aim of our study is to investigate wild type (WT) and mutant FBLN5 using functional and biophysical assays to determine the effects of the 9 AMD associated missense changes, 2 cutis laxa missense changes and 2 missense polymorphisms. **Methods** We expressed WT and mutant *FBLN5* in retinal pigment epithelial cells to determine the distribution and secretion of FBLN5. Full length and fragments of WT and mutant FBLN5 were His tagged and expressed in EBNA 293 cells. Purified protein was used in solid phase binding assays and biophysical studies using size-exclusion chromatography (pH7.4) online to multi-angle laser light scattering and circular dichroism (CD). **Results** 4 of the AMD and the 2 cutis laxa mutations led to a reduction in FBLN5 secretion. Studies of UPR activation are underway. Both AMD and cutis laxa mutations led to reduced binding affinity for elastin and the polymorphism G202R increased binding affinity. A WT fragment comprising residues 127-287 (four EGF-like repeats) dimerised and proteins containing this fragment self-associated. The hydrodynamic radius (Rh) for monomeric full-length WT FBLN5 was approximately 3nm, suggesting a compact structure. Several mutants caused increases in Rh, reflecting perturbations to the shape of FBLN5. Recently acquired CD data may reveal associated secondary structure changes. **Discussion** These results indicate missense changes in FBLN5 associated with AMD lead to changes in the structure and function of FBLN5 suggesting they are pathogenic.

1138/W

Identification of candidate genes common to bleomycin and radiation induced pulmonary fibrosis in mice. *A-M. Lemay, C.K Haston.* Dept Human Genetics, McGill Meakins-Christie, Montreal, PQ, Canada.

The genetic basis of susceptibility to pulmonary fibrosis is largely unknown. We previously identified bleomycin-induced and radiation-induced pulmonary fibrosis quantitative trait loci (QTL) in crosses of susceptible C57BL/6J (B6) mice with resistant A/J or C3H/fKam mice. We hypothesized that the genes involved in bleomycin induced pulmonary fibrosis would be the same as those of radiation-induced pulmonary fibrosis and this overlap would allow for the identification of a small number of candidate genes for further investigation. The linkage regions identified to be common to bleomycin and radiation response are on chromosomes 6, 17 and 18 (LOD= 2.4, 4.2 and 3.5). To further investigate these putative linkages, chromosome substitution strains [C57BL/6J-Chr 4A/NaJ (n=11), C57BL/6J-Chr 6A/NaJ (n=19) and C57BL/6J-Chr 17A/NaJ (n=19)], were treated with the bleomycin and the radiation protocols. The fibrosis phenotype of these strains was different from the B6 response for each treatment (% fibrosis of Chr6A=0.7 ± 0.9, Chr17A=0.2 ± 0.06, p>0.23) supporting the existence of A/J derived fibrosis protective alleles on chromosomes 6 and 17. To identify potential fibrosis candidate genes in the putative linkage intervals, gene expression studies with microarrays were completed on control and treated B6 mice. This analysis revealed 1012 genes to be differentially expressed in lung tissue in bleomycin treated mice and 118 differentially expressed genes in the study of radiation-induced pulmonary fibrosis. Of these, 48 genes were differentially expressed in both types of treatments. Pulmonary genes differentially expressed in bleomycin and radiation treated B6 mice included those of chemotaxis, proteolysis and immune response. Of the 48 genes differentially expressed in bleomycin and radiation-treated mice, 4 were located within the linkage regions. These genes were *Fkbp4* (FK506 binding protein), *Fkbp5*, *Angpt14* (angiopoietin-like 4) and *Lox* (lysyl oxidase). Genomic approaches were combined to produce a set of candidate genes which may influence susceptibility to pulmonary fibrosis regardless of the induction treatment.

1139/W

Novel EXT1 and EXT2 Gene Mutations in Hereditary Multiple Exostoses Families of Indian Origin. *Vanita. Kumar[1], K. Sperling[2], H.S. Sandhu[3], P.S. Sandhu[3], J.R. Singh[1].* 1) Centre for Genetic Disorders, Guru Nanak Dev University, Punjab, India; 2) Institute of Human Genetics, Charitè Humboldt-University, Berlin, Germany; 3) Dr. Hardas Singh Orthopaedic Hospital and Super Speciality Research Centre, Amritsar.

Background: Hereditary multiple exostoses (HME) is an autosomal dominant bone disorder, characterized by short stature and the presence of multiple benign tumors mainly at the ends of long bones. HME is genetically heterogeneous with two known genes on 8q24 (EXT1) and 11p11 (EXT2), and a third minor locus mapped to 19p (EXT3). The majority of EXT1 and EXT2 gene mutations result in premature protein truncation and loss of function. Materials and Methods: We analyzed two autosomal dominant HME families of Indian origin. Linkage analyses using fluorescently labeled microsatellite markers at the candidate gene regions was performed. Mutation analyses was carried out by bi-directional sequencing of purified PCR products. Results: We found linkage in one family to EXT1 gene and in the other family to EXT2 gene. Mutation screening in the EXT1 gene revealed a novel frame-shift mutation, in exon 1. This mutation segregated in all affected members and was absent in the unaffected family members and 60 unrelated controls. In the second family a previously unreported stop mutation, was observed in the EXT2 gene in all affected members and in none of the unaffected family members nor in 90 unrelated controls. Conclusions: Our findings expand the mutation spectrum of EXT1 & EXT2 and highlight the genetic and phenotypic heterogeneity of HME.

1140/W

Identification of Late-Onset Alzheimer's Disease (LOAD) susceptibility alleles in *VR22* and *LRRTM3* genes. *N. Ertekin-Taner[1, 2], M. Allen[1], C. Cox[1], S. Younkin[1], L. Younkin[1], M. Carrasquillo[1], F. Zou[1], D. Dickson[1], N. Graff-Radford[2], B. Boeve[3], R. Petersen[3], S.G. Younkin[1].* 1) Department of Neuroscience, Mayo Clinic Jacksonville, FL; 2) Department of Neurology, Mayo Clinic Jacksonville, FL; 3) Department of Neurology, Mayo Clinic Rochester, MN.

VR22 (α-T catenin) is an excellent functional and positional candidate LOAD gene. We previously found two SNPs within *VR22* which showed significant association with plasma Aβ levels and accounted for our linkage signal on chromosome 10. *LRRTM3* (leucine rich repeat transmembrane protein 3) resides within intron 7 of *VR22*. We genotyped 8 SNPs within *LRRTM3* and 26 in *VR22* in 3 independent LOAD case-control series. *LRRTM3* single SNPs or haplotypes did not significantly associate with LOAD. The *LRRTM3* haplotype pairs formed 25 common multilocus genotypes (MLGs). In the exploratory series, 6 protective MLGs were identified. Compared to these, the remaining *LRRTM3* MLGs were significantly risky in the exploratory series (OR = 3.91, p=0.003). Remarkably, this finding replicated in both follow-up series (ORs= 2.99, 1.97; p=0.006, 0.055). In the combined series, the OR for the risky *LRRTM3* MLGs was 2.71 (p=0.000019). We identified a set of 4 *VR22* SNPs which associated with AD (p=0.01-0.2). The 4 *VR22* SNP haplotypes did not yield significant association, but formed 15 common MLGs, two of which were protective in the exploratory series. Compared to these two protective MLGs, the remaining *VR22* MLGs were significantly risky in the exploratory series (OR=1.33, p=0.0165), with remarkable replication in both follow-up series (ORs=1.92, 1.37; p=0.0001, 0.0198). In the combined series, the *VR22* MLGs were significantly risky (p=0.00003) with OR=1.40 (1.19-1.64). These results strongly indicate the presence of LOAD susceptibility alleles within *LRRTM3* and *VR22* genes. Importantly, the *LRRTM3* and *VR22* SNPs are not in linkage disequilibrium, suggesting independent effects. Studies correlating *VR22* and *LRRTM3* MLGs with Aβ and gene expression are underway.

1141/W

Replication of DCDC2 and KIAA0319 in a single population with language disorder. *S.K. Iyengar[1], J.B. Tomblin[2], K.J. Kelsey[3], J.B. Bjork[3], L.E. Sucheston[4], B.K. Samelson[3], J.C. Murray[3].* 1) Dept Epid/Biostat, Case Western Reserve Univ, Cleveland, OH; 2) Dept Speech Pathology and Audiology, U of Iowa, Iowa City, IA; 3) Dept Pediatrics, U of Iowa, Iowa City, IA; 4) Dept Biostatistics, SUNY-Buffalo, Buffalo, NY.

DCDC2 and KIAA0319 have been repeatedly linked to dyslexia, a reading disorder (RD) characterized by poor phonological and word decoding abilities. Both genes are expressed in the brain and implicated in neuronal migration. Previously, each has been linked independently to RD in different populations. We conducted an association study of 581 second-grade children in nuclear families, with and without language-learning disabilities. Linkage disequilibrium was analyzed by genotyping five SNPs in DCDC2 and five SNPs in KIAA0319. Single SNP association analysis was performed via linear regression, accounting for sibling and family effects by the program ASSOC in the S.A.G.E. software package. All p-values presented are likelihood ratio p-values, although a Wald test was also conducted with similar effects. All SNPs in DCDC2 showed association with measures of nonword decoding in kindergarten (p-values 0.001 to 0.05). The SNP, rs807701, which showed the most significant p-value for nonword decoding, was also significantly associated with single word reading (p= .003). For KIAA0319, two SNPs were significantly associated with performance or non-verbal IQ (rs12193738, p=.002; rs9393572, p=.02). The SNP rs12193738 was also associated with reading comprehension (p=.003), phonological memory (p=.003), with marginal association for spoken language, phonological awareness, and nonword decoding. Phenotypes associated with DCDC2 are closely related to phonological abilities and consistent with core features of dyslexia. Phenotypes associated with KIAA0319 are more diverse, but include aspects of language associated with meaning. It is hypothesized that these reading-related loci may affect slightly different neurodevelopmental pathways and contribute to different profiles of reading impairment. This is the first time both DCDC2 and KIAA0319 have been associated with reading and other cognitive linguistic measures in the same sample.

1142/W

Genes showing accelerated evolutionary properties associate with communication abilities in humans. *K.J. Kelsey[1], J.B. Tomblin[2], J.B. Bjork[1], S.K. Iyengar[3], L.E. Sucheston[4], B.K. Samelson[1], J.C. Murray[1].* 1) Dept Pediatrics, The University of Iowa, Iowa City, IA; 2) Dept Speech Pathology and Audiology, U of Iowa, Iowa City, IA; 3) Dept Epidemiology and Biostatistics, Case Western Reserve, Cleveland, OH; 4) Dept Biostatistics, SUNY-Buffalo, Buffalo, NY.

Evolutionary acceleration is a property ascribed to complex human traits such as speech, language, and reading. We examined two positively selected regions, the gene ASPM and the human accelerated region (HAR) HAR1, for association with language. HARs are segments of the genome considered to have undergone strong, recent, positive selection, suitable for the detection of human-specific traits. HAR1 is a composite of two RNA genes, HAR1F and HAR1R, with HAR1F showing strong expression in the human developing neocortex. ASPM is a gene involved in the regulation of brain size, with mutants causing microcephaly. We conducted association studies of 581 second-grade children in nuclear families with a wide range of speaking and reading abilities. Five SNPs were genotyped in ASPM and seven around HAR1. Single SNP association was performed via linear regression, accounting for sibling and family effects by ASSOC in the S.A.G.E. software. Haplotype analyses were performed for the binary trait SLI using the Pedigree Disequilibrium Test (PDT) using Unphased. Single word reading showed the strongest single association with ASPM (rs107377686, p= .008). This SNP, along with rs6700180, are part of a significant two SNP haplotype (p=.03). Other traits that also showed association were: speech sound production proficiency, reading comprehension, and nonverbal IQ. For HAR1, multiple SNPs were associated with reading comprehension, nonverbal IQ, single word reading, speech sound proficiency, phonological awareness, reading decoding, and phonological memory. Of these, rs6011605 and rs6089838 showed the best signals (p=.008 for three measures). Combined into a two SNP haplotype, a significant association with spoken language was found (p=.00003). The association of ASPM and HAR1, two evolutionarily accelerated regions, with cognitive linguistic measures provides an impetus to additional studies of their roles in neurodevelopment.

1143/W

Identification of a novel locus (SPG34) responsible for a complicated form of hereditary spastic paraplegia associated with amyotrophy of the hand muscles. *J.A. Reed[1], H. Patel[1], C. Windpassinger[2], M.A. Patton[1], M. Auer-Grumbach[2], P. Hedera[3], A.H. Crosby[1].* 1) Department of Medical Genetics, St George's University of London, London, United Kingdom; 2) Institute of Medical Biology and Human Genetics, Medical University of Graz, Graz, Austria; 3) Department of Neurology, Vanderbilt University, 465 21st Avenue South, 6140 MRB III, Nashville, United States.

The hereditary spastic paraplegias (HSPs) are an extremely genetically heterogeneous group of disorders. Mutations in BSCL2 encoding seipin, a molecule of unknown function, are causative of around 40% of cases of a complicated form of HSP associated with amyotrophy of the small hand muscles. The same BSCL2 mutations have also been found in other patients with related motor neuron degenerative conditions involving hand muscle wasting. We have undertaken a genomewide screen in three families without BSCL2 mutations but with affected individuals presenting within the clinical spectrum of HSP accompanied by amyotrophy of the hand muscles/dHMNV. Following extensive exclusion mapping a single region compatible with linkage in two of these families as well as two further families was identified. This region, located on chromosome six is situated between markers D6S262 and AL035604, is supported by a maximum multipoint LOD score of 4.81 and has been designated SPG34. Sequencing of candidate genes located within this region has so far failed to reveal any potentially pathogenic sequence alterations.

1144/W

Major locus for Centrotemporal Sharp Waves in Rolandic Epilepsy families maps to chromosome 11p. *L.J. Strug[1], T. Clarke[2], B. Ball[2], P.L. Murphy[2], D.A. Greenberg[1], D.K. Pal[2].* 1) Dept Biostatistics, Columbia Univ, New York, NY; 2) Dept Epidemiology, Columbia University, New York, NY.

Introduction: Centrotemporal sharp waves (CTS) are the EEG hallmark of rolandic epilepsy (RE). CTS are also found in many other neurodevelopmental disorders including autism. RE and its characteristic EEG trait is presumed to be genetic. Although not yet established, the inheritance of RE appears complex while the inheritance of CTS appears autosomal dominant. We conducted a linkage study of CTS in RE families to determine a region of the genome that contributes to the etiology of CTS in RE families. **Methods:** We ascertained 40 families for linkage through a single RE proband. By definition, all RE probands have the CTS trait. Their siblings (age 4 to 16 years) underwent sleep-deprived EEG to determine their CTS status. Observations from individuals without an EEG, or those remaining awake without showing CTS were treated as unknown in the linkage analysis. We used GENEHUNTER and a modified version of LIPED to perform multipoint and two-point linkage analysis, respectively. We analyzed our families assuming dominant (gene frequency = 0.006) and recessive (gene frequency = 0.1) modes of inheritance, maximizing over penetrance. **Results:** We observed a maximum multipoint lod score of 4.30 at marker D11S914 on chromosome 11, assuming a dominant mode of inheritance, no heterogeneity, and 50% penetrance. The maximum two-point lod score at D11S914 was 3.1. **Conclusions:** A locus at chromosome 11p12-p13, inherited dominantly with incomplete penetrance, plays a major role in determining the presence of CTS in RE families. This locus overlaps that recently reported in a genomewide screen for autism (Szatmari et al, 2007). We will conduct fine mapping studies to localize the gene responsible for the strong linkage signal.

1145/W

Genetic Association Analysis of Pyruvate Carboxylase, a Positional Candidate Gene for Acute Insulin Response: The IRAS Family Study. *P.A. Antinozzi[1], N.D. Palmer[1], C.D. Langefeld[1], M. Bryer-Ash[2], D.W. Bowden[1].* 1) Wake Forest Univ., Winston Salem, NC; 2) Univ. of California, Los Angeles, CA.

Impaired pancreatic β-cell function is a hallmark of type 2 diabetes. Acute insulin response (AIR) is a quantitative measure of first phase insulin secretion in response to glucose stimulation. IRAS Family Study previously reported linkage of AIR in 284 African American (AA) subjects (21 pedigrees) on 11q and replicated this finding in an additional 214 AA subjects (22 pedigrees). Combined analysis yielded a bimodal peak with a LOD-1 support interval from 54-84cM (LOD=2.77 at 58cM and LOD=2.54 at 76cM). Among more than 300 annotated genes, pyruvate carboxylase (PC) was identified as a strong positional candidate. PC catalyzes the conversion of pyruvate to oxaloacetate, the initial reaction of gluconeogenesis and is involved in neurotransmitter synthesis and insulin secretion. The PC gene is encoded by two transcripts; a large, 110kb transcript found to be highly abundant in gluconeogenic tissues in animal models and a short 59kb transcript. Based on coverage and LD metrics from the HapMap Yoruban dataset, 36 SNPs were chosen for genotyping on 605 AA participants. In the Yoruban dataset, 20 of 37 SNPs with MAF >5% genotyped in HapMap for PC captured 88.0% of SNPs with r2 > 0.8 (mean r2=0.94). Using SOLAR, 4 and 6 SNPs were associated (P<0.05) with AIR in MGS set 1 and 2 respectively. This exceeds the number expected by chance (1.9). In the combined analysis, three SNPs were significantly associated with AIR (P=0.007-0.02). Of these SNPs, rs7119676 was highly significant (P=0.007) and was also associated using the Quantitative Pedigree Disequilibrium Test which is robust to population stratification. Variation at this locus confers a protective effect following an additive model with the variant having a 1.5X increased AIR (1335 vs. 914 pmol/L). This SNP is located ~3kb upstream of exon 1 of the short transcript. Based on these findings, it is proposed that this SNP, or a linked variant, could modulate the expression of the short PC transcript and confer a protective advantage which could compensate for deficiencies in individuals with impaired pancreatic β-cell function.

1146/W

Common genetic variation in *CD46* may be associated with susceptibility to *Neisseria meningitidis*. *D.C. Crawford[1], S.M. Zimmer[2], R. Lynfield[3], Q. Yi[4], C. Shephard[4], M. Wong[4], M.J. Rieder[4], R.J. Livingston[4], D.A. Nickerson[4], C.G. Whitney[5], N.E. Messonier[5], J. Lingappa[6].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Department of Medicine, Division of Infectious Diseases, Emory University, Atlanta, GA; 3) Emerging Infections Unit, Minnesota Department of Health, St. Paul, MN; 4) Department of Genome Sciences, University of Washington, Seattle, WA; 5) Department of Medicine, University of Washington, Seattle, WA; 6) CDC, Atlanta, GA.

N. meningitidis is an important cause of bacterial meningitis, with a U.S. incidence of ~1 case per 100,000 individuals. A combination of host, pathogen, and environmental factors likely determines host susceptibility. *CD46* (46.9kb) located on 1q32 has been identified as a host receptor for *N. meningitidis*. To determine if *CD46* variation alters susceptibility or disease characteristics, we used the population-based Active Bacterial Core Surveillance in Minnesota to identify 22 cases of meningococcal disease occurring between 1997-2000. Case-patients were cross matched to the state newborn testing program database; 44 anonymous age, race and birth hospital-matched controls were also identified. Half the case patients were female and the mean age was 5.7 years (range 1-26 years). For genetic variation discovery, 66 blood spot DNAs were re-sequenced as well as 77 reference DNAs. A total of 269 diallelic sites were identified among 143 DNA samples. tagSNPs were chosen from European-American reference samples (n=23; MAF>5%; r^2>0.80), and tests of association were performed among European-descent cases (16) and controls (32). Among the 15 tagSNPs tested, only SNP 6420 (rs41317049) was significantly associated with cases status for both the additive and dominant models (Fisher's exact; p<0.05). The G allele frequency was 0.19 in cases versus 0.08 in controls, and 37.5% of cases had either the GT or GG genotype compared with 12.5% of controls. Intronic SNP 6420 is in perfect linkage disequilibrium (r^2= 1) with intronic SNP 14634 (rs7545126), so it is plausible that either SNP could be the risk conferring marker for susceptibility to *N. meningitidis* if replicated in subsequent studies.

1147/W

Association of tag SNPs in neuronal UCPs with cranial-cervical dystonia. *J. Jamiyansuren[1,2], R. Kaji[1], K. Maeda[1], K. Yasuno[3,4], S. Matsumoto[1], S. Makino[2], G. Tamiya[2].* 1) Department of Neurology and Neuroscience, Tokushima University Graduate School of Medicine, Tokushima 770-8503, Japan; 2) Division of Human Molecular Genetics, Department of Neurology and Neuroscience, Tokushima University Graduate School of Medicine, Tokushima 770-8503, Japan; 3) Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan; 4) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan.

Dystonia is a syndrome characterized by sustained muscle contractions, producing twisting, repetitive, and patterned movements, or abnormal postures. We performed a case-control study in sporadic cranial-cervical dystonia (CCD) in Japan using tag SNP markers in the mitochondrial uncoupling protein genes, *UCP2, UCP4* and *BMCP1/UCP5* that are expressed in various brain tissues and may exert a neuroprotective effect against increased oxidative stress and calcium dysregulation. We found modest associations between CCD and some tag SNPs in *UCPs*, all of which were statistically significant even after correcting for multiple comparisons. Moreover, we investigated the synergistic interaction between *UCPs* in CCD by using the multifactor dimensionality reduction (MDR) method and the logistic regression analysis. Our findings suggest that neuronal *UCPs* have a modest but important involvement in the genetic etiology of CCD as well as schizophrenia we have previously reported. This is the first report of the association between CCD and neuronal *UCPs*.

1148/W

Evidence for *BACH2* in chromosomal region 6q14-6q16.3 with nonsyndromic cleft lip and palate. *A. Mostowska[1], T.H. McHenry[2], M.E. Cooper[2], M. Govil[2], D.R. FitzPatrick[3], V.J. Vieland[4], M.L. Marazita[2], J.C. Murray[1].* 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA; 3) University of Edinburgh, Edinburgh, U.K; 4) Columbus Children's Research Institute, Columbus, OH.

Isolated cleft lip with or without cleft palate (NSCL/P) is a common congenital anomaly in humans, the etiology of which is complex and associated with both genetic and environmental factors. Prior linkage scan analysis from 7 different NSCL/P populations identified a substantial probability of linkage to 6q14-6q16.3 region (two-point PPL=0.43, multipoint PPL=0.88; multipoint maximum HLOD=3.0). To fine map this region, 65 SNPs in candidate genes were selected and genotyped on 275 multiplex families from Southeast Asia. TDT analysis revealed modest associations with SNPs in two genes: *BACH2* (p=0.02 for rs1065273, rs9359876, rs404256; and p=0.04 for rs10455512) and *EPHA7* (p=0.02, rs535926). Pairwise and multilocus haplotype analyses, in sliding windows up to 5 SNPs, also revealed a significant transmission distortion for different combination of markers. The highest departure from random sharing was observed for markers located in *BACH2* gene. Sequence analysis of *BACH2* in 96 NSCL/P cases revealed a number of mutations and novel gene variants specific only for affected individuals including 163delEEDE or Val820Met. The number of DNA changes identified in cases was statistically higher than in controls. To confirm these findings sequencing analysis was performed in 96 NSCL/P cases from North America, revealing significant results, as well. BACH2 is an oxidative stress-responsive transcription factor containing a basic leucine zipper BTB domain and cytoplasmic localization signal. It functions as a positive regulator of cell death and may act as a tumor suppressor. We also show that in mice, *Bach2* is specifically expressed in craniofacial structures during development. This report provides evidence for an association of chromosomal region 6q14-6q16.3 with NSCL/P and indicates that BACH2 may be a novel transcription factor playing a role in etiology of this common anomaly. NIH grants:R37-DE08559,R01-DE016148,P50-DE016215.

1149/W

Genetic Determinants of Patent Ductus Arteriosus in Term Infants. *P.M. Patel, P.A. Romitti, J.M. Dagle, J.C. Murray.* Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA.

Background: Patent ductus arteriosus (PDA) is the second most common form of congenital heart disease. Recent work on the molecular basis of other forms of congenital heart disease has suggested that a significant fraction of them are inherited in mendelian or near-mendelian fashion with a large contribution from de novo mutations. Objective: Our goal is to identify genetic variations that are present in specific candidate genes associated with PDA in term infants as either common variants contributing to complex causes or rare variants with a strong individual effect. Methods: Our candidate gene selection was based on published reports of genes known to cause syndromic forms of PDA, and on expression studies of genes identified for their role in pathways that normally regulate PDA closure. The genes analyzed include those associated with syndromes with the highest prevalence of PDA and those involved in pathways regulating the closure of PDA. Using DNA samples from 161 term infants from the Iowa site of National Birth Defect Prevention Study (148 triads, 13 diads, total 470 persons), we performed association studies using an initial starting panel of 55 single nucleotide polymorphisms to determine allelic variation in 13 genes representing the syndromic PDAs and 4 genes representing the pathway genes. The candidate genes were evaluated for allelic variation using TaqMan assays and the data was analyzed using family-based transmission disequilibrium test analysis. Results: Statistical analysis revealed that 3 SNPs (TGFBR1rs10760671, TGFBR2rs934328, PTGISrs493694) achieved borderline statistical significance with the conservative Bonferroni correction (P value = 0.001), demonstrating that allelic variation in these genes may play a role in term PDA. Conclusions: These findings suggest a strong association between a variation in the TGFBR and PTGIS genes and the occurrence of PDA in term infants. Additional analysis with other families and polymorphic markers, as well as evaluation of potential candidate genes by sequence analysis, could confirm the identification of one or more genes playing a role in ductal closure.

1150/W

Molecular Analysis of Types 1 and 2 Usher Syndrome in Iranian Patients. *K. Kahrizi[1], N. Meyer[4], G. Assadi Tehrani[1], W.J. Kimberling[4], N. Sadeghpour[1], N. Bazazzadegan[1], M. Mohseni[1], M. Jaberi[2], K. Jalalvand[1], S. Arzhangi[1], H. Emamjomeh[3], H. Najmabadi[1], R.J.H. Smith[4].* 1) Genetics Research Ctr, GRC, USWR, Tehran, Tehran, Iran; 2) Retinitis Pigmentosa Institute, Tehran, Iran; 3) Research Center of Ear, Nose, Throat, and Head and Neck Surgery, Tehran Iran; 4) Molecular Otolaryngology Research Laboratories , Department of Otolaryngology Head and Neck Surgery , University of Iowa, Iowa, IA, United States.

Usher syndrome (USH) is a heterogeneous group of diseases that collectively are the most frequent cause of deaf-blindness. Clinically classified as Type 1, 2 or 3 based on auditory, vestibular and ophthalmologic criteria, Usher syndrome Type 1 and 2 are the most common, with Usher syndrome Type 3 being rare outside of Finland. The objective of this study was to determine the prevalence of Usher syndrome Types 1 and 2 in Iran. Thirty Iranian families with Usher clinical symptom were studied for allele segregation consistent with linkage to the following Usher loci: USH1B, USH1C ,USH1D USH1F and USH2B. One family mapped to USH1B, three families mapped to USH1D, one family mapped to USH1F and one family mapped to USH2C. Mutation analysis of MYOVIIA (USH1B) demonstrated homozygosity for 448C>T: R150X in affected persons and in the USH2C family, a single mutation was found in VLGR1. The remaining families are still being studied. Key words: usher syndrome, Iran, USH1F, USH1B.

1151/W

Development of a high-throughput linkage analysis system employing 100K/500K SNP data. *Y. Fukuda[1], Y. Nakahara[1], Y. Momose[1], H. Date[1], Y. Takahashi[1], J. Goto[1], K. Hara[2], M. Nishizawa[2], E. Nakamura[3], H. Adachi[3], S. Tsuji[1].* 1) Department of Neurology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan; 2) Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan; 3) Dynacom Co., Ltd, Kanagawa, Japan.

During the recent decade, microarray-based SNP (Single Nucleotide Polymorphism) data are becoming more widely used as markers for linkage analysis in identification of loci for disease-associated genes. Although microarray-based SNP analyses have drastically reduced genotyping time and costs compared with microsatellite-based analyses, applying these enormous data to linkage analysis programs is a time consuming step, thus necessitating a high throughput platform. We have developed a linkage analysis system for microarray-based SNP data. In this system, Affymetrix 100k/500k SNP chip data can be directly imported and passed to pair-wise (mlink) or multipoint (allegro) linkage analysis programs. The system provides all parameter setting functions that are pre-included in the original mlink and allegro programs. Various marker-selecting functions are implemented to avoid the effect of typing-error data or to eliminate uninformative data, where users set threshold for call rate, HWE test and evaluation of MAF (minor allele frequency). Furthermore, inter-marker distance can be flexibly chosen to adopt markers that are not in linkage disequilibrium (LD) each other. We used this system for the linkage analysis of familial multiple system atrophy (MSA), and found that the results using 100k SNP data were comparable or superior to those obtained from microsatellite markers. General personal computers are sufficient to execute the process, as runtime for whole genome analysis was less than a few hours even in multipoint analysis (approximately 100kb of inter-marker distance) or in the case of a family including consanguineous loops. This system can be widely applied for linkage analysis using microarray-based SNP data and there one can expect high-throughput and reliable linkage analysis.

1152/W

Homozygosity mapping of autosomal recessive primary microcephaly (MCPH) in 15 consanguineous families and identification of a new (MCPH7) locus. *A. Parsian[1], M. Cleves[1], A.J. Parsian[1], M. Watts[1], S.M. Elsayed[2], E. Elsobky[2], A. Jankhah[3].* 1) Dept Pediatrics, Univ Arkansas Medical Sci, Little Rock, AR; 2) Medical Genetic Center, Cairo, Egypt; 3) Shiraz Medical Genetic Counseling Center, Shiraz, Iran.

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by global reduction in brain size affecting mostly the cerebral cortex. The individuals with MCPH suffer from mental retardation of variable degrees. MCPH is a heterogeneous disorder and at least six known genetic loci (MCPH1-6) have been found in humans. Among all, MCPH5, caused by the mutations in ASPM that encodes the human homologue of fly abnormal spindle gene (asp), is the most common. We screened 15 consanguineous families with microsatellite markers in the MCPH1-6 regions. Homozygosity mapping of the affected individuals from these families and linkage analysis identified the linkage of two families to MCPH2 and three to MCPH3. The remaining 10 families were not linked to any of the MCPH1-6 regions. We mapped the MCPH3 locus to a 1.55 cM region of chromosome 9q34 in three separate MCPH3 affected families. We sequenced the open reading frame of the CDK5RAP2 gene using genomic DNA from affecteds in our MCPH3 linked families and did not detect any mutation. Further fine mapping of the 9q34 region identified a new locus (MCPH7) near the marker D9S1881. The best candidate gene in this region is Nek6 that is required for mitotic progression of human cells. Our linkage results also strongly suggest the existence of additional MCPH locus elsewhere in the genome.

1153/W

Screening of TMC1 Gene Mutations in DFNB7(11) Locus in Autosomal Recessive Non-syndromic Hearing Loss Iranian Population. *N. Bazazzadegan[1], N. Meyer[2], K. Kahrizi[1], M. Mohseni[1], P. Imani[1], N. Nikzat[1], S. Arzhangi[1], M. Sayfati[1], K. Jalalvand[1], J. Malbin[1], K. Javan[1], M. Farhadi[3], R.J.H. Smith[2], H. Najmabadi[1].* 1) Genetics Research Center, GRC/USWR, Tehran, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology Head and Neck Surgery, University of Iowa, Iowa, IA, United States; 3) Research Centre of Ear, Nose, Throat, and Head and Neck Surgery, Iran university of Medical sciences, Tehran, Iran.

Mutations in the transmembrane channel-like gene 1 (TMC1) cause prelingual autosomal recessive (DFNB7/11) and postlingual progressive autosomal dominant (DFNA36) nonsyndromic hearing loss suggesting that this protein plays an important role in the inner ear. These loci map to the same interval on 9q13-q21. The TMC1 protein is predicted to contain 6 transmembrane domains and to have cytoplasmic orientation of N and C termini. Mutations in this gene have been reported in North America in a family with autosomal dominant inheritance, Sudan, also in our two neighbor countries Pakistan and Turkey. Therefore we decided to study this locus in our population. Thirty nine families with autosomal recessive and one family with autosomal dominant non-syndromic hearing loss that include two or more affected children were screened for DFNB7(11) locus by linkage analysis. These families originated from different ethnic groups of Iranian population and were negative for GJB2 and GJB6 mutations in locus DFNB1 . We used D9S301, D9S175, D9S1876 and D9S1837 STR (short Tandem Repeat) markers for this study. Three out of forty families were linked to this locus. Mutation screening of TMC1 gene in these families revealed a homozygous framshift mutation (P.N150kfrx26) in one of the recessive families and a heterozygous mutation (G417R) in dominant family. Mutation detection for the other recessive family is undergoing. We concluded that after DFNB1 and DFNB21 mutations, TMC1 gene mutations are responsible for the most prevalent cause of non- syndromic hearing loss in Iranian population. Key words : linkage analysis, Iran, TMC1, DFNB7(11).

1154/W

Mutation Spectrom of Pendred Syndrome in Iranian Population. *M. Mohseni[1], K. Kahrizi[1], F. Azizi[2], C. Nishimura[3], N. Bazazzadegan[1], A. Dehghani[1], M. Sayfati[1], M. Taghdiri[1], P. Jamali[1], A. Daneshi[4], R.J.H. Smith[3], H. Najmabadi[1].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Endocrine Research Center , Taleghani hospital, Tehran, Iran; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology Head and Neck Surgery, University of Iowa, Iowa, IA, United States; 4) Research Center of Ear, Nose, Throat, and Head and Neck Surgery, Tehran, Iran.

Mutations in the SLC26A4 gene in DFNB4 locus is responsible for syndromic (Pendred syndrome) and non-syndromic hereditary hearing loss(HHL). In many populations mutations in this gene have been reported as a second cause of HHL. The objective of this study was to investigate the prevalence of SLC26A4 mutations in our HHL consanguineous families. After completing clinical evaluation the signed consent form was taken from each family. We included 80 families with two or more affected individuals, who have been referred to Genetics Research Center (GRC). All families had previously been tested negative for the DFNB1 locus were candidate for homozygosity mapping using STRs for DFNB4 locus. Families localized to this region were subjected to complete DNA sequencing. Eleven out of eighty families were mapped to DFNB4. Sequence analysis of eleven linked families revealed eight mutations (T420I, 1197delT, G334Y, R409H, T721M, R79X, S448L, L445W) . The T420I , G334V and R79X were novel mutations, we couldn't find any mutation in four linked families. We didn't detect any non syndromic individual with mutation in our study. We have been able to identify mutation in SLC26A4 gene only in 7 out of 80 families (8.75%). we detected in 11 families some degrees of diffuse or nodular goiter, three out of 11 families showed thyroid function impairment and in five of 11 families positive prechlorate discharge test. All of affected had normal temporal bone scan. This investigation, demonstrated that the SLC26A4 gene mutation is the most prevalent syndromic hereditary hearing loss in Iran. This result is in accordance with reports from other countries. Key words: DFNB4, SLC26A4 gene, hereditary hearing loss, Pendred, PDS.

1155/W

Hereditary Deafness in Iran. *H. Najmabadi[1], C. Nishimura[2], K. Kahrizi[1], N.C. Meyer[2], N. Bazazzadegan[1], J.L. Sorensen[2], M. Mohseni[1], Y. Riazalhosseini[1], M. Malekpour[1], G. Assadi tehrani[1], A. Daneshi[3], M. Farhadi[3], P. Imani[1], A. Anousheh[1], A. Nazeri[1], S. Abedini[1], N. Nikzat[1], S. Arzhangi[1], R.J.H. Smith[2].* 1) Genetic Research Ctr, Univ Social Welfare/Rehab, Tehran, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa, IA, United States; 3) Research Center of Ear, Nose, Throat, and Head and Neck Surgery, Iran university of Medical sciences, Tehran, Iran.

Genetic testing for deafness in Iran is well established. The population is extremely heterogeneous, which means that ethnic-specific data are required. We have generated much of these data by screening over 2000 families segregating autosomal recessive non-syndromic deafness (ARNSD). All patients were screened for mutations in GJB2 and GJB6 (DFNB1), and if no mutations were identified, haplotypes were reconstructed by typing three short tandem repeat polymorphisms flanking 20 known ARNSD loci. In a subset of families, genome-wide linkage analysis was completed. For approximately 30% of families, we have been able to establish a genetic cause for deafness. Over half have mutations in GJB2, and after GJB2, mutations in SLC26A4 and TECTA are most commonly detected. We have also found mutations in MYOVIIA, TMPRS53, MYO15A, VLGR1, USH1C and TMC1. In addition, we have described a new syndrome, a contiguous gene deletion syndrome that involves both deafness and infertility in males. The data from the Iranian population attest to its diversity and contribute to the current body of knowledge regarding the deafness of genetics. Key Words: Hereditary hearing loss, Iran, Linkage,.

1156/W

A comparison of allele frequencies estimates in 6,174 SNPs genotyped in HapMap and Seattle SNPs. *A.D. Skol[1], M.L. Feolo[2].* 1) Sec Genetic Med, Univ Chicago, Chicago, IL; 2) NCBI, National Library of Medicine, NIH, Bethesda, MD.

We examined the accuracy between probe-based genotyping conducted by The HapMap (HM) project, and genotypes derived by sequencing conducted by SeattleSNPs (SS). SS genotypes were obtained by targeted sequencing of genic regions in 23 CEPH Utah individuals. HapMap genotype data came from probe-based genotyping of the 60 CEPH Utah founders. A subset of subjects was genotyped in both groups on 5,128 SNPs, allowing us to test for genotyping inconsistencies. 1,828 SNPs were genotyped in ≥ 17 subjects by both HM and SS. Of the 6,174 SNPs, 2,932 were polymorphic in ≥ 1 sample. We identified 103 SNPs (1.7%) for which ≥ 1 individual's genotype was called differently by HM and SS. Of these, 68 have a single discrepant subject, 19 have two discrepant subjects, and 16 have ≥ 3 discrepancies. Of the 38,458 opportunities to detect discrepancies, we found only 258 discrepant genotypes (0.7%). We tested for allele frequency differences between the SS and HM samples using a standard Chi-squared test of independence. The distribution of p-values, which we expect to be uniformly distributed between 0 and 1, shows an excess of significant results at the .0001 and .001 levels. We also discovered that of the 103 SNP with ≥ 1 discrepant genotype, 24 are monomorphic in HM, but have at least one heterozygous SS individual. The converse occurs only 5 times. When there is no genotype discrepancies this imbalance is not observed. We determine empirically if heterozygotes are being overcalled by sequencing or undercalled by conventional genotyping by examining the linkage disequilibrium pattern between the SNP in question and those in the surrounding region. In summary, we find that data quality of both sequence data from SS and genotype data from HM is of very high quality. A small proportion of SNPs may appear monomorphic when using conventional genotyping methods when a SNP is present in the primer sequence or due to limitations of the genotype calling algorithm. As more genes are resequenced such SNPs can be identified, allowing either the redesign of primers or substitution of a SNP in linkage disequilibrium with the problem SNP.

1157/W

TUNA (Testing UNtyped Alleles) reveals new associations with type 2 diabetes in Mexican Americans. *M.G. Hayes[1], W. Wen[2], Y. Sun[3], A. Pluzhnikov[1], C.L. Hanis[4], N.J. Cox[1,3], D. Nicolae[1,2].* 1) Medicine; 2) Statistics; 3) Human Genetics, The University of Chicago, Chicago, IL; 4) Human Genetics Center, The University of Texas Health Sciences Center, Houston, TX.

Initial investigations (Hayes et al., 2007, *Diabetes*) in our genomewide association study (GWAS) identified several susceptibility genes for type 2 diabetes (T2D) in Mexican Americans (MA) from Starr County, Texas. Although the Affymetrix 100K mapping array directly interrogates only ~95K SNPs in the MA sample, given a reference database like the HapMap and the knowledge of the LD patterns in it, it is possible to use the SNPs genotyped on the array to conduct association tests in the remainder of the untyped variation in the human genome. Using the TUNA (Testing UNtyped Alleles) statistical framework (Nicolae, 2006, *Genetic Epidemiology*) for this procedure, we estimated unobserved allele frequencies as a linear combination of observed haplotype frequencies. The set of untyped variants to be tested is found using a multi-locus measure of LD, M_D, and haplotype frequencies from the European (CEU) and Asian (ASN) HapMap samples (we independently considered the LD patterns in these two populations due to the admixed nature of the MA population) to yield χ_1^2 distributed statistics under the null hypothesis of no association. TUNA is relatively quick (hours to overnight), and increased the number of SNPs examined from ~95K SNPs passing quality control thresholds to >800K SNPs. Interrogating the imputed SNPs reveals several new associations with T2D not detected among the SNPs typed on the 100K array. For example, *MAGI2,* shows marginal evidence of association using the typed SNPs on the 100K array (best χ_1^2=7.274), but yielded χ_1^2>20 with imputed SNPs regardless of which HapMap population (CEU or ASN) is used in the calculations. This gene is of particular interest since it shows evidence of replication in the Broad/Lund/Novartis Diabetes Genetics Initiative GWAS. We are currently genotyping these imputed SNPs and others identified as highly associated with T2D in the MA for confirmation and proof of principle of this approach.

1158/W

A Genome-wide Scan for Estimated Glomerular Filtration Rate (eGFR): The Family Investigation of Nephropathy and Diabetes (FIND). *R.P. Igo, The FIND Consortium.* Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH.

Diabetic nephropathy (DN) is a leading cause of mortality and morbidity in patients with type 1 and type 2 diabetes, accounting for almost half of all cases of end-stage renal disease in the Western world. The multi-center FIND Consortium aims to identify genes for DN by studying quantitative traits of kidney function, including the eGFR, in diabetic individuals from four populations: African American (AA), American Indian (AI), European American (EA) and Mexican American (MA). A genome-wide scan containing more than 5500 autosomal single-nucleotide polymorphism markers (average spacing of 0.6 Kosambi cM) was carried out on 1235 pedigrees (3972 diabetic participants, including 6.7% with type 1, 55.5% type 2, and 37.8% unknown) ascertained for DN. eGFR was calculated using the four-variable Modification of Diet in Renal Disease (MDRD) equation. We report here the results of genome-wide linkage analysis for eGFR.

The strongest peaks from an initial linkage scan, performed without covariates using Haseman-Elston regression, were on chromosome 10p in EA families (27 cM, $p = 1.9 \times 10^{-4}$) and on 20q in MA families (57 cM, $p = 3.5 \times 10^{-5}$). Additional linkage peaks were observed on chromosome 1q (259 cM, $p = 4.7 \times 10^{-4}$) and 7p in MA pedigrees (78 cM, $p = 1.0 \times 10^{-3}$) and on chromosome 15q in EA pedigrees (11 cM, $p = 1.2 \times 10^{-3}$). The latter overlaps a previously reported locus for eGFR in an independent EA sample (Leon et al. (2002) *Nephrol. Dial. Transplant.* **22,** 763). Suggestive evidence for linkage on chromosome 2q in both AA (188 cM, $p = 1.1 \times 10^{-3}$) and EA (184 cM, $p = 6.0 \times 10^{-3}$) samples contributed to the strongest overall genetic signal (188 cM, $p = 2.6 \times 10^{-3}$). After adjusting for body-mass index and duration of diabetes at enrollment, an additional peak on chromosome 3p was found in EA families (108 cM, $p = 7.5 \times 10^{-4}$), near a locus reported for DN in an AA sample (Bowden et al. (2004) *Kidney Int.* **66,** 1517). These findings both identify novel genetic factors for eGFR in diabetes and replicate previously identified loci for DN phenotypes.

1159/W

Digenic Inheritance of Apparent Autosomal Dominant Keratoconus in a Large Australian Pedigree. *K.P. Burdon[1], D.J. Coster[1], J.C. Charlesworth[2], J.E. Craig[1].* 1) Dept Ophthalmology, Flinders University, Bedford Park, SA, Australia; 2) Southwest Foundation for Biomedical Research, San Antonio, Texas, USA.

Keratoconus is a debilitating, blinding disease characterized by progressive asymmetrical thinning of the cornea. The onset is typically in early adulthood and over 30% of corneal grafts are attributable to keratoconus. It is a complex disease and multiple linked loci have been described, although only one gene has been reported (VSX1). A large four generation pedigree with autosomal dominant keratoconus was identified by the treating physician undertaking corneal grafting in multiple family members. Additional affected and unaffected family members were recruited and underwent a full ophthalmic examination and medical history. A genome wide linkage scan was conducted using the Affymetrix 10K SNP array, with multipoint parametric linkage analysis conducted in MERLIN. Analysis under a fully penetrant dominant model did not reveal any linkage. With the penetrance in heterozygotes reduced to 70%, two peaks were identified on 1p36.23-36.21 and 8q13.1-q21.11, with LOD scores of 1.9 and 2.0 respectively. Haplotype analysis revealed that all affected individuals carried identical haplotypes at both loci, while unaffecteds carried only one or neither of the haploytpes. Digenic linkage analysis was undertaken in GENEHUNTER-TWO LOCUS. The maximum LOD score of 3.4 was observed at 19.1cM on chromosome 1 and from 84.4 to 93.5cM on chromosome 8. Non-parametric analysis revealed a maximum NPL score of 7.8 with a p-value of 0.00024 at the same location as the parametric analysis. Thus, although the pedigree appears to demonstrate simple autosomal dominant inheritance, the disorder is likely digenic. Ingenuity Pathways Analysis was used to help prioritise candidate genes by looking for reported interactions between transcripts or proteins located under the two peaks. An interaction between SPSB1 (reportedly expressed in cornea) and TCEB1 (expressed in fetal eye and anterior segment) was identified, making these genes a high priority for follow-up. Given the digenic inheritance observed, this pedigree may provide a link between simple inherited forms of keratoconus and complex sporadic cases.

1160/W

Generalized epilepsy with febrile seizures plus: linkage analysis in three families from Southern Italy. *S. Carrideo[1], G. Incorpora[2], F. Annesi[1], E.V. De Marco[1], G. Provenzano[1], F.E. Rocca[1], L. Pavone[2], A. Labate[1,3], A. Gambardella[1,3], G. Annesi[1].* 1) Inst Neurological Sci, CNR, Mangone, Italy; 2) Department of Paediatrics, University of Catania, Italy; 3) 3Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Generalized Epilepsy with Febrile Seizures Plus (GEFS+) is a genetic disorder transmitted as an autosomal dominant trait with incomplete penetrance. GEFS+ is characterized by febrile seizures, that persist beyond the age of six, and by heterogeneous afebrile seizures that may include tonic-clonic, myoclonic, atonic seizures and absence. So far GEFS+ has been associated with mutations in SCN1A, SCN2A, SCN1B genes (encoding respectively the alpha 1, alpha 2 and beta 1 voltage-gated sodium channel subunits) and GABRG2 gene (encoding the GABAA receptor gamma subunit). The aim of this study is to better define the genetics of GEFS+ by analyzing 3 large families from Southern Italy. We enrolled 3 unrelated families with GEFS+. The diagnosis was made in accordance with the International League Against Epilepsy criteria. DNA was extracted from peripheral blood samples after informed consent. To investigate the role of the known genes we performed a linkage study on 22 affected and 17 non affected subjects belonging to these three families. We used microsatellite markers selected from those available in the NCBI data base. The following chromosome regions were examined: 2q24-33 (SCN1A-SCN2A), 19q13 (SCN1B), 5q31-33 (GABRG2). Two point linkage analysis was performed by LINKAGE. Linkage analysis allowed us to exclude the involvement of SCN1A and SCN2A genes in all these families. As regarding the remaining genes negative LOD score values (less than -2) were obtained for all informative markers in two families, while the study is still in progress in the third family. In two of our families the results showed no association between GEFS+ and the genes already described for this disease while more studies are needed in the third family. Our results provide further evidence for the high level of genetic heterogeneity associated with GEFS+ suggesting the involvement of at least one more gene in this syndrome.

1161/W

Evidence of a quantitative trait locus for energy and macronutrient intakes on chromosome 3q27.3 in the Quebec Family Study (QFS). *A. Choquette[1,2,4], S. Lemieux[3,4], A. Tremblay[2,4], Y.C. Chagnon[5], C. Bouchard[6], M.C. Vohl[1,3,4], L. Perusse[1,2].* 1) Lipid Research Center, CHUQ-CHUL Pavilion, Québec, Canada; 2) Social and Preventive Medicine Department, Division of Kinesiology, Laval University, Québec, Canada; 3) Food Science and Nutrition Department, Laval University, Québec, Canada; 4) Institute of Functional Foods and Nutraceuticals, Québec, Canada; 5) Robert-Giffard Research Center, Québec, Canada; 6) Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA.

Background: Poor dietary habits are associated with an elevated risk of obesity. Little is known about the genes influencing dietary energy and nutrient intakes, despite evidence that they are influenced by genetic factors. Objective : To identify chromosomal regions harboring genes affecting energy, carbohydrate, lipid and protein intakes through a genomewide linkage analysis. Design : Energy intake (EI) as well as intakes of carbohydrate (CHO), lipid (LIP) and protein (PROT) were assessed in 836 subjects from QFS using a 3-day dietary record. A total of 443 markers were genotyped and tested for linkage with age- and sex-adjusted dietary intakes and macronutrient intakes expressed as percent (%) of total energy intake using the Haseman-Elston method. A maximum of 454 pairs from 217 nuclear families were available for analysis. Results : The strongest evidence of linkage was found on chromosome 3q27.3 at marker D3S1262 for EI (p = 0.0000003). This marker was also linked with CHO (p = 0.00083) and LIP (p = 0.000029). The peak linkages for CHO, LIP and PROT were found on chromosomes 18q22.3 (p = 0.000074), 5q15 (p = 0.000009) and 10p14 (p = 0.000003), respectively. Evidence of linkage was also found on 20q11.21 for %CHO (p = 0.00036), 20q13.12 for %LIP (p = 0.00091) and 19q12 for %PROT (p = 0.00086). Conclusion : These results provide evidence for the presence of a QTL influencing total caloric intake as well as CHO and LIP intakes on chromosome 3q37.3. This region of the human genome has been shown to be linked to obesity in previous studies, which suggests the presence of a gene involved in the etiology of obesity through its influence on dietary intake.

1162/W

Genome-wide scan for malaria resistance and susceptibility genes in an Amazonian population. *R.G.M. Ferreira[1], R.C. Pagotto[2], M.J. Menezes[1], C.E. Kawamata[1], E.P. Camargo[1], H. Krieger[1].* 1) Dept Parasitology, University of Sao Paulo, Sao Paulo, SP, Sao Paulo, Brazil; 2) University of Rondonia, RO, Brazil.

The biochemical pathways involved in the pathogenesis of the parasite that causes malaria and the human host mechanisms of defense against the infection are not well know up to the present days. Epidemiologic studies, as well genetic studies, suggest the existence of genetic components related to the host innate resistance/susceptibility to malaria (Hill A.V.; Annu. Rev. Genet., 2006; 40:469-86.). To search for these genes, a genome-wide scan was conducted on a sample of 182 individuals, belonging to 34 nuclear families from Portuchuelo, Rondonia state, Brazil. Portuchuelo(8º37'S, 63º49'W) is a small riverine population with less then 200 individuals, located at the right bank of Madeira River, which is a hipo-endemic area for malaria (Ferreira R.G.M. *et al* Hum Biol., 2002 74(4):607-14).

The sample was genotyped using 108 STRs markers along 22 autossomes, with a mean distance of 24cM from each other. Those markers where obtained from Marshfield markers map (Broman K.W. *et al* Am. J. Hum. Genet. 1998 Apr 63:861-689). The software SimWalk2 (Sobel E. *et al*Am. J. Hum. Genet., 2002; 70:496-508) was used to check mendelian segregation of the alleles in families.

The reported number of malarial episodes, corrected by age and sex, was used as the affection phenotype. This phenotype showed expected association with Duffy- individuals (Ferreira *et al*, unpublished data) as well as biological driven inheritance, indicating its epidemiological importance. Linkage analysis were conducted using the software Solar 2 (Almasy L. *et al* Am. J. Hum. Genet. 1998 62:1198-1211).

Despite the small size of the sample, multipoint linkage analysis showed a peak of linkage at the short-arm of chromosome 4, between 0 and ~50cM with a lod-score suggestive of linkage (lod-score=2.1). (FAPESP, CNPq).

1163/W

Non-syndromic cleft lip with or without cleft palate (CL/P): Multipoint posterior probability of linkage (PPL) analysis sequentially updated over phenotypic subgroups reveals a Philippines-specific linkage to a region on chromosome 6q. *M. Govil[1], S. Daack-Hirsch[2], A.C. Lidral[3], V.J. Vieland[4], J.C. Murray[3], M.L. Marazita[1].* 1) Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA; 2) College of Nursing, The University of Iowa, Iowa City, IA; 3) Department of Pediatrics, University of Iowa, Iowa City, IA; 4) Columbus Children's Research Institute & Ohio State University, Columbus, OH.

Non-syndromic CL/P, the most commonly occurring congenital craniofacial anomaly, presents a challenge for testing and identifying contributory genes due to its complex etiology and ethnicity-specific prevalence. Sequentially updating over population of origin, a two-point genome-wide PPL analysis of 487 ethnically diverse families from 8 study populations estimated that there was an 80% probability of at least one CL/P risk gene located in specific regions of chromosomes 1, 2, 6, 9 and 12 encompassing about 50cM total (Govil et al, ASHG, 2005), with a 43% probability of a gene on chromosome 6 alone. A multipoint PPL analysis of these select regions with a three-point sliding windows approach indicated a striking 88% probability of a gene linked to a 14cM region on 6q14.1-6q16.1 (Govil et al, ASHG, 2006). In both these analyses, the Philippines population, with 258 families, provided the strongest signal, with a 41% (two-point) and 80% (multipoint) PPL for the 6q14.1-6q16.1 region. Since CL/P has considerable phenotypic variability and is also considered to be etiologically distinct from isolated cleft palate (CP), we subdivided the 258 Filipino families into 4 non-overlapping cleft phenotype groups (PG). Sequentially updating over PG provides a second peak in the 6q25.2 region with a 2-point PPL of 28%, where the cleft lip only (CL) PG has a PPL of 35%. The peak imputed PPL for the 6q24.3-6q25.3 region is 47% with sequentially updated three-point sliding windows. While the previously reported results identify our primary region for further analysis, the results presented here indicate the possibility of a second region on chromosome 6 specific to the Philippines population. NIH grants: R37-DE08559, P50-DE016215, R03-DE017167, R01-DE014667, K02-DE015291.

1164/W

Genome-wide SNP Linkage Analysis of Korean Multiplex Schizophrenia Families. *K.S. Hong[1, 2], H-H. Won[2], E-Y. Cho[2], H.O. Jeun[2], S-S. Cho[2], Y-S. Lee[3], D.Y. Park[1], Y.L. Jang[1], K-S. Choi[5], D. Lee[1], M-J. Kim[2], S. Kim[2], J.W. Kim[2, 4].* 1) Dept Psychiatry, Samsung Med Ctr, Sungkyunkwan Univ Sch Med, Seoul, Korea; 2) Samsung Biomedical Research Institute, Seoul, Korea; 3) Yong-In Mental Hospital, Yong-In, Korea; 4) Dept Laboratory Medicine & Genetics, Samsung Med Ctr, Sungkyunkwan Univ Sch Med, Seoul, Korea; 5) Dept Psychiatry, Eulji University Sch Med, Taejeon,Korea.

The present study reports the results of a genome-wide SNP linkage scan for schizophrenia in the Korean population. Fifty-six multiplex schizophrenia families were analyzed. Clinical evaluations on all subjects were performed by raters of a single research team. Multipoint non-parametric linkage analysis was performed, and empirical simulations were generated to determine genome-wide significance. Eight chromosomal regions yielded NPL Z scores above 2.0 for broad and narrow phenotype classes. We found genome-widely significant evidence of linkage for schizophrenia to chromosome 2p24.3 (NPL Z=3.18), 6q27 (NPL Z= 2.90), 3q24 (NPL Z=2.74), and 18q22.3 (NPL Z=2.59). Four other chromosomal regions, i.e., 13q12.3, 20p12.2, 4p14, and 1p36.12, were found to have NPL Z scores higher than 2.0. Although linkage to these loci has not received prominent attention in studies on Caucasian families, multiple overlaps were observed between our loci (on 2p, 3q, and 13q) and linkage peaks generated from extended families of various isolated populations. Fine mappings and the detection of candidate genes within these regions are warranted.

1165/W

Investigating Linkage to Chromosome 10 in Familial Interstitial Pneumonia (FIP). *A.L. Wise[1,3], M.C. Speer[3], M.P. Steele[3], L.H. Burch[1], A. Herron[3], J.E. Loyd[4], K.K. Brown[5,6], J.A. Phillips III[4], S.H. Slifer[3], M.I. Schwarz[5,6], D.A. Schwartz[1,2].* 1) National Institute of Environmental Health Sciences, Research Triangle Park, NC; 2) National Heart, Lung, and Blood Institute, Bethesda, MD; 3) Duke University, Durham, NC; 4) Vanderbilt University School of Medicine, Nashville, TN; 5) National Jewish Medical and Research Center, Denver, CO; 6) University of Colorado Health Sciences Center, Denver, CO.

The Idiopathic Interstitial Pneumonias (IIPs) are complex conditions, with limited treatment options and unknown etiology. Through a whole genome microsatellite screen for FIP (the familial form of IIP), two regions of interest on chromosome 10 (maximum multipoint LOD score 2.3) and chromosome 11 (LOD score 3.0) were identified. Given the complex nature of both IIP and FIP, we sought to determine if accounting for the chromosome 11 locus and/or phenotypic classification could improve evidence for linkage to chromosome 10. To begin with, families were classified into two phenotypic groups: homogeneous families, with only idiopathic pulmonary fibrosis (IPF) (N=42), and heterogeneous families, with multiple IIP phenotypes including at least one case of IPF (N=40). After phenotypic stratification, ordered subset analysis (OSA) was performed using chromosome 11 family-specific LOD scores as the covariate. Fine-mapping of chromosome 10 revealed 2 linkage peaks in all 82 families strongly defined by phenotypic classification upon stratification (LOD scores 1.5 and 2.1). Homogeneous families showed evidence of linkage to the centromeric peak (LOD score 1.8), while heterogeneous families exhibited evidence of linkage to the telomeric peak (LOD score 1.6) as well as a third peak seen in only the heterogeneous families (LOD score 1.7). Applying OSA, lower chromosome 11 family-specific LOD scores also maximized linkage to chromosome 10 within a subset of 39 of the 82 families (LOD score 3.3, p=0.01) and 22 of the heterogeneous families (LOD score 3.6, p=0.006). Thus, our investigations support possible genetic heterogeneity within FIP, with evidence for linkage to chromosome 10 strengthened in families with lower chromosome 11 LOD scores and a heterogeneous phenotype.

1166/W

Mapping and identification of genes underlying autosomal dominant non-syndromic sensorineural hearing loss in Chinese families. *H. Yuan[1], J. Cheng[1], Q. Sun[1], H. Sun[1], P. Dai[1], D. Han[1], X. Liu[3], L. He[2].* 1) Institute Of Otolaryngology, Chinese PLA General Hospital, Beijing 100853, China; 2) Bio-X Life Science Research Center, Shanghai Jiao Tong University, Shanghai 200030, China; 3) Department of Otolaryngology, University of Miami, Miami, FL 33136 USA.

In this study, we have identified two novel mutations in two Chinese DFNA families. A maximum two-point LOD score of 6.69 at theta=0 was obtained for marker D14S1040 in family SD-Z001. Haplotype analysis placed the locus within a 7.6 cM genetic interval defined by marker D14S1021 and D14S70, overlapping with the DFNA9 locus on chromosome 14q12-q13. DNA sequencing of coding exons and exon/intron boundaries of the COCH gene identified a c.1625G>A mutation in exon 12 that co-segregates with auditory dysfunction in the pedigree. The mutation results in a predicted C542Y substitution at an evolutionarily conserved cysteine residue in the vWFA2 domain of cochlin. The predominant feature of these Chinese families is that all the affected subjects harboring COCH mutations in the vWFA2 domain do not suffer the vestibular symptoms during their life time. A comprehensive vestibular assessment reveals only subtle vestibular hypofunction in some affected members of these families. In family NMG-L024, the disease locus was mapped to a 12 cM region of chromosome 7p15 between marker D7S629 and D7S526, with a maximum two point LOD score of 5.39 for D7S2457, overlapping with DFNA5 locus. A novel heterozygous mutation,IVS8+4A>G, in the splice donor site of intron 8 was identified in this family. Messenger RNA studies indicated that the identified mutation leads to skipping of exon 8 in the mutant transcript. The IVS8+4A>G mutation is predicted to create a shift in the reading frame and introduce a stop codon at position 372, resulting in a prematurely truncated DFNA5 protein. Thus far, a total of four mutations have been identified in DFNA5 families, all of them result in skipping of exon 8 at the mRNA level. This is the first mutation that is identified in intron 8 in DFNA5 family. Our findings provide further support for the hypothesis that DFNA5-associated hearing loss is caused by gain-of-function mutation.

1167/W

Familial idiopathic scoliosis and the IRX gene family. *C. Justice[1], N.H. Miller[2], B. Marosy[3], D. Behneman[1], A.F. Wilson[1].* 1) Genometrics Section, IDRB, National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland; 2) University of Colorado, The Children's Hospital, Denver, Colorado; 3) Institue of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Familial idiopathic scoliosis (FIS) is characterized by a lateral curvature of the spine present in otherwise normal individuals that affects 2 to 3% of the population. The original sample was comprised of 202 families with at least two individuals with scoliosis. Prior to analysis, three subgroups were determined including: most likely mode of inheritance (XLD vs. AD), families with at least two members with kyphoscoliosis, and families with at least two members with triple curves. Linkage analysis was performed in each subgroup with 391 STRP markers. Linkage analysis of the kyphoscoliosis subgroup (7 families, 53 individuals) identified candidate regions on chromosomes 5 and 13. The region on 5p13 (~3 Mb) contained only three genes, all belonging to the Iroquois homeobox (IRX) gene family. Three other IRX genes were located on 16q, in a region also linked to FIS in our AD subgroup.

To compare the distribution of other gene families in linked vs. non-linked regions, FIS-linked regions were defined as being ± 5 Mb from two consecutive p-values < 0.025 (26 regions ,15% of the genome). A BLAT search was performed with the IRX1 mRNA sequence, and 65% of homologous loci were in regions linked to FIS. Four other genes, three on chromosome 5 and one on chromosome 12, of similar size to IRX1, also underwent a BLAT mRNA homology search. The number or regions linked to FIS ranged from 17% to 36%, substantially less that for the IRX gene family.

1168/W

Linkage disequilibrium mapping of common myopia susceptibility loci using an LD map for a replicated linkage region at 3q26. *T. Andrew[1,2], N. Maniatis[3], T.D. Spector[2], C.J. Hammond[2].* 1) EPH, Imperial College, London, United Kingdom; 2) Twin Research & Genetic Epidemiology Unit. St Thomas' Hospital, London; 3) 2Department of Epidemiology & Public Health, Imperial College, London. 3Human Genetics Division, Southampton General Hospital, University of Southampton, Southampton.

Purpose: Common myopia has a strong heritable component (Hammond et al 2001) and affects ~25%-61% of the population. In this study we aim to map susceptibility alleles that contribute to myopia. Methods: In a genome-wide linkage study, evidence of linkage to chromosome 3q26 was observed (LOD 3.7) using 221 healthy dizygotic (DZ) female twins measured for myopia using a high-precision autorefractor (Hammond et al 2004). In a replication study, evidence of linkage to 3q26 is also observed (LOD 1.9) using 485 DZ pairs based upon a spectacle prescription from their optometrist. Both measures are on a continuous scale, with a cohort 1-99th percentile range of -10 (myopic) to +6.5 (hyperopic) dioptres. For the fine mapping stage of the study, we defined 241 genetically "enriched" cases (individuals with < -1 dioptres and a twin pair mean of =<-0.75 dioptres) and 257 "super controls" (individuals with >+1 dioptres and a twin pair mean of >+1 dioptres). Based upon previous work (Maniatis et al 2002 and 2007), we defined a genetic LD map using HapMap Phase II data. The map is defined in LD units (LDU), discriminates blocks of conserved LD and has additive distance and locations monotonic with physical (kb) and genetic (cM) maps. We use an innovative and efficient study design, in which 3-6 SNPs per LDU for a range of common allele frequencies were placed evenly across the LD map, resulting in a total of ~2300 SNPs capturing most of the common genetic variation (MAF>=0.05) in the ~25MB 3q26 region. Results and conclusions: Preliminary analyses indicate 2 short kb regions are significantly associated with myopia. Work is underway to replicate these.

1169/W

Inference of population structure using arbitrarily linked multilocus genotype data. *L. Jin[1,2], Y. Wang[1], W. Fu[1].* 1) Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, China; 2) CAS-MPG Partner Institute for Computational Biology, SIBS, CAS, Shanghai, China.

STRUCTURE analysis plays an important role in revealing the genetic structure of populations. However, such analysis is only applicable to those markers that are not in strong linkage disequilibrium and therefore, the full amount of information from genome-wide scan can not be exploited. To overcome this obstacle, we describe a grade of membership models (GoM) clustering method using principle components (PC) to infer population structure and assign individuals to population. The population parameters and individuals' admixture proportion to each population is simultaneously estimated. The principle component transformation allows our method to model arbitrarily linked genetic markers. The application of the method includes exposing hidden population structure in the sample, analyzing individual's admixture proportion, acting as the first step in structure association correction in case-control association study, determine the relation of nominal populations and so on. We test the software on three dataset and demonstrate the superiority of this method over the existing ones.

1170/W

Association analysis RANKL/RANK/OPG genes with cross-sectional geometry at the femoral neck in Caucasian families. *H.W. Deng[1,2,3,4], H. Shen[1], D.H. Xiong[3], R.R. Recker[3], C. Papasian[1], Y.J. Liu[1].* 1) Orthopedic Surg/Basic Med Sci, Univ Missouri, Kansas City, Kansas City, MO; 2) School of Life Science and Technology, Xi'an Jiaotong University, Xi'an , Shaanxi 710049, P. R. China; 3) Osteoporosis Research Center, Creighton University, Omaha, NE 68131, USA; 4) College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China.

The molecular mechanisms underlying the crosstalk between stromal/osteoblast cells and hematopoietic cells have recently been elucidated by finding a new cytokine system, which is composed of a ligand - RANKL; its specific receptor - RANK; and its decoy receptor - OPG. Bone geometry, in addition to bone mineral density (BMD), is a key factor in bone strength, which is the ultimate intrinsic determinant of fracture risk. In this study, we aimed to investigate if the polymorphisms in the RANKL, RANK, and OPG genes contribute to variation in bone geometry, and if there exist gene-gene interactions among these three genes that are involved in a functional pathway. We performed family-based association analyses by genotyping 41 SNPs (an average density of one SNP per 4kb) in a sample of 405 Caucasian nuclear families comprising 1873 subjects. We conducted analyses using single SNP- and haplotype-based association test (FBAT) for association with five hip geometric variables, namely, cross-sectional diameter (CSA), cortical thickness (CT), endocortical diameter (ED), sectional modulus (Z), and buckling ratio (BR). In addition, we performed gene-gene interaction analyses using multianalytic approaches such as the restricted partition method (RPM) and a newly developed LD-based statistic to detect gene-gene interaction between two unlinked loci. The most significant associations were found between RANKL and CSA, CT and BR (P = 0.02 - 0.002). Haplotype analyses further supported the association observed in single SNP analyses. Significant gene-gene interactions were also observed among the OPG, RANK and RANKL genes. Our findings suggest that genetic variants in genes involved in the RANKL/RANK/OPG bone remodeling pathway are strongly associated with variation in bone geometry.

1171/W

Identification of ancestrally informative regions in Latinos using whole genome SNP data. *S. Kim[1], R. Jiang[2], C. Shtir[2], R. Varma[2,3], P. Marjoram[2], J. Wall[1].* 1) Institute of Human Genetics, UC San Francisco, San Francisco, CA; 2) Department of Preventive Medicine, Keck School of Medicine, USC, Los Angeles, CA; 3) Doheny Eye Institute and Department of Ophthalmology, Keck School of Medicine, USC, Los Angeles, CA.

Standard admixture mapping requires the identification of ancestrally informative markers (AIM), which often incur additional experimental costs in genome-wide association studies. Having a method for conducting admixture mapping that does not require AIMs would be both easier and cheaper than the current protocols. Here we explore the feasibility of using new methods for estimating genetic ancestry directly from whole genome SNP sets, such as the Affymetrix 500K. We identified regions suggestive of high Native American ancestry in our sample of Latinos from the Los Angeles Latino Eye Study. These regions may serve as candidate regions that may harbor susceptibility genes for traits with higher incidence rates in Native Americans compared to Europeans.

1172/W

Suggestive evidence for linkage of 5-Year Change in Bone Mineral Density to Chromosome 6q: The San Antonio Family Osteoporosis Study. *J.R. Shaffer[1], C.M. Kammerer[1], J.M. Bruder[2], S. Cole[3], T.D. Dyer[3], L. Almasy[3], J.W. MacCluer[3], J. Blangero[3], R.L. Bauer[2], B.D. Mitchell[4].* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Texas Health Science Center at San Antonio, San Antonio, TX; 3) Southwest Foundation for Biomedical Research, San Antonio, TX; 4) University of Maryland, Baltimore, MD.

Rapid bone loss in later life, particularly in those who have achieved low peak bone mineral density (BMD) in their earlier years, is a major contributor to osteoporosis and bone fracture. While cross-sectional studies have identified putative quantitative trait loci (QTLs) influencing variation in BMD, such studies cannot adequately distinguish loci affecting loss of BMD with age from those affecting the acquisition of peak bone mass occurring in young adulthood. To this end, we measured areal BMD (g/cm^2) of the hip by dual-energy x-ray absorptiometry at two time points and calculated 5-year annualized BMD change in 243 Mexican Americans (ages 45-65, 35% men) from 34 extended kinships (138 sibling pairs, 112 first-cousin pairs, and 86 other relative pairs). We then performed genome-wide linkage analysis of BMD change using a 10 cM density scan. We adjusted for the following covariates: sex, baseline BMD, baseline weight, menopausal status, and interim change in weight. The additive residual heritability for hip BMD change was 0.53 (p = 0.006). We observed suggestive evidence for linkage to chromosome 6q for hip BMD change (multipoint LOD = 2.5, cM = 103) near marker D6S1056. This locus was not implicated in our previously reported genome-wide linkage screen for cross-sectional BMD from this sample, and has not been reported in previous studies of BMD in other populations. This region may harbor one or more genes affecting the rate of BMD loss with age. Identifying the genes and pathways involved may provide important targets for therapeutic intervention to prevent age-related bone loss.

1173/W

The HIV Elite Controller Study: a genome-wide association study to identify variants involved in HIV viral control and disease progression. *P.I.W. de Bakker[1], F. Pereyra[2], L. Davies[1], A. Rothchild[2], L. Gianniny[1], B. Block[2], B. Baker[2], N.P. Burtt[1], R.R. Graham[1], R.M. Plenge[1], B.D. Walker[2], HIV CONTROLLER CONSORTIUM.* 1) Broad Institute of Harvard and MIT, Cambridge, MA; 2) Partners AIDS Research Center, Massachusetts General Hospital, Boston, MA.

The HIV ELITE CONTROLLER STUDY (www.elitecontrollers.org) is a collaborative effort between academia and the community to study untreated HIV infected people who are able to maintain viral load (VL) at or below the limits of detection. The goal of this collaboration is to identify the key viral, host genetic and immunologic contributions to this extraordinary outcome of infection. While the vast majority of untreated HIV infected individuals exhibit evidence of progressive viral replication and CD4+ T-cell depletion, approximately 1 patient in 300 is able to control replication at low levels without the need for antiretroviral (ARV) medications. These so-called elite controllers have VL<50 copies/mL, and represent the extreme tail of an otherwise normal distribution of viral load in the HIV infected population. To test the hypothesis that host genetic factors influence innate and adaptive immunity and durable suppression of HIV infection, we are conducting a genome-wide association study in 300 elite and 300 viremic controllers (<2000 copies/mL) as cases and >900 individuals with high VL (in the absence of ARV, ultimately requiring therapy) selected from a cohort of the AIDS Clinical Trial Group as controls. We are genotyping these individuals using the Illumina HumanHap650Y platform. We present the results of the association analysis and highlight analytical strategies we have taken to correct for population stratification and admixture. Although this initial scan has good power to detect common alleles of large effect (odds ratio >2.0; as seen for HLA risk alleles in autoimmune diseases), power is limited to detect more modest effects (OR<1.5; as seen for complex traits such as type 2 diabetes). Efforts are underway to recruit the estimated 2000 or more persons in the US that fit the HIV controller criteria, with additional international collaborations adding to the numbers, to improve power.

1174/W

Genome-wide linkage analysis for circulating levels of inflammatory markers in the Quebec Family Study (QFS). *S.-M. Ruchat[1,5], J.-P. Després[2], S.J. Weisnagel[1,5], Y.C. Chagnon[3], C. Bouchard[4], L. Pérusse[1,5].* 1) Social and Preventive Medicine, Laval University, Quebec, Quebec, Canada; 2) Quebec Heart Institute, Hopital Laval Research Centre, Quebec, Canada; 3) Laval University Robert-Giffard Research Center, Beauport, Quebec, Canada; 4) Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA; 5) Lipid Research Center, CHUL Research Center, Quebec, Canada.

Background: Adipose tissue synthesizes and secretes a wide range of biologically active molecules considered as inflammatory markers which dysregulation in obesity plays a role in the development of insulin resistance and vascular disorders. Thus, finding genes that influence circulating levels of inflammatory biomarkers may provide insights into genetic determinants of obesity-related metabolic diseases. Objective: Search for genes influencing plasma levels of adiponectin (APM1), C-reactive protein (CRP), interleukin-6 (IL6) and tumor-necrosis factor-alpha (TNFA) through a genome-wide linkage analysis. Design: Fasting plasma levels of APM1, CRP, IL6 and TNFA were measured in 764 subjects from QFS. APM1, IL6 and TNFA were measured by ELISA (R&D System Inc., Minneapolis, Minnesota) and CRP by nephelometry (BN Prospec, Dade Behring). After log10 transformation, phenotypes were adjusted for age and sex and tested for linkage with a total of 443 markers using the Haseman-Elston method. A maximum of 393 sibpairs from 211 nuclear families were available for analyses. Results: The peak linkages were found on chromosomes 9q34.3 for APM1 (markerD9S158; nominal p-value = 0.000001), 12q24.2 for CRP (D12S375; 0.0004312), 17q11.2 for IL6 (D17S1294; 0.0000133) and 4p15.2 for TNFA (D4S2397; 0.0000122). Significant evidence of linkage ($p < 0.0001$) was also found on chromosomes 1p36.3 (D1S468; 0.0000305), 3q13.33 (D3S3023; 0.0000446), 14q32.2 (D14S1426; 0.0000097) and 15q21.1 (D15S659; 0.0000013) for APM1, 11q25 (D11S2359; 0.0000136) for IL6 and 5q35.3 (D5S408; 0.0000834) for TNFA. Conclusion: These results suggest that several QTLs can influence plasma levels of inflammatory markers. The genes underlying these QTLs need to be identified.

1175/W

Loci associated with successful aging in the Amish. *P.J. Gallins[1], J.L. McCauley[2], L. Jiang[2], A.E. Crunk[2], M. Creason[1], L. Caywood[1], D. Fuzzell[2], C. Knebusch[2], C.E. Jackson[3], J.R. Gilbert[1], M.A. Pericak-Vance[1], J.L. Haines[2], W.K. Scott[1].* 1) University of Miami, Miami, FL; 2) Vanderbilt University, Nashville, TN; 3) Scott & White, Temple, TX.

Successful aging (SA) involves maintaining cognitive and physical function, being socially engaged throughout the lifespan, and avoiding disease and disability. Several components of SA have demonstrated heritability in different samples: longevity, upper extremity strength, lower extremity function, and retention of cognitive ability. The oldest Amish communities of Indiana and Ohio were founded in the mid-1800s by few individuals and remain socially and genetically isolated. Isolation and a relatively homogeneous environment make the Amish a suitable population for identifying complex trait loci. We surveyed cognitively intact Amish age 80 and over, collecting DNA and subjective and objective measures of function, cognition, life satisfaction, and social support. Over 300 individuals have been enrolled in the study and 217 were included in a whole-genome SNP linkage screen (Illumina Linkage Panel IVb). All individuals can be linked back to common ancestors through 11 generations. 68 individuals met criteria for SA (cognitively intact, not depressed, satisfied with life, little self-reported limitation in activities of daily living or musculoskeletal function, in the top 1/3 of the sample on a lower-extremity physical function battery, and having adequate social support). 5,645 SNPs were analyzed for association with SA using the CC-QLS method (Bourgain et al., AJHG 2003; 73:612-26), which performs an adjusted chi-square test of association in related cases and controls, adjusting for correlated data using pairwise kinship coefficients. SNPs on 5 chromosomes were associated with SA (when using a fairly liberal screen threshold of $p<0.001$, chosen to correspond to a lod score of 2 in a whole genome screen): 1p, 3p, 6q, 15q, 16q. The region on 15q is notable because it confirms our preliminary analysis on a subset of 107 individuals. These results suggest that several regions of the genome might harbor loci that influence successful aging in the Amish, particularly the region on chromosome 15q.

1176/W

Whole genome association studies of rheumatoid arthritis and replication of identified susceptibility loci. *X. Ke[1], W. Thomson[1], A. Barton[1], S. Eyre[1], A. Hinks[1], J. Bowes[1], R. Donn[1], S. Hider[1], I.N. Bruce[1], A.G. Wilson[2], A. Morgan[3], P. Emery[3], A. Carter[4], S. Steer[5], L. Hocking[6], D.M. Reid[6], D. Strachan[7], P. Wordsworth[8], J. Worthington[1], YEAR consortium.* 1) arcEU, University of Manchester, UK; 2) School of Medicine & Biomedical Sciences, University of Sheffield; 3) Academic Unit of Musculoskeletal Disease, Chapel Allerton Hospital, Leeds; 4) Academic Unit of Molecular Vascular Medicine, University of Leeds; 5) Clinical and Academic Rheumatology, Kings College Hospital, London; 6) Bone Research Group, Dept. Medicine & Therapeutics, University of Aberdeen; 7) Division of Community Health Sciences, St George's, University of London; 8) Nuffield Department of Orthopaedic Surgery Nuffield Orthopaedic Centre, Oxford.

The arcEU is part of the Wellcome Trust Case Control Consortium (WTCCC), conducting whole genome association scans on 7 common diseases including rheumatoid arthritis (RA). In the study, 2,000 Caucasian RA samples were genotyped using the Affymetrix 500K chip and compared with 3,000 common controls. 9 SNPs associated with RA at p=5x10-5-1x10-7, excluding known susceptibility variants of HLA and PTPN22, were identified. These 9 SNPs, located within or close to genes like IL2RA, IL2RB, PODXL and TNFAIP3/OLIG3, were subjected to a replication study. Potentially functional SNPs within these genes were also tested. The replication cohorts comprised 5,063 RA cases and 3,849 controls from the UK, providing at least 80% power to almost all the SNPs being replicated at $p<0.05$. One SNP which located between TNFAIP3 and OLIG3 was found to be significantly associated with RA in the replication study (OR=1.24, 95% CI 1.15-1.33, trend $p<1.9x10-8$). Stratified analysis revealed patients positive for rheumatoid factor and anti-CCP had a much stronger association with this SNP. Association to SNPs located in or near PODXL (OR=1.09, 95% CI 1.03-1.19, trend $p < 0.0067$), IL2RA (OR=1.09, 95% CI 1.01-1.18, trend $p < 0.04$) and IL2RB (OR=1.12, 95% CI 1.03-1.21, trend $p < 0.0069$) genes were also replicated. Fine mapping with these genes and further replication efforts with other potential SNP variants identified in the initial analysis will be carried out.

1177/W

The ATP-binding cassette transporter 2 (TAP2) gene is strongly associated with Systemic Lupus Erythematosus (SLE). *P.S. Ramos[1], L. Bera[1], P.M. Gaffney[2], K.L. Moser[2].* 1) Dept Medicine, Univ Minnesota, Minneapolis, MN; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK.

SLE is a systemic autoimmune disease characterized by antibody production against nuclear antigens. The interferon (IFN) pathway is clearly implicated in disease pathogenesis, as shown by the overexpression of IFN-inducible genes observed in SLE and other autoimmune diseases. We are currently analyzing the interferon regulatory factor 2 (IRF2) gene for its possible contribution to disease susceptibility. Given the potential role of multiple IFN pathway genes in disease predisposition, we chose single nucleotide polymorphisms (SNPs) from several genes known to interact with IRF2 and tested them for association with SLE in our collection of 453 Caucasian families. We used the standard Transmission Disequilibrium Test (TDT) on ATP-binding cassette transporter 1 (TAP1) (mean r2=0.87), TAP2 (mean r2=0.71), tapasin (or TAP binding protein, TAPBP) (mean r2=0.26), tumor protein p53 (TP53) (mean r2=0.53), IRF2 binding protein 1 (IRF2BP1) (mean r2=1.0) and IRF2BP2 (mean r2=0.75). Several SNPs in TAP2 showed evidence for association, the strongest effect being found with a SNP that localizes in the 3'UTR and mRNA of the gene ($P = 1.33x10-6$). This is the largest cohort studied to date that implicates TAP2 in SLE predisposition. Additional analyses are currently underway to replicate this finding and determine if this effect is independent of the MHC. No evidence of association was found for the other genes screened in this study. Further analyses are warranted to pinpoint the exact mechanism by which this variant might affect TAP2 function and consequent antigen presentation, thus contributing to SLE predisposition.

1178/W

Significant linkage on chromosome 5q35 for the disorganization dimension of schizophrenia revealed by quantitative trait linkage analysis. *D. Avramopoulos[1,2], J. Mcgrath[1], M. Mohseni[2], V.K. Lasseter[1], P. Wolyniec[1], M.D. Fallin[3], K.Y. Liang[3], D. Valle[2], G. Nestadt[1], A.E. Pulver[1].* 1) Dept. Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 3) Bloomberg School of Public Health, Johns Hopkins Univ, Baltimore, MD.

Schizophrenia is a frequent heritable brain disorder with a peak onset between 18 and 35 years. Many linkage scans, two of which from our group, have attempted to locate genes for schizophrenia using varying research strategies. Apparent lack of consistency among scans may reflect the underlying genetic heterogeneity of schizophrenia, also likely reflected in its phenotypic heterogeneity. Latent structure analytic methods have been used to characterize this phenotypic heterogeneity, and the resulting latent classes or factors are considered more homogeneous aspects of the schizophrenia phenotype. We hypothesized that such factor scores might better reflect the individual's genetic makeup than the schizophrenia diagnosis alone, factors showing higher heritability presumably being more influenced by genes and thus more appropriate for linkage analysis. Using 73 items available for our collection of 1199 schizophrenia patients we performed a principal components factor analysis and developed a statistically supported nine factor model consistent to a large extent with previous reports. We used scores on factors with heritability > 0.3 as quantitative traits for a genome scan on 116 multiplex Caucasian pedigrees. We obtained a LOD score of 4.1 on chromosome 5q35 for the disorganization factor. This is a region previously reported by multiple linkage studies, in which however there was no significant linkage to the binary disease phenotype in our pedigrees. Our approach successfully reduced genetic heterogeneity providing significant linkage in a replicated region. Inconsistency in linkage findings might be in part due to studying less specific phenotypes.

1179/W

Locus for autosomal dominant keratoconus identified on chromosome 13. *B.A. Bejjani[1], D. Winters[1], M. Rydzanicz[1], A. Molinari[2], J.A. Pitarque[2], K.L. Mackay[1], K. Lee[3], R.A. Lewis[3], S.M. Leal[3], M. Gajecka[1].* 1) Health Research & Education Center, Washington State Univ, Spokane, WA; 2) Hospital Metropolitano, Quito, Ecuador; 3) Baylor College of Medicine, Houston, TX.

Keratoconus (KC) is a non-inflammatory thinning and anterior protrusion of the cornea that results in steepening and distortion of the cornea, altered refractive powers, and altered visual acuity. We identified and investigated an Ecuadorian cohort in which KC without other ocular or systemic features is transmitted as an autosomal dominant trait with incomplete penetrance. We sequenced the coding exons for the KC candidate genes, VSX1 and SOD1 in affected individuals from these Ecuadorian families and in ethnically matched controls and excluded VSX1 and SOD1 as candidate genes for KC in this population. Next, we performed a genome-wide linkage analysis on 18 Ecuadorian families (77 affected individuals, 67 unaffected and 11 individuals with unknown status) using fluorescent markers with an average spacing of 5 cM, spanning the genome. We excluded previously defined KC loci on chromosomes 2p24, 3p14-q13, 5q14.3-q21.1, 15q and 16q22.3-q23.1. One of the largest families (KC-014) had 22 available DNA samples, of which 11 samples were from individuals affected with KC. A new locus was identified on chromosome 13 for family KC-014 with a maximum 2-point LOD score of 2.8 and multipoint NLP score of 9.9.

1180/W

Suggestive evidence for linkage to a chromosome 13 locus influencing serum IGF-1 levels and body weight in cystic fibrosis mice. *J.C. Canale-Zambrano[1], S.M. Cory[2], C.K. Haston[1].* 1) Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada; 2) McGill Centre for Bioinformatics, McGill University, Montreal, Quebec, Canada.

Cystic fibrosis (CF) is the most common, fatal genetic autosomal recessive disease affecting young Caucasians. CF is a multi-organ disease affecting primarily the lungs and the digestive system. As a consequence of intestinal disease, CF patients suffer from failure to thrive and present low body weight relative to the non-CF population. CF mice present the phenotype of low body weight and have been shown to have low serum levels of insulin-like growth factor-1 (IGF-1), as has been clinically reported in CF patients. In addition, we have recently shown CF body weight phenotype to be dependent on intestinal crypt proliferation and apoptosis. The objective of this study is to use CF mice to identify candidate modifier genes influencing body weight in CF disease. To identify these factors we phenotyped a population of 12 week-old CF mice for body weight, serum IGF-1 and crypt changes and conducted a genome-wide mapping study in an F2 population (B6xBALB) of CF mice. Concentrations of serum IGF-1 levels and body weight showed a phenotypic correlation (r=0.55, p=0.03) in female F2 CF mice. These phenotypes were suggestively linked to chromosome 13 with a LOD score of 2.2 for IGF-1 and a LOD score of 2.5 for body weight. At this locus the presence of B6 allele in an F2 mouse decreases the serum levels of IGF-1 in male mice (*Balb/Balb* 475 ng/ml vs *B6/B6* 343 ng/ml; p = 0.01). A QTL for body weight has been described to be at the location for non-CF mice; however, no QTL has been described for IGF-1 in non-CF animals suggesting this locus to be specific to CF mice. The mapping of intestinal crypt changes is ongoing. We conclude that a locus for serum levels of IGF-1 was co-incident with the body weight in CF mice on chromosome 13, providing a possible mechanism linking both phenotypes.

1181/W

Usage of a novel algorithm to rank candidate regions and genes after a high-density SNP genotyping study of families with a hypertrichosis insulin resistance disorder (Rosai-Dorfman-like).. *S.T. Cliffe[1,2], T. Roscioli[1,2,3], M. Wong[4], A. Darmanian[5], G. Peters[5], M.F. Buckley[1,2], R. Lindeman[1,2,6].* 1) Molecular and Cytogenetics Unit, Department of Haematology and Genetics, Prince of Wales Hospital, Sydney, Australia; 2) Centre for Vascular Research, University of New South Wales, Sydney, Australia; 3) Sydney South West Integrated Genetics Service, Royal Prince Alfred hospital, University of Sydney, Australia; 4) Department of Allergy, Immunology and Infectious Diseases, The Children's Hospital Westmead, Sydney, Australia; 5) Department of Cytogenetics, The Children's Hospital Westmead, Sydney, Australia; 6) School of Medical Sciences, University of New South Wales, Sydney, Australia.

We report the analytical procedure used to examine 55, 721 SNP genotypes in two consanguineous Lebanese-Australian families with an autosomal-recessive Rosai-Dorfman-like disorder. Traditionally STR markers have been used to detect regions of homozygosity, however high-density SNP genotyping is becoming more prevalent due to advantages in both cost and resolution. The binary nature of SNP alleles however accords low marker informativeness, which can be mitigated by using a much greater density of markers than a typical STR marker study. This can potentially result in a greater resolution, but requires more sophisticated analysis to detect significant regions of identity by descent.

Markers were scored based on a range of criteria, which included marker heterozygosity in the proband's parents and siblings. A sliding window scoring homozygous segments in the probands was then employed to identify 40 candidate regions spanning 36 Mb in total. These regions contained 237 known genes that were ranked for significance using expression patterns, functional analysis and genome data-mining. The most significant candidate genes have been selected for further molecular testing.

1182/W

A high-density SNP genome-wide linkage search of 206 families identifies susceptibility loci for chronic lymphocytic leukemia. *L.R. Goldin[1], G.S. Sellick[2], R.W. Wild[2], S.L. Slager[3], D. Catovsky[4], N. Caporaso[1], R.S. Houlston[2].* 1) Genetic Epidemiology Branch, Divsion of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; 2) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 3) Division of Biostatistics, Dept. of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; 4) Section of Haemato-oncology, Institute of Cancer Research, Sutton, Surrey, UK.

Chronic lymphocytic leukemia (CLL) and other B-cell lymphoproliferative disorders display familial aggregation. To identify a susceptibility gene for CLL we assembled families from the major European (ICLLC) and American (GEC) consortia to conduct a genome-wide linkage analysis of 101 new CLL pedigrees using a high-density single nucleotide polymorphism (SNP) array and combined the results with data from our previously reported analysis of 105 families. Here we report on the combined analysis of the 206 families. Multipoint linkage analyses were undertaken using both non-parametric (model-free) and parametric (model-based) methods. After the removal of high linkage disequilibrium SNPs we obtained a maximum NPL of 3.02 (P= 1.3 x 10-3) on chromosome 2q21.2. The same genomic position also yielded the highest multipoint heterogeneity LOD (HLOD) score under a common recessive model of disease susceptibility (HLOD = 3.11; P= 7.7 x 10-5) which was significant at the genome-wide level. In addition, 2 other chromosomal positions 6p22.1 (corresponding to the major histocompatibility locus) and 18q21.1 displayed HLOD scores >2.1 (P < 0.002). None of the regions coincided with areas of common chromosomal abnormalities frequently observed in CLL. These findings provide direct evidence for Mendelian predisposition to CLL and evidence for the location of disease loci.

1183/W

Genome-wide linkage analysis finds significant imprinting effects on obesity in HyperGEN. *C. Gu[1], J. Zhou[1], K. North[2], R.H. Myers[3], Y.J. Sung[1], S.C. Hunt[4], D.K. Arnett[5], D.C. Rao[1].* 1) Washington University in St Louis, St Louis, MO; 2) University of North Carolina, Chapel Hill, NC; 3) Boston University, Boston, MA; 4) The University of Utah, Salt Lake City, UT; 5) University of Alabama, Birmingham, AL.

Obesity is a complex metabolic disorder affecting millions; both genetic and non-genetic risk factors may contribute to its development and manifestation. Recent reports suggest that imprinting effects might play an important role in its development. We performed a genome-wide linkage analysis incorporating imprinting effects at 380 microsatellite markers genotyped by Marshfield Genotyping Service in a sample of white and black families from the HyperGEN study sponsored by NHLBI. There were 2,105 white subjects (1,003 men and 1,102 women in 884 nuclear families) and 2,300 blacks (843 men and 1,457 women in 951 nuclear families). NHLBI unisex clinical guidelines for obesity (http://www.nhlbi.nih.gov/guidelines/obesity/sum_clin.htm) were used to classify subjects as "obese" or "non-obese." BMI are available for all whites (29.0±4.8 in men and 29.1±6.8 in women) and almost all blacks (29.5±6.3 in men and 33.4±8.1 in women). Using a computer program implementing parametric imprinting models (GENEHUNTER-IMPRINTING, Strauch et al. AJHG, 66(6):1945-57, 2000), we performed analysis separately in both race groups. Using the maximum LOD (MOD) score approach, we found significant linkage to obesity on Chromosome 2 (MOD = 4.20 at 30.3 cM near GGAA20G10) and Chromosome 10 (MOD = 3.44 at 22.1 cM near AFM063XF4) in blacks, of which the linkage on Chromosome 10 was heavily influenced by genetic imprinting (imprinting index = 0.48). In whites, we also found significant linkage to obesity on Chromosome 6 (MOD = 3.35 at 166.9 cM near AFM242ZG5) and Chromosome 19 (MOD = 3.54 at 26.8 cM near GATA21G05), both of which were influenced by imprinting (imprinting index = 0.49 and -0.82, respectively). There were other suggestive signals (MOD ≥ 2.0) in both samples. We are currently performing additional analyses using different definitions of "obese" or quantitative phenotypes, such as waist to hip ratio or percent of body fat.

1184/W

A gene locus for nephrotic syndrome on chromosome 13q21. *C.N. Vlangos[1], S. Heeringa[1], B. Hinkes[1], R. Gbadegesin[1], J. Liu[1], G. Nurnberg[3], P. Nurnberg[3], F. Hildebrandt[1,2].* 1) Dept. of Pediatrics, University of Michigan, Ann Arbor, MI; 2) Dept. of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Gene Mapping Centre, Max Delbrück-Centrum, Berlin, Germany.

Nephrotic syndrome (NS) is defined by proteinuria, edema, hypoalbuminemia, and hyperlipidemia. Genetic causes of NS include recessive mutations in the nephrin (*NPHS1*), podocin (*NPHS2*), *PLCE1* (*NPHS3*), and *LAMB2* genes. Dominant mutations causing NS have been reported in the *CD2AP*, *ACTN4*, *TRPC6*, and *WT1* genes. In order to identify novel genes causing autosomal recessive NS we performed a total genome search for linkage to a novel NS locus using 50k SNP Affymetrix DNA microarrays. DNA from 8 consanguineous multiplex families and 55 consanguineous simplex families was analyzed. In 5 families we detected the presence of only one or two distinct peaks of homozygosity per family, which did not overlap between families. This excludes a shared locus between these families and, therefore, these families were excluded "*a posteriori*" from linkage calculations. When the 3 consanguineous multiplex families with 3 (F325) and 2 (F1085, F341) affected children were calculated together for linkage analysis, a significant maximum parametric LOD score (LODmax=3.3) was obtained (NPLmax=10.7), thereby identifying a new gene locus (SRN4) for NS on chromosome 13q21.2-q21.32. Haplotype mapping was performed by aligning SNP haplotypes from the 3 multiplex and 55 consanguineous simplex cases at the SRN4 locus. This alignment identified 4 simplex cases with overlapping homozygosity at the SRN4 locus. The SRN4 locus is delimited by family F325 within a 7.7 Mb critical genetic interval by heterozygous markers rs1486946 and rs10492594. This 7.7 Mb critical interval contains only 5 candidate genes: *DIAPH3* (diaphanous homolog 3), *TDRD3* (tudor domain-containing protein 3), *PCDH20* (protocadherin-20), AK127969 (unannotated), and *PCDH9* (protocadherin-9). Direct DNA sequencing of the five candidate genes revealed no disease causing mutations. Further analysis of putative genes in the region is currently being performed to identify a novel genetic cause of nephrotic syndrome at the SRN4 locus.

1185/W

Susceptibility loci for blood pressure detected in adults from the Samoan islands. *K. Aberg[1], G. Sun[2], S.R. Indugula[2], D. Smelser[2], Q. Zhang[2], R. Deka[2], D.E. Weeks[1,3], S.T. McGarvey[4].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 3) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 4) International Health Institute, Brown University, Providence, RI.

Using variance component linkage analysis, we performed genome-wide scans for systolic (SBP) and diastolic (DBP) blood pressure in sample sets from Samoa (S) and American Samoa (AS), respectively. The two sample sets have a common population history but, in contrast to S, the environment in AS has been extensively modernized during the last decades. In an attempt to explore gene-environmental interaction we investigate a combined dataset including both S and AS. In total, we studied 71 families including 1156 adults (>18 years of age) phenotyped for SBP and DBP and genotyped for 368 microsatellite markers. We used SOLAR/Loki to calculate multipoint LOD scores. To explore gene-environmental interaction we created a material life style index (MLSI) to measure individual modernization. The MLSI, education, alcohol and cigarette consumption, physical activity, age, sex, agesex, age^2, age^2sex and body mass index (BMI) were included as covariates. When adjusting the traits in the 3 datasets for significant covariates, the heritability estimates for SBP and DBP ranged from 0.16-0.31 and 0.10-0.22, respectively. Interestingly, neither MLSI, education nor physical activity were associated with SBP or DBP. Suggestive LOD scores were detected for SBP (LOD≥2.18) on 18q22 in the combined dataset and in AS, and for DBP (LOD=2.05) on 2p25 in AS.

About 16% of our sample was treated for hypertension and was excluded from our quantitative study. We are currently performing qualitative analysis of individuals with hypertension (or treated for hypertension) vs. non-hypertensive individuals. In addition, we are carrying out genome-wide bivariate linkage analysis for SBP and DBP.

1186/W

Family-based association analyses of positional candidate genes from a region showing significant linkage to atopic rhinitis on chromosome 3q. *C. Brasch-Andersen[1,2], A. Haagerup[3], K. Brøsen[1], J. Vestbo[4], T.A. Kruse[2].* 1) Clinical Pharmacology, Institute of Public Health, University of Southern Denmark, Odense, Denmark; 2) Dept. of Biochemistry, Pharmacology and Genetics, Odense University Hospital, Denmark; 3) Dept. of Paediatrics, Sygehus Viborg, Denmark; 4) Dept. of Cardiology and Respiratory Medicine, Hvidovre Hospital, Denmark.

Allergic rhinitis is a common disease of complex inheritance and is characterized by mucosal inflammation caused by allergen exposure. It often affects patients with other coexisting manifestations of allergy, suggesting overlapping disease etiology. We have previously shown significant evidence for linkage to chromosome 3q13.31 for rhinitis, atopy and atopic rhinitis after finemapping the region suggested by a genome-wide scan using Danish atopy sib pair families. Highest identity by descent sharing at the locus was seen for the phenotype atopic rhinitis. The region of 'maximum LOD score - 1.5' spans approximately 3 centiMorgan and harbors only two genes; Growth Associated Protein 43 (GAP43) and Limbic System- Associated Membrane Protein (LSAMP). Both genes seem to be involved in neural growth. One sib from all pairs with atopic rhinitis used in the linkage analysis (n=76) were screened for polymorphisms using denaturing high-performance liquid chromatography (DHPLC). All exons and exon-intron boundaries of GAP43 and LSAMP were screened. DHPLC analyses revealed a polymorphism in the untranslated region of exon 3 in GAP43 and a single nucleotide polymorphism (rs14360) was identified by sequencing. Furthermore, a GT-repeat polymorphism was identified in intron 1 of LSAMP by DHPLC and sequencing. Both polymorphisms were genotyped in the full sample containing 1021 individuals in 159 Danish families. Screening of the genes did not reveal any polymorphisms in the coding regions of GAP43 and LSAMP. Association between genotypes and rhinitis, atopy and atopic rhinitis were analysed using the Family-Based Association Test (FBAT) program but failed to show association (p>0.05). As linkage analysis strongly suggests a susceptibility gene for rhinitis at 3q13.31 further analysis of the region is needed to identify susceptibility variants.

1187/W

A Fine-Mapping analysis of the MHC region in IgA Deficiency. *R.C. Ferreira[1,4], W. Ortmann[1,4], P.K. Gregersen[2], L. Hammarström[3], T.W. Behrens[1,4].* 1) University of Minnesota, Minneapolis, MN, USA; 2) Feinstein Institute for Medical Research, Manhasset, NY, USA; 3) Karolinska University Hospital Huddinge, Stockholm, Sweden; 4) Current address: Genentech, Inc., South San Francisco, CA, USA.

IgA Deficiency (IgAD) is the most common primary immune deficiency in humans, with an estimated prevalence of approximately 1/500 in Caucasians. Although the genetic basis of IgAD is well established, the characterization of the susceptibility loci is still an ongoing process. Previous studies have shown association of major histocompatibility complex (MHC) haplotypes with IgAD, and we have recently shown that *MSH5*, located in the central MHC region, is a strong candidate gene for IgAD.

The MHC, located in human chromosome 6, contains a high density of genes expressed in the immune system, and is characterized by a high level of linkage disequilibrium (LD). In order to identify all the genetic variation contributing to the MHC signal in IgAD, the International MHC Autoimmunity Genetics Network (IMAGEN) initiated a fine mapping effort of the MHC in IgAD, using a 1,230 SNP panel spanning the entire MHC region. These SNPs were chosen to tag all common European haplotypes across the MHC.

Here we report the results from the fine mapping effort of the MHC in a cohort of 264 Swedish IgAD patients and 657 matched controls. We found a very high level of association across the MHC, with the main peak of association being located at SNP rs3134942, located between the MHC class II and class III regions ($P = 2.1 \times 10^{-16}$). In total, 116 SNPs showed P values $< 10^{-8}$. Haplotype analysis showed that the strongest signals derived from an extended HLA-A1-B8-DR3 haplotype. Conditional regression analysis was then used to characterize DR3-independent SNP variation contributing susceptibility to IgAD. This analysis identified SNPs that tag for the ancestral DR2 haplotype as strongly protective for IgAD. These data identify common risk and protective MHC haplotypes for IgAD in Swedish cases, and are consistent with the hypothesis that several genes in the MHC, including *MSH5*, contribute to IgAD risk.

1188/W

Identification of a novel chromosome 14 systemic lupus erythematosus (SLE) susceptibility gene. *A. Hellquist[1], C.M. Lindgren[1], S. Koskenmies[2], P. Onkamo[2], E. Widén[2], H. Julkunen[3], U. Saarialho-Kere[2,4], A. Wong[5], D.S. Cunninghame-Graham[5], T.J. Yyse[5], K. Kivinen[1], T. Skoog[1], L. Berglind[6], V. Mäkelä[6], G. Assadi[1], M. Zucchelli[1], M. D'Amato[1], J. Kere[1,6].* 1) Karolinska Institute, Stockholm, Sweden; 2) University of Helsinki, Helsinki, Finland; 3) Peijas Hospital and Helsinki University Hospital, Helsinki, Finland; 4) Karolinska Institutet at Stockholm Söder Hospital, Stockholm, Sweden; 5) Imperial College, Hammersmith Hospital, London, UK; 6) Karolinska University Hospital, Huddinge, Sweden.

SLE is a chronic autoimmune inflammatory disease characterized by autoantibody production and tissue injury. SLE exhibits a complex pattern of inheritance involving multiple genes as well as an environmental component. Despite the identification of several loci and candidate genes, most of the genetic background remains unexplained as yet. We have previously identified suggestive linkage on chromosomes 5p, 6q25-q27, 14q21-q23 and HLA on 6p (Koskenmies et al JMG 2004). Further, an excess sharing of a haplotype on 14q and excess transmission of a haplotype on 6q were shown after additional markers were added in the suggestive regions of linkage (Koskenmies et al EJHG 2004). Using a dense microsatellite and single nucleotide polymorphism (SNP) mapping approach we have identified a novel and previously poorly characterized gene within the 14q21-q23 locus associated to SLE in two independent material family materials of Finnish and British origin. The UK material in particular showed strong association to one marker located in the 5' of the gene (T=131, U=71, p= 0.000024, OR=1.8, CL@95% 1.6-2.1). This marker, although not significantly associated, showed the same over transmission trend in the Finnish sample set (T=15, U=8, p=0.14, OR=1.9, CL@95% 0.8-4.4). The gene, C14SLEC1, is expressed at low levels in many tissues, including monocytes, and may be involved in adhesion based on similarity to other adhesion molecules. By treating THP-1 monocytes with inflammatory cytokines we found upregulation of C14SLEC1 by tumor necrosis factor alpha (1.9 times, p = 0.008) and gamma-interferon (2.2 times, p = 0.008), suggesting that C14SLEC1 may be regulated through a proinflammatory pathway.

1189/W

Using disease subtypes to reduce phenotypic heterogeneity: the example of TGFBR3 and pulmonary emphysema. *C.P. Hersh, F.L. Jacobson, R. Gill, A.A. Litonjua, J.J. Reilly, E.K. Silverman.* Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Background: Chronic obstructive pulmonary disease (COPD) is a heterogeneous syndrome, including emphysema, chronic bronchitis, and small airway disease. Using specific disease subtypes may avoid problems with phenotypic heterogeneity that have complicated many previous COPD genetics studies. **Methods:** Genome-wide linkage analysis was performed in 44 extended families of probands with severe, early-onset COPD and in a stratified analysis of 34 of these pedigrees with emphysema-predominant COPD probands. Emphysema was assessed by chest computed tomography scans. Emphysema candidate genes were selected in regions that showed stronger evidence for linkage in the stratified analysis. Association analysis of positional candidate genes was performed in 949 individuals from 127 extended pedigrees and in a case-control study, comparing 389 cases with severe emphysema to 472 control smokers without lung disease. **Results:** Genome-wide linkage analysis identified a region on chromosome 1p with suggestive evidence of linkage for lung function in families of emphysema-predominant probands (LOD score 2.89 in emphysema-predominant families vs. 1.85 in all families). Association testing of five positional candidate genes revealed replicated association with the transforming growth factor beta receptor-3 (TGFBR3) Ser15Phe SNP (p=0.01 in the family-based study, p=0.02 in the case-control study). **Conclusions:** Stratified linkage analysis followed by association testing has identified TGFBR3 as a potential susceptibility gene for the emphysema subtype of COPD. Published human microarray and murine linkage studies have also demonstrated the importance of TGFBR3 in emphysema and lung function, and our group and others have previously found association of COPD and related traits with TGFB1, a ligand for TGFBR3.

Support: NIH grants HL080242, HL71393, HL075478, U01HL065899, P01HL083069; a grant from the Alpha-1 Foundation; an American Lung Association Career Investigator Award; and a Clinical Innovator Award from the Flight Attendant Medical Research Institute.

1190/W

Genome-wide screen for sex-specific fertility genes in humans. *G. Kosova[1], T. Hyslop[2], C. Ober[3].* 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Biostatistics Section, Thomas Jefferson University, Philadelphia, PA; 3) Dept. of Human Genetics, University of Chicago, Chicago, IL.

Infertility is a major health problem, affecting 15% of couples world-wide. Previous genetic studies of infertility in humans have been largely limited to candidate gene approaches. To more broadly survey the genetics of human fertility, we have conducted genome-wide linkage and association mapping using 1500 autosomal markers (STRPs and SNPs) in the Hutterites, a founder population of European descent that proscribes contraception and has large family sizes. We considered two measures of fertility. The first (reproductive capacity) is the number of births, corrected for the years from marriage until the last birth, wife's birth year and wife's age at first birth; the second (reproductive rate) is the number of births per year of marriage, corrected for the same covariates as above. Reproductive capacity was heritable in women (H^2=0.52, s.e. 0.24) whereas reproductive rate was heritable in men (H^2=0.85; s.e. 0.23). We mapped each trait in women and men, respectively, using homozygosity-by-descent linkage and association methods (Abney et al. AJHG 2002;70:920). Genome-wide significance is assessed by a permutation test. Three linkages reached genome-wide significance in men: chromosome 4q encompassing *PCDH10*, chromosome 6p at the location of *HLA-G*, and chromosome 9p at the type I interferon cluster. Homozygosity at each locus was associated with reduced birth rate in men. In women, four associations reached genome-wide significance, including an intronic SNP in *CFI*, two intronic SNPs in *ITGAM*, and an STRP in an intergenic region on chromosome 16q. Homozygosity at each locus was associated with reduced family size in women. Further studies showed that homozygosity for the associated *ITGAM* allele is also associated with increased pregnancy loss rates (P=0.036) in Hutterite women participating in a prospective study. This is the first genome-wide study of fertility in humans, and our results suggest that there are distinct and diverse processes affecting this trait in males and females. Supported by HD21244.

1191/W

Hepatic lipase variants have sex-specific associations with metabolic syndrome and its risk factors in the NHLBI Family Heart Study. *M.F. FEITOSA[1], R.H. Myers[2], I.B. Borecki[1].* 1) Washington Univ, St. Louis, MO; 2) Boston Univ, Boston, MA.

Metabolic syndrome (MetS) is a clustering of abdominal obesity, high triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), high blood pressure (BP) and elevated fasting glucose (GLU) levels, and is a major risk factor for diabetes and cardiovascular disease. The gene for hepatic lipase (LIPC) resides on15q21 and the enzyme plays a major role in lipoprotein metabolism. However whether LIPC variants influence risk of MetS and its related phenotypes has not been explored. We selected 19 tag SNPs across 593 kb of LIPC that were typed in 433 families (2,192 subjects) to evaluate associations to MetS (defined by National Cholesterol Education Program), central obesity (waist circumference, (WC) and waist-to-hip-ratio (WHR)), glucose metabolism (GLU, insulin (INS), and HOMA), lipid profile (TG, HDL-C, LDL-C, total cholesterol, APOA-1, and APOB), and BP (SBP and DBP). Family-based methods were used for both qualitative (e.g., MetS and dichotomizations of the quantitative variables at clinically-relevant thresholds) and the actual quantitative phenotypes. Significant associations were observed mainly in the large first intron for most of these phenotypes, many of which exhibited sex-specific effects. SNP associations were found in women (vs. men, MetS: p=0.0013 vs. p=0.3314, TG: p=0.0040 vs. p=0.3613, HDL-C: p=0.0022 vs. p= 0.7109, and WC: p=0.0190 vs. p=0.8295), as well as in men (vs. women, MetS: p=0.0279 vs. p=0.365, GLU: p=0.0163 vs. p=0.573, SBP: p=0.0235 vs. p=0.977). Similarly, sex-specific association patterns were found between several SNPs and quantitative traits (e.g. for women vs. men, HDL-C: p=0.00018 vs. p=0.1964, WHR: p=0.00674 vs. p=0.6660, GLU: p=0.00714 vs. p=0.71003, INS: p=0.00304 vs. p=0.1197, HOMA: p=0.00195 vs. p=0.1252, TG: p=0.0022 vs. p=0.5514). Associated haplotypes in different LD blocks were identified, which exhibited sex-specific effects suggesting that not only might there be more than one region in LIPC with variants influencing MetS traits but that these variants have differing effects depending upon the sex of the individual.

1192/W

IL10 SNPs Modify the Effect of Dust Mite Exposure on Allergy and Asthma Exacerbations. *G.M. Hunninghake[1,4], M. Soto-Quiros[2], J. Lasky-Su[1,3], L. Avila[2], N. Ly[1,4], J. Sylvia[1], B. Klanderman[1,4], B. Raby[1,4], D. Gold[1,4], S. Weiss[1,4], J.C. Celedon[1,4].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Hospital Nacional de Ninos, San Jose, Costa Rica; 3) Harvard School of Public Health, Boston, MA; 4) Harvard Medical School, Boston, MA.

Background: SNPs in IL10 (a candidate gene for asthma) may modify the effect of dust mite allergen on allergy and asthma exacerbations. Methods: We genotyped 11 SNPs in IL10 in 428 Costa Rican children with asthma and their parents; 6 of these SNPs were also genotyped in 483 families of 502 white children with asthma in CAMP. The family-based association test (FBAT) approach was used to test for interaction between IL10 SNPs and dust mite allergen on serum IgE to dust mite in Costa Rica (not measured in CAMP). We then assessed whether IL10 SNPs modify the effect of dust mite exposure on asthma exacerbations in both studies. Results: Parental genotypes were in HWE for all SNPs in both studies. Three SNPs in IL10 (rs1800896, rs3024492, and rs3024496) significantly modified the relation between dust mite exposure and IgE to dust mite in Costa Rica (P for interaction <0.0001 for SNP rs1800896). For each of these SNPs, homozygosity for the minor allele was associated with increased levels of IgE to dust mite with increased dust mite exposure. These results remained robust to "bootstrapping" using population-based regression methods. Using logistic and Poisson regression, we found that homozygosity for the minor allele of each of the three SNPs above was associated with increased risk for occurrence (~3-44 fold increase) and frequency (~25-36% increase) of asthma exacerbations among children exposed to >10 ug/g of dust mite allergen in Costa Rica. Similar results were obtained for two of these SNPs (rs1800896 and rs3024496)among children in CAMP. Conclusions: IL10 SNPs modify the effect of dust mite allergen levels on dust mite sensitization and asthma exacerbations. Our findings may help reconcile conflicting findings on dust mite exposure and asthma and allergies.

This work was supported by grants HL66289, U01 HL065899, P01 HL083069 and F32HL083634 from the National Institutes of Health.

1193/T

Haploview: A computational tool for analysis and visualization of whole genome association data. *JB. Maller[1,2], D. Bender[1,2], J. Barrett[3], S. Purcell[1,2], MJ. Daly[1,2].* 1) Center for Human Genetics Research, Massachusetts General Hospital, Boston MA; 2) Broad Institute of Harvard and MIT, Cambridge MA; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford UK.

Recent advances in technology have resulted in the generation of orders of magnitude more SNP genotype data. This increase has created a need for computational tools which can efficiently analyze and effectively visualize these large data sets. The linkage disequilibrium and haplotype analysis package Haploview has been extended to be a part of a complete set of tools for such whole genome association analysis. Here we describe the new functionality and integration with other tools which enable this type of analysis.

1194/T

Candidate regions for susceptibility genes linked to synaesthesia: results of a whole-genome scan. *J.E. Asher[1, 2], J.A. Lamb[3], S. Baron-Cohen[2], D. Brocklebank[1], E. Maestrini[4], L. Addis[1], M. Sen[1], P. Bolton[5], S. Rahman[6], H. Waine[2], A.P. Monaco[1].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Department of Psychiatry, University of Cambridge, Cambridge, UK; 3) Centre for Integrated Genomic Medical Research, University of Manchester, Manchester, UK; 4) Dipartimento di Biologia, Universita di Bologna, Bologna, Italy; 5) Department of Child and Adolescent Psychiatry, Institute of Psychiatry, London, UK; 6) Institute of Neurology, University College London, London, UK.

Synaesthesia is a neurodevelopmental condition affecting 0.05 - 1% of the population in which a stimulus in one sensory modality triggers an automatic, consistent response in another modality (e.g. sound triggers the perception of colour) or a different facet of the same modality (e.g. black text triggers the perception of colour). Previous familiality studies indicate a strong genetic component and several pedigree analyses support a single-gene X-linked dominant mode of inheritance. Here we report the results of the first genome scan for susceptibility genes linked to synaesthesia. A whole-genome linkage scan using 410 microsatellite markers (mean inter-marker distance = 9.05cM) was performed in 43 families (n = 196). NPL analysis using Merlin 1.1a detected 14 potential candidate regions on 11 chromosomes with LOD scores > 1. Fine-mapping with additional microsatellites (mean inter-marker distance = 5.00cM) provided additional evidence for 12 candidate regions on 10 chromosomes, with 4 regions showing LOD scores > 2 and 2 regions showing LOD scores > 2.3 (maximum LOD = 2.37, p = 0.0005). We are performing a family-based analysis to investigate the presence of locus heterogeneity. Preliminary results from an HLOD analysis using Merlin indicate strong evidence for linkage to chromosome 2. No support was found for linkage to the X-chromosome; in addition, we have identified the first confirmed cases of male-to-male transmission of synaesthesia. Together with existing data, these results indicate that synaesthesia is most likely a complex disorder with substantial locus heterogeneity.

1195/T

Genome wide linkage of a large serbian family with GEFS+. *F. Annesi[1], G. Provenzano[1], S. Carrideo[1], A.J. Ristic[3], S. Jankovic[3], G. Maksimovic[3], B. Gnjatovic[3], I. Petrovic[3], N. Vojvodic[3], D. Sokic[3], A. Gambardella[2], G. Annesi[1].* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Institute of Neurology, Clinical Centre of Serbia, Belgrade.

Generalized epilepsy with febrile seizures plus (GEFS+) is an autosomal dominant epilepsy syndrome with incomplete penetrance characterized by heterogeneous phenotypes within the same family. The most common phenotypes include febrile seizures (FS) and febrile seizures plus, in which FSs persist beyond 6 years of age or associated with afebrile, mostly generalized or more rarely partial, seizures. Four genes associated with GEFS+ have been identified: SCN1A, SCN2A, SCN1B and GABRG2. In addition some GEFS+ loci have been reported but the causative genes have not yet been identified. We conducted a genome-wide linkage study in a large multigenerational Serbian family showing a clear dominant Mendelian inheritance pattern of GEFS+. The GEFS+ Serbian family shows 20 affected individuals over four generations. Patients with GEFS+ express a variable phenotype combining febrile seizures, afebrile generalized seizures and partial seizures. Genomic DNA was obtained on 22 living members (11 affected, 11 unaffected). Initially we excluded SCN1A, SCN2A, SCN1B, GABRG2 genes and known loci with linkage analysis. Subsequently, a genome wide scan was conducted genotyping 382 microsatellite markers. Two point parametric linkage analysis was performed by MLINK (LINKAGE 5.1), LOD score values were calculated by assuming a disease-allele frequency of 0.001, equal allele frequencies and autosomal dominant inheritance with 0.90 penetrance. Some regions with LOD scores above 1 were found in parametric two-point linkage analysis. Loci suggestive of linkage were genotyped with additional markers and haplotype analysis of implicated regions is in progress. Our linkage data clearly exclude all known loci and genes for GEFS+ demonstrating genetic heterogeneity of this syndrome. Genome scan revealed linkage at some chromosomal loci and analysis of candidate regions is underway.

1196/T

Fine mapping of the Blmpf2 lung fibrosis locus in bleomycin-treated congenic mice. *F.D Depault, C.H Haston.* Human Genetics, McGill University, Montreal, Quebec, Canada.

Bleomycin is a drug used to treat cancers, which produces pulmonary fibrosis (p-f) in up to 10% of patients. From clinical studies it is suggested that the development of p-f has a genetic component. Inbred strains of mice differ in their propensity to develop p-f following bleomycin treatment: the B6 strain develop fibrosis while those of the C3H (C3) strain do not develop any disease. This phenotype variability serves as a model to address our hypothesis that genetic factors influence the development of p-f. We have previously used a QTL approach and identified two genomic regions that modify the development of p-f in B6 x C3 bleomycin-treated mice. The present study focuses on the chr.11 Blmpf2(B-2)locus, which produces a drastic diminution of p-f when carrying the C3 alleles at that position. However, the QTL locus remains large (25.9 Mb) and requires further mapping. Therefore, we created a congenic lineage, which have a B6 background except inside the B-2 locus that contains the C3 allele. By intercrossing the recombinant animals, we created 13 sublines carrying different length of the C3 allele. By identifying the position of C3 alleles that result in p-f reduction in congenic bleomycin-treated mice, we have succeeded in defining a resistant locus which contains 50 genes. It is postulated that the candidate gene expression will be divergent between the B6 and C3 strains in the lung. Our previously completed RNA microarray datasets inform us that one gene is differentially expressed and maps inside the reduced B-2 locus. To confirm the action of this gene in p-f development, we derived four additional sublines with recombination in the vicinity of the candidate gene. By the fine mapping of B6/C3 recombination breakpoints, we propose to narrow down this locus to a length that is amenable to molecular analysis. Ultimately, the candidate genes will be functionally studied to confirm whether they encode transcripts influencing lung fibrosis. Susceptibility genes discovered in this study may be useful for predicting a patient response to the chemotherapy side-effects and may serve as a clinical target.

1197/T

An autosomal dominant spontaneous and preterm delivery in a large multigenerations family. *R. Uppala[1], J.V. Solanki[2], U.C. Patel[2], R. Memon[1], A.K. Maiti[3], S.K. Nath[4], U. Radhakrishna[1].* 1) Green Cross Voluntary blood bank and Genetic Research Centre, Paldi, Ahmedabad-380014; 2) Department of Animal Genetics & Breeding, Veterinary College, Gujarat Agriculture University, Anand, India; 3) Sealy Center for Molecular Biology, University of Texas Medical Branch, Galveston, TX 77550, USA; 4) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, USA.

A full-term pregnancy is called when the babies are born between 38 to 42 weeks. Births that occur at less than 37 weeks of pregnancy are called premature or preterm. Preterm birth remains the leading cause of neonatal morbidity and mortality. The frequency of preterm birth (PTB) varies between populations, in the United States; ~12 percent of babies are born prematurely. The etiology of PTB is largely unknown but is believed to be complex, involving both multiple genetic and environmental determinants. It is associated with various risk factors including, intrauterine growth retardation, social, environmental, medical and genetic. There are several sporiadic cases and small families reported with PTB. We have studied one large multigenerations Indian pedigree, with an autosomal dominant mode of inheritance and a history of PTB. The family consists of 72 individuals including 11 affecteds. Family history revealed that there are fourteen preterm deliveries occurred in this family and all pregnancies were ended between 22 to 32 weeks. There were no consanguineous marriages observed in this pedigree. None of the PTB babies survived and all died immediately after birth. No other associated anomalies observed in this family. Cytogenetic analysis of three affected individuals did not show any abnormality. Markers on chromosome 19q13.4 region in the vicinity of candidate gene NALP7 was excluded by linkage and haplotype analyses. We are planning to perform a high-density genome-wide linkage analysis to identify the responsible preterm birth PTB susceptibility locus. Email: madam_fille@yahoo.com.

1198/T

Clinical diagnosis variability of progressive familial intrahepatic cholestasis (PFIC3). *M. Amyere[1], E. Wiame[2], M. Vikkula[1], E. Van Schaftingen[2], MC. Nassogne[3].* 1) Laboratory of Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 2) Laboratory of Physiological Chemistry, de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 3) Department of Pediatrics, Division of Nephrology, Université catholique de Louvain, Saint-Luc University Hospital, Brussels, Belgium.

PFIC3 is an autosomal recessive liver disorder presenting with early onset cholestasis that progresses to cirrhosis and liver failure before adulthood (De Vree et al., 1998). Several MDR3 mutations have been identified in children with PFIC3 and are associated with a low level of phospholipids in bile, leading to a high biliary cholesterol saturation index. This phenotype is characterized by elevated serum gamma-glutamyltransferase levels. Here, we describe a consanguineous family, originating from Morocco, with three children displaying liver disease with cholestasis, but normal gamma-glutamyltransferase level and normal MDR3 staining. We performed autozygosity mapping using Affymetrix SNP Chip 50K and identified a homozygous region of approximately 10 Mb at chromosome 7q21, in the three affected individuals. This region was confirmed by linkage analysis showing a maximum multipoint Z-score of 6.7. The MDR3 gene is located in this locus. Screening of MDR3 cDNA in the three affected individuals showed a homozygous mutation that changes a splice site near exon 2. Despite the normal level of gamma-glutamyl transferase activity in the serum and the normal MDR3 staining in liver sections, it may be that this mutated allele expresses a truncated, but non functional protein, which is nevertheless detectable by immunostaining. Therefore, molecular genetics testing should be performed in patients with classical PFIC3, even if MDR3 immunotest is normal to be sure of diagnosis.(miikka.vikkula@uclouvain.be).

1199/T

REDUCTION OF GENOMIC COMPLEXITY FOR RE-SEQUENCING BY REGION-SPECIFIC EXTRACTION. *J. Dapprich[1], D. Ferriola[1], M. Kunkel[1], A. Gabriel[2], M. Dunham[3].* 1) Generation Biotech, Lawrenceville, NJ; 2) Rutgers University, Piscataway, NJ; 3) Princeton University, Princeton, NJ.

Structural variation can have significant influence on the accuracy of SNP typing, sequencing and haplotype analysis. Interpretation of typing results can be affected by the underlying genomic context. Molecular analysis to determine the positions of non-fixed or copy number variable elements throughout the genome can be difficult or impossible by sequence analysis alone. Further, the assembly of short, random shot-gun sequencing reads within the context of genomic structural variation has become an acute problem for next-generation sequencing due to the presence of repetitive regions in complex genomes. Region-specific extraction (RSE) is an automated method that reduces complexity of genomic DNA by physically isolating targeted genomic elements, including flanking sequences. A coupled enzymatic hybridization and tagging process achieves single-base specificity and high capture efficiency of genomic regions. RSE is able to resolve sequence ambiguities caused by missing cis-trans linkage, copy number variation or mobile genetic elements. Here we demonstrate the selective separation and analysis of a highly homologous, duplicated gene region called MICA/MICB, located in the Major Histocompatibility Complex. This region is implicated in numerous autoimmune and other diseases such as diabetes. RSE probes were used to selectively extract the duplication containing the MICA gene from the duplication containing the MICB gene using sequence variation between the two copies. A similar approach was used to resolve homologous gene cassettes in the killer immunoglobulin-like receptor region on chromosome 19 and map the location and copy number of mobile genomic elements in yeast on DNA microarrays. RSE is directly compatible with essentially any typing method and can be carried out in a 96-well format on commercially available systems. This provides a sample preparation tool that can deconvolute complex genomic regions in a high-throughput mode by combining the flexibility of current whole genome analysis methods with the more informative content of site-directed screening methods.

1200/T

Novel combinatorial optimisation methods to partition large pedigrees for genetic analysis. *D. Brocklebank, J. Gayán, L.R. Cardon.* Dept. Statistical Genetics, WTCHG, University of Oxford, UK.

Large multigenerational pedigrees are powerful resources for the identification of genetic loci influencing traits. However, there are computational limitations to the analysis of these large families, despite the substantial advances which have been incorporated in pedigree analysis software packages. Methods for genetic analysis impose varying constraints on the complexity of the inheritance information they can analyze. The identification of computationally feasible pedigree structures for genetic analysis has emerged as a crucial factor for detecting loci influencing disease traits. Our aim here is to maximally exploit the information contained in large pedigree resources while allowing for tractable statistical analyses. We have developed two new combinatorial optimisation methods, based on simulated annealing and genetic algorithms. The algorithms search over the space of possible sub-pedigrees that conform to a given constraint imposed by a statistical test. An important feature of the methods is that they optimise the pedigree structures for information retained for analysis with direct control over the complexity of the simplified output pedigree, so that, for example, one can extract the largest sub-pedigrees that still permit practical analyses in Merlin, Allegro or SimWalk2. We apply the methods to real extended and inbred human and animal pedigrees, and show that the complexity of the pedigree structures identified by the methods strongly influences the power for linkage analysis. Maximal exploitation of the information contained in large pedigree resources, and their success, is thus expected to rely substantially on analyzing the most highly complex pedigrees that are tractable for a given analysis. Our methods aim to provide a systematic approach to extract maximum information for analysis and to advance the effective use of complex pedigree structures.

1201/T

A new statistical approach for mapping modifier genes taken advantage of family structure. *R. Secolin, C.S. Rocha, I. Lopes-Cendes.* Department of Medical Genetics, State University of Campinas, Campinas, São Paulo, Brazil.

The objective of this study was to develop and validate a new statistical strategy for mapping modifier genes using the 500,000 SNP maps (Genome-Wide Human SNP Array 5.0 - Affymetrix). Our strategy takes advantage of family structure and uses the probabilities of IBD sharing alleles in affected concordant relative pairs and the IBD non-sharing alleles in discordant sib pairs. The algorithm was implemented in R environment. In order to test for the power to detect genes of minor effect we use a set of 23 unrelated pedigrees segregating a type of epilepsy, familial mesial temporal lobe epilepsy (FMTLE). Complex segregation analysis and linkage studies have showed that FMTLE is likely to be caused by a major gene segregating in an autosomal dominant pattern; however, the presence of minor effect genes, modifying the main phenotype, was also detected. In fact, clinical variability of the disease among individuals in the same family is observed. Data from our large cohort of FMTLE individuals have demonstrated that 70% of them present **benign** epilepsy (with good seizures control), and 30% have **refractory** epilepsy (resistance to medical treatment). We simulate the scenario in which we genotyped 99 individuals from the 23 FMTLE pedigrees, generating a total of 221 concordant relative pairs and 22 discordant sib pairs. Simulated linkage data for 507,220 SNPs were calculated by LODPAL software from S.A.G.E.©. LODPAL result values were converted to *p* values and corrected for multiple tests by the False Discovery Rate (FDR). None of the 507,220 simulated SNPs resulted in *p* values less then $1x10^{-6}$. However, a test SNP simulated as linked to the **benign** epilepsy phenotype resulted in a *p* value = $1x10^{-14}$. These results showed that this new strategy of non-parametric linkage analysis have enough statistical power for mapping FMTLE modifier genes in our sample of FMTLE pedigrees. In addition, we expect truly positive results between $1x10^{-6} < p \text{ value} < 1x10^{-14}$. The SNPs genotyping step is already in progress in our laboratory.

Supported by: FAPESP.

1202/T

Data mining and comparison of measures of informativeness for ancestry in admixture mapping. *T.B. Mersha[1], H.W. Wiener[2], H.K. Tiwari[1], D.T. Redden[1,3], D.B. Allison[1, 3,4], R.C.P. Go[2].* 1) Section on Statistical Genetics, Department of Biostatistics, UAB, Birmingham, AL; 2) Department of Epidemiology and International Health, UAB, Birmingham, AL; 3) Clinical Nutrition Research Center, UAB, Birmingham, AL; 4) Department of Nutrition Sciences, UAB, Birmingham, AL.

Given the huge amount of single nucleotide polymorphism (SNP) data available from high-throughput sources such as HapMap, data mining is a reasonable approach to identify SNPs that are informative for genetic ancestry. The distribution and density of the SNPs across the genome of African and European populations were extensively investigated using three SNP databases of HapMap, Affymetrix and Illumina. We have exploited these resources by web mining the data available from each of these databases to prioritize potential candidate SNPs useful for admixture mapping. About 4 million SNPs were compared between Africans and Europeans using various measures of ancestry informativeness in use today viz. absolute allele frequency differences (δ), Fisher Information Content (FIC), Shannon Information Content (SIC), and Fixation Index (FST). Each method provides different sets of candidate ancestry informative markers (AIMs) within and across the databases. The selected SNPs represent valuable resources for admixture mapping studies. The overlap and non-overlap between selected AIMs by different measures of informativeness, and in the different platforms are discussed.

1203/T

A Genome-wide linkage analysis for urinary albumin excretion In a cohort of West Africans with type 2 diabetes. *G. Chen, A. Adeyemo, J. Zhou, Y. Chen, C. Rotimi.* Natl Human Genome Ctr, Howard Univ, Washington, DC.

Diabetic nephropathy currently accounts for approximately 50% of all new cases of chronic renal failure in USA. Among persons with type2 diabetes (T2D), increased genetic susceptibility, high blood pressure, obesity, smoking, and increased glomerular filtration are important factors that contribute to the development and progression of end stage renal disease (ESRD). However, the specific underlying genetic determinants are still unknown. We performed a genome wide linkage scan in a genome panel of 372 autosomal short-tandem repeat markers at an average spacing of 9cM in 691 T2D patients (321 sib pairs and 36 half-sib pairs). enrolled from West Africa. To identify QTLs for log Urinary albumin to creatinine ratio (ACR), multipoint variance components linkage analysis was conducted adjusting for gender, age, pulse pressure, duration of T2D and hypertension. The strongest linkage evidence to urinary log ACR phenotype was observed in 19p13.2 region with a LOD score 2.14 (nominal p-value = 0.0008 and empirical p-value = 0.0017) at 21cM, near marker D19S1034 and in 16q23 region with a LOD score 1.50 (nominal p-value = 0.0043 and empirical p-value = 0.0093) at 111cM, near marker D16S3091. In conclusion, linkage regions in 19p13.2 and 16q23 may harbor genes influencing variation in urinary ACR phenotype in West Africans with T2D.

1204/T

A genome wide scan for linkage in families with early onset Maturity onset diabetes of the young suggest a potential role for genes on chromosomes 2p 3q, 4q and 10q in glucose homeostasis. *A.L. Gloyn[1], B. Barrow[1], M. van de Bunt[1], D. Hammersley[1], M. Shepherd[2], K. Elliot[3], N.W. Rayner[3], S. Ellard[2], C.M. Lindgren[1,3].* 1) Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, Oxford, UK; 2) Peninsula Medical School, UK; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Maturity-onset diabetes of the young (MODY) is an autosomal dominantly inherited early onset subtype of non-insulin dependent diabetes. MODY causing mutations in 6 genes have been described but only explain around 87% of cases. We hypothesised that patients with a very young age of diagnosis (<15 yrs) may represent a genetically homogenous group and could facilitate identification of novel MODY genes. The aim of this study was to identify potential novel loci for further gene candidacy testing for MODY. We performed an autosomal genome wide linkage scan in 40 individuals (20 affected) from 5 families with >6 members with onset of diabetes ≤15 years. Samples were genotyped using the Illumina IV linkage panel. Extensive quality checks were performed including HapMap concordance (0.2%) within genotyping concordance (0.4%) and checking of Mendelian inconsistencies. 5,131 markers were used for linkage analysis using both non-parametric linkage analysis and a parametric autosomal dominant model taking linkage disequilibrium into account (r^2 >0.1). The highest NPL score 3.75 (pnominal <0.0001, HLOD 1.5), was observed on chromosome 10q. Three additional regions showed a NPL score >3.00 on chromosomes 2p (NPL 3.38 pnominal<0.0005, HLOD 0.83), 3q (NPL 3.10 pnominal=0.0010, HLOD 1.81) and 4q (NPL 3.00 pnominal<0.0015, HLOD 1.43). About 145 genes map to the 1 NPL drop off region of these 4 loci of interest, including strong biological candidates, potassium channels, transcription factors and cell signalling components. In conclusion, we have identified 4 novel loci which potentially harbour novel genes involved in the pathogenesis of MODY. Utilizing families with an early age of diagnosis in combination with advanced bioinformatics to prioritise candidacy, is a strategy to facilitate identification of novel disease loci by linkage mapping.

1205/T

Mapping quantitative traits in pedigrees. *J. Dupuis[1], J. Shi[2], D. Siegmund[3].* 1) Dept Biostatistics, Boston Univ SPH, Boston, MA; 2) Stanford University School of Medicine, Stanford, CA; 3) Department of Statistics, Stanford University, Stanford, CA.

In this project we are developing methods for mapping quantitative traits in moderately large pedigrees, with emphasis on the pedigree structures found in the Framingham Heart Study Cohorts. Our methods are based on the score statistic, which in contrast to the likelihood ratio statistic, can use nonparametric estimators of variability to achieve robustness of the false positive rate against departures from the hypothesized phenotypic model. We have developed simple, accurate approximations to the genome-wide false positive rate that takes account of the skewness in the statistic arising from statistical dependencies or distant relationships within pedigrees, and we develop a simple method for evaluating complex pedigrees in terms of an equivalent number of independent sib pairs. Because the score statistic is much easier to calculate than the likelihood ratio statistic, our basic mapping methods utilize relatively simple computer code in R, which performs statistical analysis on the output of any program that computes estimates of identity-by-descent (e.g., MERLIN). This simplicity also permits development and evaluation of methods to deal with multivariate and ordinal phenotypes, and with gene-gene and gene-environment interaction.

1206/T

Auriculo-Condylar Syndrome (ACS): Mapping of the first locus (ACS1)and genetic heterogeneity. *M.R. Passos-Bueno[1], C. Masotti[1], K. G. Oliveira[1], F. Poerner[2], A. Splendore[3], J. Souza[2], R. Freitas[2], R. Zechi-Ceide[4], M.L. Guion-Almeida[4].* 1) Dept Gen & Evol Biol, Univ De Sao Paulo, Sao Paulo SP, Brazil; 2) Integral Attending Center to the Cleft Lip and Palate Affected (CAIF), Curitiba, Paraná, Brazil; 3) Department of Genetics, Stanford University, California, USA; 4) Hospital de Reabilitação de Anomalias Craniofaciais, Universidade de São Paulo, Bauru, São Paulo, Brazil.

Auriculo-condylar syndrome (ACS), an autosomal dominant disorder of first and second pharyngeal arches, is characterized by malformed ears ('question mark ears'), prominent cheeks, microstomia, abnormal temporomandibular joint and mandibular condyle hypoplasia. Penetrance seems to be complete, but there is high inter and intra-familial phenotypic variation, with no evidence of genetic heterogeneity. We herein describe a new multigeneration family with 11 affected individuals (F1), in whom we confirm intrafamilial clinical variability. Facial asymmetry, a clinical feature not highlighted in other ACS reports, was highly prevalent among the patients in F1. The locus responsible for ACS is still unknown and its identification will certainly contribute to the understanding of human craniofacial development. No chromosomal rearrangements have been associated with ACS, thus mapping and positional cloning is the best approach to identify this disease gene. In order to map the ACS gene, we conducted linkage analysis in 2 large ACS families (F1 and F2, described by Guion-Almeida et al., Am J Med Genet 2002; 112:209). We have first excluded through segregation analysis the four known loci associated with disorders of the 1st and 2nd branchial archs. Next, we performed a wide genome search and we observed evidence of linkage in F2 through two-point and multipoint analysis (Lod max 3.2 at *=0). Interestingly, this locus was shown not to be linked to the phenotype segregating in F1. Therefore, our results led to the mapping of the first locus of auriculo-condylar syndrome (ACS1) and also showed genetic heterogeneity for this condition, suggesting that there are at least two loci responsible for this phenotype. CEPID/FAPESP, CNPqθ.

1207/T

A Review and Meta-analysis of Homozygosity Mapping Publications from 1987-2006. *T. Roscioli[1,2], C.G. Bell[1], M.F. Buckley[1], R. Lindeman[1].* 1) Department of Haematology and Genetics, Prince of Wales and Sydney Children's Hospitals, Sydney, NSW, Australia; 2) Sydney South West Integrated Genetics Service, Royal Prince Alfred Hospital.

Homozygosity mapping, the process by which pathogenic gene mutations are inferred based on the presence of excess homozygosity at linked marker loci, was suggested formally as a gene identification technique by Lander and Botstein in 1987. It has resulted in the identification of the genetic aetiology of many autosomal recessive disorders. There has however been no systematic review of the success of this technique. We report the results of a systematic review of 179 papers reporting 619 families published between 1987 and 2006 employing this technique. Based on this, we discuss the size of families reported, the most efficient mapping density, the properties of candidate regions and the success of gene identification in the published literature to guide future studies.

1208/T

Prediction of the linked regions and exclusion probabilitiesRequirement on family sizes in linkage analyses. *W.L. Yang[1], Z.Y. Wang[2], L.S. Wang[2], P. Huang[3], Y-L. Lau[1].* 1) Dept Paediatrics and Adol Med, LKS Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong; 2) Dept Computer Sci, City University of Hong Kong, Hong Kong; 3) Dept Biostat, Bioinfor, and Epidemiol, Medical University of SC, Charleston, SC.

Transition of genotyping methods from the use of sparse microsatellite markers to the use of high-density SNPs in linkage analyses allows accurate determination of crossover points and allele sharing status among individuals in families. This facilitates extraction of full inheritance information and making full use of family members available. For well-defined inheritance models, linkage analyses based on single families of intermediate size is feasible because of the development in genotyping technology, and necessary since large families or multiple families with homogeneous genetic causes of unknown mutations become very rare. In this study, we developed an algorithm that can predict the fractions of genomic regions that can be excluded by linkage studies based on family structure and size. Software was also developed to allow evaluation of family sizes in linkage analyses based on crossover simulations and determination of allele sharing status among family members. The program can determine, given a certain family structure and size, inheritance model and penetrance: 1). the probability that a chromosome can be excluded from consideration and 2). size distribution of the regions not being excluded when the chromosome does not contain the mutation; 3). size distribution of the true region with the causal mutation that can be determined in that family based on a number of simulations. It provides a reliable prediction tool to help determine the sufficiency of a family for a linkage analysis, or portion of the genome that can be excluded from consideration when the size of a family is too small for a complete linkage analysis. The latter is important for identifying the mutation responsible for a given family for diseases of known causes but of enormous genetic heterogeneity, and also for mutation identification of a candidate gene approach, in narrowing the list of candidates need to be screened.

1209/T

Focusing on linked pedigrees for localizing disease genes: the sumLINK statistic applied to general and aggressive prostate cancer linkage data from the ICPCG. *G.B. Christensen, N.J. Camp, ICPCG.* Dept Biomedical Informatics, Univ Utah, Salt Lake City, UT.

We propose a new genomewide linkage-based statistic, sumLINK, to identify disease-susceptibility loci. Our approach focuses only on 'linked' pedigrees (pedigree-specific LOD≥0.588; p≤0.05) to identify regions of extreme consistency across pedigrees. The sumLINK statistic is simply the sum of multipoint LOD scores for linked pedigrees at a given point in the genome. The significance of the sumLINK is assessed by a unique shuffling method that simulates the expected consistency of linked pedigrees. For each pedigree, we calculate multipoint LOD values at 1-cM intervals across the genome. All chromosomes are connected to create a loop, then the loop is broken at a random position to create a null genomewide scan for the pedigree. This procedure maintains each pedigree's potential for linkage signals across the genome, but randomizes consistency across pedigrees. For each null simulation, all pedigrees are realigned to their new starting points and the sumLINK is calculated for each cM position. The process is repeated 1000 times to determine the empirical distribution of the sumLINK for the pedigree resource. We applied the sumLINK approach to autosomal data for 1,232 pedigrees with prostate cancer (PCa) from the International Consortium for Prostate Cancer Genetics (ICPCG), and 190 ICPCG aggressive PCa pedigrees. Peaks were considered significant if exceeded fewer than 0.05 times per genome in the simulations, and suggestive if exceeded less than once per simulated genome. In the general PCa analysis, two genomewide significant regions (5p, 22q) and eleven suggestive regions were identified. In the aggressive PCa pedigrees, two significant (11p, 20q) and one suggestive (2p) loci were found. Some loci found here were also seen using standard HLOD analyses, but several have not previously been identified. An advantage of loci identified with the sumLINK approach is that they have good potential for subsequent gene localization using statistical recombinant mapping, as, by definition, there exist multiple linked pedigrees contributing to each peak.

1210/T

The Homozygosity Haplotype allows a genome-wide search for the autosomal segments shared among patients. *K. Hagiwara[1], H. Miyazawa[1], M. Kato[2], T. Awata[3,4], H. Iwasa[5,6], N. Koyama[1], T. Tanaka[1], X. Huqun[1], S. Kyo[2], Y. Okazaki[5,6].* 1) Respiratory Medicine, Saitama Medical University, Moroyama, Saitama, Japan; 2) Cardiovascular Surgery, Saitama Medical University, Moroyama, Saitama, Japan; 3) Endocrinology and Diabetics, Saitama Medical University, Moroyama, Saitama, Japan; 4) RI laboratory, Saitama Medical University, Moroyama, Saitama, Japan; 5) Functional Genomics and Systems Medicine, Saitama Medical University, Moroyama, Saitama, Japan; 6) Translational Research, Saitama Medical University, Moroyama, Saitama, Japan.

When identifying disease susceptibility genes for both single and multiple gene diseases, a promising strategy is to search patients' autosomes for shared chromosomal segments derived from a common ancestor. Such segments are characterized by the distinct identity of their haplotype. The methods and algorithms currently available have only a limited capability for determining a high-resolution haplotype genome wide. We herein introduce the homozygosity haplotype (HH), a haplotype described by the homozygous SNPs that are easily obtained from a high density SNP genotyping data. The HH represents haplotypes of both copies of homologous autosomes, allowing for direct comparisons of the autosomes among multiple patients, and enabling the identification of the shared segments. The HH successfully detected the shared segments from members of a large family with Marfan syndrome that is an autosomal dominant single gene disease. It also detected the shared segments from patients with model multigene diseases originating from common ancestors who lived 10-25 generations ago. HH is therefore considered to be useful for the identification of disease susceptibility genes in both single gene diseases and multigene diseases.

1211/T

Genome-wide association study identifies new HSCR loci. *M. Garcia-Barcelo[1], C. Tang[2], S. Cherny[2,3], P. Sham[2,3], P. Tam[1].* 1) Dept Surgery, Univ Hong Kong, Hong Kong; 2) Dept Psychiatry, Univ Hong Kong, Hong Kong, Hong Kong; 3) Genome Research Centre, Univ Hong Kong, Hong Kong.

Hirschsprung's disease (HSCR, aganglionic megacolon) is a developmental disorder characterised by the absence of the enteric ganglia along a variable length of the intestine. HSCR exhibits significant clinical and genetic heterogeneity and has greater prevalence in males. Its incidence varies among populations, being more frequent in Asians (2.8 per 10,000 life births). HSCR mostly presents sporadically, although it can be familial (≈20%). The gene encoding a receptor tyrosine-kinase (*RET*) is the major HSCR gene, although coding sequence mutations (CDS) only account for 7%-35% of the sporadic and up to 50% of the familial HSCR cases. Reduced penetrance of *RET* CDS mutations, lack of genotype-phenotype correlation, and a highly frequent low penetrance locus in *RET* intron 1 indicates that the disease likely results from the interaction of several yet unknown susceptibility loci. A major feature of phenotypes with a complex pattern of inheritance is the segregation of multiple predisposing loci with cumulative effects. HSCR is as an oligogenic entity being genetically dissected and used as a paradigm for the study of polygenic/complex diseases. To find additional HSCR loci, two independent genome-wide screenings on Caucasian and Chinese populations are being carried out on the International HSCR Consortium set of patients. Initial data obtained from genotyping 72 Chinese HSCR trios using the GeneChip Mapping 500K Set (Affymetrix) revealed suggestive susceptibility loci on 7q31.2, 5q34, 18q12.2, and Xq27.3 chromosomal regions, in addition to 10q11.2 (*RET*). Our data provide support for *RET* to be the most important locus for HSCR, with several SNPs reaching significant p-values. Analyses of interactions among these loci and a follow up on the most significant SNPs on 192 HSCR cases and 192 controls will be presented.

1212/T

Graphical synthesis of association results and neighboring linkage disequilibrium. *E. Jorgenson, M. Kvale, J.S. Witte.* Epidemiology & Biostatistics, Univ California, San Francisco, San Francisco, CA.

Promising findings from association studies are commonly presented with two distinct figures: one giving results of the association study, and the other indicating linkage disequilibrium between genetic markers in the region(s) of interest. For example, a number of recent genome-wide association studies have presented their most compelling results in this manner. Usually, this means plotting p-values on a negative log base 10 scale and displaying a linkage disequilibrium map beneath those results. Ideally, these results would be combined together, and indicate how linkage disequilibrium affects the observed association signal and help localize the causal variant(s).

Here we present a method for displaying both association results and linkage disequilibrium between genetic markers in the same figure. Using this method, we are able to plot both the association results and the expected association results for each genetic marker conditional on their linkage disequilibrium with other markers in the region. It is then possible to test whether the association result for a given maker significantly exceeds the expected association due to linkage disequilibrium with other markers. Software that can efficiently handle dense genotype data over large regions is available on our website.

1213/T

Genome-Wide Association Studies for Complex Diseases Using the Quebec Founder Population. *B. Paquin, H. Fournier, J. Raelson, P. Van Eerdewegh, P. Croteau, Q. Nguyen, J. Segal, S. Briand, N. Paquin, B. Stojkovic, V. Bruat, S. Debrus, S. Kebache, R. Little, J. Hooper, A. Belouchi, T. Keith.* Genizon BioSciences, St-Laurent, QC, Canada.

To date we have completed 10 GWAS for complex diseases using the Quebec founder population (QFP). Based on permutation studies, we have evidence for genome-wide significance for many association signals across the various studies, indicating that we have achieved well-powered GWAS with relatively small sample sizes (~500 cases and 500 controls). Key to success was the use of the QFP coupled with high quality genotyping and efficient, automated pipelines for genetic analyses. The QFP is an ideal population for genetic studies, characterized by an initial bottleneck of 2,600 effective French founders in the 17th century followed by rapid population expansion in genetic isolation, resulting in a relatively homogeneous population of 6 M individuals. Using an Illumina-based genotyping platform, we generated over 2 B genotypes with call rates >99% and reproducibility and accuracy >99.7%. High-quality genotyping data are crucial for the reliable identification of association signals (simulation studies show signals may be missed by degrading data quality). To efficiently process and analyze the vast amount of data, we have built an automated genetic analysis pipeline, GeneSys, which consists of a suite of optimized genetic analysis components including data cleaning, haplotype estimation, association analyses (both haplotype and single marker), and data visualization using a customized version of GBrowse. We show that some loci were detected in the genome scan exclusively from haplotypes and that haplotype analyses increase power for gene detection in some regions. Using our pipeline, a complete GWAS including genome-wide significance based on 500 permutations can be completed within one week. We perform sub-phenotype and gender-specific analyses and conditional analyses for the detection of gene-gene interactions. The identified genes are used to infer a GeneMap that consists of networks of interacting disease genes and their biological pathways. Our process is shown using examples from various studies.

1214/T

Marker-marker correlation, population stratification and significance thresholds in genome-wide association studies. *J. Shi[1], A. Whittemore[2], J. Webster[3], D. Stephan[3], D. Levinson[1].* 1) Psychiatry & Behavior Science, Stanford University, Stanford, CA; 2) Health and Research Policy, Stanford University, Stanford, CA; 3) Neurogenomics, Translational Genomics Research Institute, Phoenix, AZ.

Permutation tests are used to obtain empirical significance levels in case-control genome-wide association (GWA) studies. Using gene expression data, Efron demonstrated that correlation among tests can lead to overdispersion or underdispersion of test statistics relative to the expected normal distribution, and suggested correcting for this effect to avoid spurious findings or power loss. We evaluated whether a similar effect could be observed in GWA data. We studied data for ~300,000 SNPs (after QC filtering) from each of two European-ancestry control populations, creating replicate datasets by randomly selecting cases and controls from a population. The uncorrected (unconditional) 5% genome-wide significance threshold (b) was determined across replicates. Then, for each replicate, the conditional genome-wide p-value (GWP) of b was computed, using a novel logistic regression method to adjust for the central proportion of Z-scores (computed based on the theoretical distribution) falling within 1 SD of the mean. For the theoretical 5% significance threshold, the conditional GWP ranged from 2% to 10% depending on the central proportion. The variance of the conditional GWP for a given b is predicted mathematically by the squared average marker-marker genotypic correlation (tau-squared). Population stratification (PS) can cause correlation among unlinked markers. In both datasets, after correcting for PS (Eigenstrat), tau-squared was reduced by 80%, and the central proportion no longer predicted GWP. In these European-ancestry GWA datasets, marker-marker correlations are sufficiently weak that the potential effect of distribution dispersion can be ignored, if an adequate correction for PS is applied.

1215/T

Fine-mapping of genome-wide breast cancer association study. *M.S. Udler[1], K.A. Pooley[2], A.M. Dunning[2], P.D. Pharoah[2], D.G. Ballinger[3], J.P. Struewing[4], R. Luben[1], S. Ahmed[2], C.S. Healey[2], Search Collaborators[2], C.A. Haiman[5], P. Brennan[6], C.Y. Shen[7], D. Kang[8], D.R. Cox[3], E.A. Ostrander[9], B.A.J. Ponder[10], D.F. Easton[1].* 1) Department of Public Health and Primary Care, University of Cambridge, UK; 2) Department of Oncology, University of Cambridge, UK; 3) Perlegen Sciences, Inc., Mountain View, CA; 4) Laboratory of Population Genetics, NCI, Bethesda, MD; 5) Department of Preventive Medicine, Keck School of Medicine, USC, Los Angeles, CA; 6) International Agency for Research on Cancer, Lyon, France; 7) Institute of Biomedical Sciences, Academia Sinica, Taipeii, Taiwan; 8) Seoul National University College of Medicine, Seoul, Korea; 9) Cancer Genetics Branch, NHGRI, Bethesda, MD; 10) CR-UK Cambridge Research Institute, UK.

Genome-wide association (GWA) studies utilize linkage disequilibrium (LD) between Single Nucleotide Polymorphisms (SNPs) in a population to identify genetic variants that are associated with increased risk of disease. Since SNPs located close to each other on a chromosome may be correlated, it is often difficult to determine which is the causal SNP. We have applied statistical techniques for fine-mapping to susceptibility loci identified in a breast cancer GWA study. The three strongest associations from the study were in *FGFR2*, *TNRC9*, and *MAP3K1*, located in LD blocks on chromosomes 10, 16, and 5, respectively. Here we investigate the use of single and multiple SNP analyses as well as haplotype analyses using data from 8,792 cases and 8,200 controls from five studies of European and Asian descent. For the haplotype analyses, an Ancestral Recombination Graph-based approach (Margarita[1]) and a clustering algorithm (HapCluster[2]) were utilized. Through these approaches, the number of candidate causal SNPs was considerably decreased. In *FGFR2*, of 117 SNPs in the region, all but six were excluded at odds of 100:1 after applying these methods. In this case, logistic regression following genotype imputation provided a straightforward analytical approach, and haplotype-based analysis did not provide additional precision. [1]Minichiello MJ, Durbin R. Am J Hum Genet. 2006;79:910-22. [2]Waldron ERB, et al. Genetic Epidemiology. 2006;30:170-9.

1216/T

The impact of haplotype Size, diversity and frequency on haplotype blocking inference. *C.H. Chen[1], C.C. Chang[1], S. Shete[2], C.S.J. Fann[1].* 1) Intitute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Dept of Epidemiology, The University of Texas M. D. Anderson Cancer Center.

While direct molecular haplotyping technologies are timing and costly, statistical algorithms to estimate haplotypes based on genotype data have been proposed to facilitate genetic analysis. Most methods estimate haplotypes and haplotype frequency within a pre-determined halotype block (segment). However, the boundaries of haplotype blocks change when sample size, marker density, and ethnicity of the genotype data vary. It is essential to examine the stability of the estimated haplotype block. In this study, we defined a block similarity index to quantify the difference among haplotype block estimates based on various data sets. To initiate the robustness of the block boundaries, we conducted a simulation based on the genotype data of MHC region from the YRI and CHB samples of the HapMap project. The first step was to determine haplotype blocks by inter-markers LD measure D'. Haplotypes and haplotype frequencies were estimated within each haplotype block. Haploview was used to carry out the above calculation. The estimated haplotypes and their frequencies were then used to simulate genotype data using SimPed. Next, the simulated genotype data were analyzed using Haploview as in the first step. Finally, the block similarity index was used computed to compare the haplotype blocking of the original and simulated data sets. A decay of the block similarity index suggested an instability of the estimated haplotype block. In addition, block length and haplotype diversity were also considered in the comparison. Our results showed that the use of different cutoffs of D', (0.5, 0.6, 0.7), had little impact on defining block boundaries. For blocks with lower haplotype diversity, the block similarity index was higher when the block length increased and lower for shorter blocks. However, for blocks with high haplotype diversity, the trend held if the number of haplotypes is small. In summary, the robustness of a haplotype block, in terms of the block similarity index, was related to its block length, number of haplotyes, and haplotype diversity.

1217/T

Mutual Information Theory Based Multilocus LD Measure and Its Application to Haplotype Block Partitioning. *L. Zhang[1,2], J.F. Liu[1], HW. Deng[1,2,3].* 1) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shanxi 710049, P.R. China; 2) Departments of Orthopedic Surgery and Basic Medical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108; 3) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China.

In association studies, it is helpful to evaluate the pattern of linkage disequilibrium (LD) across the human genome for partitioning haplotype block as well as searching for disease genes. Commonly used pairwise measures for assessing LD between two loci, such as D' and r2, may lose power in either using multilocus data or precisely describing LD patterns. Meanwhile, most existing multilocus LD measures, such as Normalized Entropy Difference (NED), do not consider the LD heterogeneity in the genome. Consequently, a unified LD measure for multiple loci may result in an ambiguous LD boundary. Additionally, these existing multilocus LD measures can not handle distant regions which may render long range LD patterns. In this study, we proposed a novel multilocus LD measure based on mutual information theory. The measure can largely overcome the drawbacks of both pairwise and multilocus previous LD measures. Using mutual information, our proposed measure describes LD pattern between two multilocus regions each as a compact unit. As such, the proposed measure can better characterize LD patterns between two arbitrary regions. We further applied this LD measure to haplotype blocks partitioning using both simulation and empirical data sets. The results show that the developed LD measure herein has distinct advantages over both traditional pairwise and multilocus LD measures. Compared with the other measures, our LD measure is more robust, which can capture well the LD information between neighboring regions as well as regions with a long distance from each other. Furthermore, haplotype block boundaries can also be precisely detected via our proposed method.

1218/T

Genetic locus associated with white blood cell count in the Health, Aging, and Body Composition Study. *M.A. Nalls[1], J.G. Wilson[2], N.J. Patterson[3], A. Tandon[3], J. Zmuda[4], S. Huntsman[5], D. Hu[5], B. Beamer[6], K. Patel[1], J. Files[2], M. Akylbekova[2], C. Hardy[2], H. Taylor[2], D. Reich[3], T.B. Harris[1], E. Ziv[5].* 1) L.E.D.B., National Institute on Aging, Bethesda, MD; 2) Jackson Heart Study, Jackson, MS; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 5) Department of Medicine, University of California-San Fransisco, San Fransisco, CA; 6) Division of Geriatric Medicine, Johns Hopkins University, Baltimore, MD.

Background: White blood cell count (WBC) is an important clinical marker that varies among different racial/ethnic groups. African Americans are known to have lower WBC than European Americans. We surveyed the entire genome for loci underlying this difference in WBC using admixture mapping, taking advantage of the fact that African Americans are a population with West African and European ancestry. **Methods:** We analyzed data from African American participants in the Health, Aging, and Body Composition Study. All 863 individuals were genotyped at 1322 single nucleotide polymorphisms that were pre-selected to be informative for African vs. European ancestry and span the entire genome. We used the markers to estimate genetic ancestry in each chromosomal region and then tested the association between WBC and genetic ancestry at each locus. **Results:** We found a locus on chromosome 1q strongly associated with WBC levels ($p<10^{-42}$). The strongest association was with a marker known to affect the expression of the Duffy blood group antigen. Participants who had both copies of the common West African allele had mean WBC of 4.9 (SD 1.3); participants who had both copies the common European allele had mean WBC of 7.1 (SD 1.3). This allele explained ~20% of the variation in WBC. **Conclusions:** We used admixture mapping, a novel method for conducting genetic association studies, to find a region on chromosome 1q that was significantly associated with WBC. Additional studies are needed to determine the biological mechanism of this effect and its clinical implications.

1219/T

PPLD: Extension of the PPL framework to detect trait-marker LD and estimate D' in general pedigree structures. *Y. Huang[1], L. Brzustowicz[3], V. Vieland[1,2].* 1) Ctr for Quant & Comp Biology, Columbus Children's Res Inst, Columbus, OH; 2) Dept Pediatrics, Ohio State University, Columbus, OH; 3) Dept Genetics, Rutgers University, Piscataway, NJ.

Linkage disequilibrium (LD) analyses are frequently used to conduct fine mapping once a genomic region of interest is identified and to identify potentially causal SNPs. Under the framework of the PPL (Posterior Probability of Linkage), our group previously developed a statistic called the LD-PPL, which measures the evidence of linkage while allowing for trait-marker LD. Our new statistic, the Posterior Probability of LD given Linkage (PPLD), an extension of the LD-PPL, directly measures the evidence for (or against) LD conditional on linkage. In current applications, we set the prior probability of LD ($D'\neq;0$) given linkage at 2%; (and the prior probability of linkage at 2%;), and apply uniform weight over D' for small recombination values. Like the LD-PPL, the PPLD is on the probability scale with possible values ranging from 0 to 1. The posterior mode of the PPLD provides an estimate of D'. We have evaluated the behavior of the PPLD in application to a set of medium to large pedigrees originally ascertained for a study of schizophrenia. Using a variety of disease models, we simulated data containing either one or two susceptibility loci and a set of 10 flanking SNP markers exhibiting a range of LD (D' from .14 to .86) with the susceptibility locus. Compared with two other family-based association methods, the PPLD has higher power to detect LD, and the estimate of D' is quite accurate even with moderate sample sizes and in the presence of two causal SNPs. Because the PPL and the PPLD are on the same scale, we now have a ready mechanism for sequentially updating the posterior map (of potential trait-gene locations) obtained from linkage analyses with LD evidence obtained from fine-mapping or WGA data, in a mathematically rigorous manner. Of special note is that the method applies immediately, without any modification, to trio or case-control data structures, enabling us to assess the overall evidence for linkage and/or LD based on multiple data sets involving different pedigree structures.

1220/T

SNP tagging for fine-mapping association analysis of the Extended MHC region in a T1D association study. *E. Luczkowski[1], M. Klinker[1], J. Basken[1], S. Bolte[3], S. Twigger[2], S. Ghosh[1].* 1) Max McGee Center, Medical College of Wisconsin, Milwaukee , WI; 2) Human Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 3) GE Healthcare, Wauwatosa, WI.

The advent of whole-genome association chip sets has made single nucleotide polymorphisms (SNPs) association studies for an entire cohort of individuals possible by using tagSNP approaches to improve time and cost effectiveness. Many common diseases, such as type 1 diabetes have an extensive history of linkage studies, which have now given way to large association studies, which reinforce the link between specific genomic regions and disease. Given that in many cases the low SNP density of association panels will not usually elicit specifically associated genes in gene-dense areas with large LD blocks, further fine-scale tagSNP work and sequencing is necessary to discover the exact genes driving the association. To begin a fine mapping study we have taken the extended MHC region SNPs (Chr6:25,760,400-33,772316) of the CEU HapMap dataset and collated them for tagging. We have analyzed the SNP set, despite the shortcomings of missing genes or few regional tags and have produced a tag SNP set. The creation of the tagSNP set was completed by parsing the available HapMap data of the extended MHC region into 8, 500kb overlapping regions and submitting them to the Tagger server. Collating this list brought us to 3299 SNPs. This data set does not tell us anything directly about our ability to tag HLA alleles. This set of tagSNPs also does not take into account other methodologies for choosing tag SNPs. Further investigation into other tagging methodologies (Haplotype tagging, Bayesian tagging) is in progress to create the most comprehensive tag set for the extended MHC region. This work will offer researchers a clear methodology to fine mapping association studies via tagSNP techniques for any disease and genomic region of interest.

1221/T

KBAT: Kernel-based association test. *H.C. Yang[1], H.Y. Hsieh[2,3], C.S.J. Fann[2,3].* 1) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 3) Institute of Public Health, Yang-Ming University, Taipei, Taiwan.

Disease gene association mapping is a powerful tool for positional cloning of genes susceptible to complex disorders. We propose a kernel-based association test (KBAT) which is a composite function of "p-values of single point association tests" and "kernel weights related to intermarker distances and/or linkage disequilibrium". KBAT is a general form of many existed test statistics. This method can be applied to study candidate genes as well as scan whole chromosomes by incorporating a sliding window procedure with a proposed selector of optimal window sizes. We evaluated performance of KBAT by using comprehensive simulation studies which considered evolutionary parameters, disease models, sample sizes, kernel functions, test statistics, windows attributes and genetic/physical maps. The results of simulations with 10,000 simulation replications for each condition showed that KBAT had high test power and well controlled type 1 error compared with many existed methods. In addition, KBAT was also applied to study a large authentic data set of alcoholism dependence (COGA) provided by GAW14. Results of the genomewide analysis not only confirmed previous findings but also identified some novel regions. In summary, strengths of KBAT are multi-folds: (1) Robust to inclusion of nuisance markers; (2) Scale-invariance to map scale; (3) Accommodated to different study designs; (4) Amenable to pooled DNA association mapping and allelic imbalance detection; (5) Applicable to meta-analysis. Utilities of the proposed methods are integrated in user-friendly software KBAT.

1222/T

Approximate inheritance reconstruction using high density typing. *H. Yao, K. Markianos.* Program in Genomics, Children's Hospital, Boston, MA.

We present an approximate solution to the estimation of identity by descent probabilities in extended pedigrees and genetic isolates. We rely on the high information content of newer technologies such as high density SNP chips and re-sequencing. Traditionally, simultaneous consideration of hundreds of tightly linked markers requires application of the Lander-Green algorithm or the use of MCMC sampling methods. The approach we present here uses the Lander-Green algorithm only for local inheritance re-construction and error correction (within nuclear families). It relies on pedigree structure and population constraints to estimate sharing among two or more individuals. Estimation of identity by descent coefficients is computationally intensive but grows linearly with the number of individuals and it is well within currently available computer power. Although this is not an optimal approach, the high information content of the new generation of markers makes the results indistinguishable from the exact calculation in several cases of practical interest. We present the advantages and limitations of this approach using simulated as well as real 500k SNP data sets.

1223/T

Phenotypic and genetic characterization of a family with autosomal dominant autoimmunity resembling autoimmune polyendocrine syndrome type 2. *A. Ballarini[1], A. Näke[1], A. Herr[2], L. Senenko[1], K. Engel[1], M. Gahr[1], F. Rüschendorf[3], N. Hubner[3], M. Lee-Kirsch[1].* 1) Klinik für Kinder- und Jugendmedizin, Technische Universität Dresden, Dresden, Germany; 2) Institut für Klinische Genetik, Technische Universität Dresden, Dresden, Germany; 3) Max-Delbrück-Centre for Molecular Medicine, Berlin-Buch, Germany.

Autoimmune diseases affect 3-5% of the general population and are due to defects in the development or the maintenance of self-tolerance mechanisms. Despite the identification of common gene variants (e.g. in CTLA-4) and genes causing monogenic autoimmune syndromes (e.g. AIRE/APECED and FOXP3/IPEX), the genetic basis of autoimmunity remains largely unknown.

We describe a non-consanguineous German family with 22 members, 5 of which are affected with one or more of the following organ-specific autoimmune diseases: type 1 diabetes, thyroid disease, Addison's disease, or celiac disease. Moreover, 5 additional members were found to have autoantibodies only. There was no hypoparathyroidism or candidiasis and a mutation in AIRE was excluded. Including all individuals with evidence for autoimmunity, pedigree analysis suggests a dominant trait resembling autoimmune polyendocrine syndrome type 2. In search for genes involved in autoimmunity we performed a SNP-based genome-wide linkage analysis on 15 family members. In parallel, we investigated changes at the transcriptional level using the GeneChip U133 plus 2.0 arrays in $CD4^+CD25^+$ regulatory T cells isolated from affected individuals and controls. Parametric linkage analysis led to the identification of two loci with suggestive linkage on chromosomes 3 and 4 with LOD-scores of 2.45 and 2.4, respectively. Bioinformatic analysis of gene expression data revealed several differentially expressed genes that are involved in cell proliferation, migration, and cytokine production.

These findings may contribute to our understanding of the molecular mechanisms underlying this familial form of autoimmunity and may also provide insight into the pathogenesis of common complex organ-specific autoimmune diseases. .

1224/T

Mapping Quantitative Trait Loci of Complex Traits Based on Zygotic Linkage Disequilibrium. *S. Wu, T. Liu, J. Yang, J.S. Yap, W. Hou, R.L. Wu.* Department of Statistics, University of Florida, Gainesville, FL.

Linkage disequilibrium-based mapping that capitalizes on historical recombinant events has proven to be powerful for detecting quantitative trait loci (QTLs) that control a complex trait in a natural population. This approach, founded on the non-random association between markers and QTL at the gametic level, requires the population mapped to be in Hardy-Weinberg equilibrium (HWE), which may not be a case for many genetically informative isolated populations. Here, we present a new QTL mapping approach based on linkage disequilibria at the genotypic or zygotic level by accommodating the deviation from HWE. This approach allows joint or separate estimation of Hardy-Weinberg disequilibrium at individual loci, gametic and non-gametic linkage disequilibria, trigenic, and quadrigenic linkage disequilibria between the markers and QTLs. By testing these different types of disequilibria, we generalized framework for inferring the existence of the underlying QTL for a complex trait. We performed simulation studies and real data analyses to investigate the statistical properties of this approach and validate its utilization. This approach will open a general gateway for studying the detailed picture of the genetic architecture of quantitative variation in natural populations.

1225/T

Ignoring Temporal Trends in Genetic Effects Substantially Reduces Power for Detection of Quantitative Trait Loci. *G. Shi[1], D.C. Rao[1, 2].* 1) Division of Biostatistics, Washington University, St. Louis, MO; 2) Departments of Genetics and Psychiatry, Washington University, St. Louis, MO.

As individuals grow from birth, many physiological and biological changes take place in the pathways underlying human diseases, increasing risk for many diseases as a result of accumulating changes with age. Biological evidence of temporal trends in genetic effects have been demonstrated in animal studies. We propose a systematic variance components linkage analysis method incorporating temporal trends in QTL effects as well as in polygenic effects. Heritabilities are no longer constant over all ages, but instead are functions of the appropriate ages and depend in turn upon a few additional unknown temporal trend parameters. Using the generalized variance component model, we evaluate the gain in power of the linkage test in the presence of temporal trends. In our extensive simulations, we find that ignoring this gene-by-age interaction, when present, substantially reduces power thereby jeopardizing gene discovery. On the other hand, modeling such trends explicitly enhances gene discovery for complex traits. For example, when the average (over age) QTL heritability is 0.10 and the peak QTL heritability is 0.4, the empirical power is only 43% when trends are ignored, which increases to over 99% when the temporal trend is appropriately modeled.

1226/T

Ignoring Imprinting Effects Can Severely Jeopardize Detection of Linkage. *Y.J. Sung[1, 2], DC. Rao[1, 2, 3].* 1) Division of Biostatistics, Washington University in St Louis, St Louis, MO; 2) Department of Psychiatry, Washington University in St Louis, St Louis, MO; 3) Department of Genetics, Washington University in St Louis, St Louis, MO.

Genes with imprinting (or parent-of-origin) effects inherited from the mother express differently than those inherited from the father. Some genes that affect development and behavior in mammals are known to be imprinted. We have developed parametric linkage analysis with imprinting and implemented it in the lm_twoqtl program in the MORGAN package. The program offers computationally tractable analysis of general pedigrees with many markers. To study the impact of imprinting on linkage analysis, we simulated data sets where imprinting contributes 0%, 25%, 50%, and 75% of the variance of a QTL effect. To study misspecification of imprinting, we analyzed each data set with all 4 different models. The correct model with imprinting provided the highest lod scores with all max lod scores over 4. The incorrect model with no imprinting provided the lowest lod scores with max lod scores of 2.23, -1.13, and -5.21 with 25%, 50%, and 75% imprinting, respectively. Cases with max lod > 3 from the correct model with imprinting and max lod < -2 from the incorrect model with no imprinting occurred in 60 out of 100 cases. Models with misspecified imprinting produced lod scores intermediate between those of the models with correct imprinting effects and no imprinting. These simulations show that accounting for imprinting can substantially improve linkage detection.

1227/T

Genetic Mapping and Characterisation of Non-Syndromic X-Linked Mental Retardation in a Saudi Family. *H. Abalkhail[1], Z. Al-Hassnan[2], M. Faiyaz Ul-Haque[1], N. Sakati[2], M. Al-Owain[2], A. Tbakhi[1], M. Al-Dosari[3], F. AL-Sharief[1], E. Faqieh[2].* 1) Department of Pathology & Lab Medicine, King Faisal Special Hospital and Research center, Riyadh, Saudi Arabia; 2) Department of Medical Genetics, King Faisal Special Hospital and Research center, Riyadh, Saudi Arabia; 3) Department of Neuroscience, King Faisal Special Hospital and Research center, Riyadh, Saudi Arabia.

Abstract: X-linked mental retardation (XLMR) is a genetically and clinically heterogeneous disorder affecting approximately 1 in 1000 males. Clinically, XLMR exists in syndromic and non-syndromic forms. In syndromic forms, mental retardation is associated with other neurological, behavioral, and/or metabolic abnormalities, while in the non-syndromic forms, the only consistent phenotypic manifestation is mental retardation. We examined a large Saudi family with non-syndromic X linked mental retardation recruited for this study. We carried-out a preliminary X chromosome screening using DNA from a large Saudi family with 4 affected males in two different sib ships, connected through healthy females. All affected family members were examined by a clinical geneticist; families with a known diagnosis for mental retardation, male-to-male transmission, fragile-X syndrome or an abnormal G-banded karyotype were excluded from study. Following an X chromosome scan with the use of 48 microsatellite markers (ABI PRISM® linkage mapping set v2.5) distributed along the X chromosome, we successfully identified a recombination in the four affected family members flanked at 7.22 cM between the proximal DXS8043 (Xq27.3) and the distal DXS8087 (Xq28) that corresponded to a physical distance of 8.7 Mb. Haplotype analysis was performed manually and a shared haplotype was identified in all affected individuals. To further refine the initial region (~7.22 cM), additional markers were investigated at a higher resolution in order to perform multipoint / two point linkage analysis and to identify a potential disease locus. To our knowledge, this is the first clinical study of its kind carried-out on Saudi population.

1228/T

Impact of DNA Source in the Illumina Golden Gate (GG) Assay. *Y. Tsai, O. Osimokun, I. McMullen, J. Zhang, J. Romm, E. Pugh, K. Doheny, C. Boehm.* Center for Inherited Disease Research (CIDR) and Genetic Resources Core Facility SNP Center, IGM, JHUSOM, Baltimore, MD.

We have been providing SNP genotyping services using Illumina GG chemistry since 2004. Here we present performance statistics by DNA source for 53 custom and linkage projects released since summer 2005. The table below shows performance for DNAs from many labs obtained from several commonly-used sources. Samples with lower call rates and lower genotype quality scores are considered failed and data is not returned on them.

DNA source	Number of Samples	Overall Sample Failure	Number of Projects	Range of Failure by Project
Blood	40,110	2.63%	36	0 - 9.4%
Cultured Cells	19,651	1.29%	16	0 - 7.7%
Whole Genome Amplified (WGA)	3,645	5.46%	12	0 - 84.6%
Buccal	3,282	2.80%	5	0 - 16.7%

Blind duplicates supplied by the investigator were used to calculate genotype reproducibility by DNA source. Rates were overall very good (Blood: 99.9968%; Cultured cells: 99.9971%; WGA: 99.9522%; Buccal cells: 99.9937%). However, there is a distinct drop in reproducibility in WGA samples. Within DNA sources samples with higher call rates had higher reproducibility. Conclusion: DNA source made a difference in success of genotyping using the GG chemistry. Sources typically considered high quality (blood, cell lines) yielded higher genotype data quality as measured by reproducibility and call rates. The utility of WGA samples for genotyping was highly variable from project to project, ranging from sample failure rates of 0% to 84%.

1229/T

Extreme sampling may improve power of admixture mapping for quantitative traits. *D. Hu, E. Ziv.* Dept Medicine, Univ California, San Francisco, San Francisco, CA.

Admixture mapping can be used to map regions for complex traits in populations of mixed ancestry. Admixed populations consist of 2 or more ancestral populations which have mixed recently. The advantage of admixture mapping is that the recent admixture creates long range linkage disequilibrium between markers that have allele frequency differences in the ancestral populations. The main limitation of admixture mapping is that power depends strongly on the allele frequency difference of the causative variant(s). Admixture mapping has no power if the allele frequency of the causative variant(s) is identical in the ancestral populations. For quantitative traits, the power to detect a locus by association mapping may be enhanced by sampling the extremes of the distribution. We evaluate the power to detect a quantitative trait locus by admixture mapping using an "extreme sampling" approach (eg sampling from the top and bottom deciles of a distribution). We find that power remains considerably high even for loci with relatively modest allele frequency differences (0.15 - 0.2). In addition, this design is relatively immune to variation in the contribution of ancestral populations. Furthermore, in some cases, the power is greater for less common alleles with the same effect size and the same allele frequency difference. For example, assuming a marker with an allele frequency of 0.4 and 0.5 in 2 ancestral populations (allele freq difference 0.1) and an additive effect of 0.67/SD, with a sample size of 500/group sampled from the top and bottom deciles, we expect ~31% power to detect an effect with alpha 0.00005. Using the same assumptions about effect size, sample size, alpha and sampling scheme, we expect ~76% power to detect a marker with an allele frequency of 0.025 and 0.125 in 2 ancestral populations (allele freq difference 0.1). Thus, we conclude that admixture mapping for quantitative trait loci may benefit substantially from extreme sampling. Since many variants with allele frequency <0.15 in one population are often absent in other populations, this design may be an efficient approach to detecting such variants.

1230/T

A comparison of the fine mapping accuracy of several similarity measures in the framework of Bayesian Partition Models allowing for unphased genotypes. *YQ. Luo[1], SH. Won[1], RC. Elston[1], TS. Park[2].* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Department of Statistics, Seoul National University, Korea.

Linkage disequilibrium fine mapping can be performed using coalescent-based or haplotype-clustering-based approaches. We have demonstrated, in our previous work, that the current implementation of the latter approach in the Bayesian Partition Models (BPM, Waldron et al. [2006]) is at least as efficient in localization as the coalescent-based approaches, while being much more computationally manageable. The performance of the BPM framework depends on the choice of similarity measures between haplotypes, based on which the haplotypes are clustered. A good similarity measure should be able to capture the evolutionary events well. We compare several similarity measures proposed in the literature in other context, as well as some combinations thereof, in terms of their fine mapping accuracy in the BPM framework. We also extend the BPM framework to handle phase-unknown genotypes via a Metropolized Gibbs algorithm. A non-informative prior is employed so that the estimates are approximately equal to maximum likelihood estimates (MLE). An extensive simulation study shows that our new algorithm improves the accuracy of causal SNP localization.

1231/T

Non-syndromic Cleft Lip and Palate: Linkage and candidate genes analysis of multigenerational families. *S.K. Nath[1], U. Ratnamala[2], S. Han[1], S. Beiraghi[3], K. Ewing[1], D. Mandhyan[1], K. McElreavey[4], L. Bartoloni[5], GS. Antonarakis[6], SE. Antonarakis[5,7], U. Radhakrishna[2,5].* 1) Dept Arthritis & Immunology, Oklahoma Medical Res Fndn, Oklahoma City, OK; 2) Green Cross Voluntary Blood Bank and Genetic Research Center, Ahmedabad, India; 3) Division of Pediatric Dentistry, University of Minnesota, Minneapolis, USA; 4) Department of Reproduction, Fertility and Populations, Institut Pasteur, Paris; 5) Department of Genetic Medicine and Development,; 6) Department of Orthodontics, School of Dental Medicine; 7) Geneva University Hospitals, University of Geneva Medical School, Geneva.

Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common congenital craniofacial birth defects, affecting 1 in 700-1,000 newborns in the United States each year. Its highest prevalence rates are in Native Americans and Asians. Various independent association and linkage studies of different populations have identified 11 loci with evidence of linkage for syndromic and/or NSCL/P at various chromosomal regions, however pathogenic mutations have been identified in 4 of these 11 loci. Majority of these reports were made using small nuclear families. However, our recently published three large multi-generational NSCL/P families identified significant evidence of linkage at 13q33.1-34 (Am J Hum Genet 79:580-5, 2006) and 18q21.1 (Am. J. Hum. Genet, 81:180-8, 2007) for markers rs1830756 (NPL=5.57; P=.00024; LOD = 4.45) and rs728683 (NPL =43.33 and P =.000061; nonparametric LOD =3.97 and P =.00001) respectively. We have analyzed another large multi-generational Indian family UR057 with NSCL/P. The family consists of a total of 204 individuals including 18 affecteds (12 males & 6 females). The phenotype of affecteds ranged from unilateral to bilateral NSCL/P. A high-density genome-wide linkage analysis using Affymetrix microchips are currently being processed, however, the results are inconclusive. We present linkage results using family UR057 as well as combined analysis using all the other families for positional candidate genes at 13q33.1-34 and 18q21.1. We discuss the implications of using the large multi-generational families in NSCL/P gene identification perspective.

1232/F

Candidate gene screening of a locus on chromosome 14 and analysis of anticipation in familial Ménière disease. *M.E.S. Bailey[1], Y. Lowe[1], A.W. Morrison[2], G.A.J. Morrison[3].* 1) Div Molecular Genetics, IBLS, Univ Glasgow, Glasgow, U.K; 2) Dept. of Otolaryngology, Royal London Hospital, London, U.K. (formerly); 3) Dept. of Otolaryngology, Guy's and St Thomas' Hospitals, London, U.K.

Ménière disease (MD) is a late-onset, multifactorial disorder characterised by episodic hearing loss, tinnitus, and rotatory vertigo and associated with a significant reduction in quality of life. Its aetiology may involve endolymphatic hydrops, whereby increased hydrostatic pressure in the endolymph compartment probably leads to dysfunction and destruction of cochlear and vestibular hair cells. Approx. 7% of cases are familial, and our series consists of 46 confirmed families with two or more cases of definite MD. A genome scan using 18 of these MD families yielded significant evidence for linkage (HLOD 4.19, $\alpha = 55\%$, 80% penetrance) to a region on chromosome 14q. A critical region of 5Mbp containing 16 identified genes has been defined in 14q21.2-q21.3. Ranking of these genes as positional candidates has been carried out using PROSPECTR and SUSPECTS, and screening of each candidate for mutations is ongoing, with 5 of the genes completed so far. Novel polymorphisms have been recognised, but no likely causative variants. We have also carried out an analysis of age-of-onset anticipation. The mean difference in AOO between parent-offspring generations was 16.5yrs in all suitable MD families in our series. Corrections to the analysis to reduce known ascertainment bias factors yield results that are consistent with real anticipation. We have embarked on a screening programme of potentially expanding microsatellite repeats in the critical region; all screened thus far appear invariant in the patient set and this is currently being confirmed in individuals from the general population. There is no evidence so far for an expanding repeat being responsible for the disorder in the chr.14-linked MD families in this sample. The identity of the predisposing gene at this locus thus remains elusive. Its identification should suggest candidates to screen in the remaining families and illuminate pathways contributing to susceptibility to the more common, sporadic cases of MD.

1233/F

Molecular pathology of deafness due to mutation in *PMP22*. *M.J. Kovach[1], V.E. Kimonis[2], T.A. Carver[1], B. Andrews[1].* 1) Biological & Environmental Sciences, University of Tennessee, Chattanooga, TN; 2) Division of Genetics and Metabolism, University of California Irvine Medical Center, Irvine, CA.

Charcot-Marie-Tooth disease (CMT) is an autosomal dominant disorder characterized by progressive peripheral neuropathy caused primarily by a duplication of the PMP22 gene. A clinical and genetic variant of CMT associated with profound and progressive sensorineural deafness was described in a large family from Illinois. Molecular analysis identified a unique point mutation in the gene instead of the common duplication. PMP22 is a member of the family of Growth arrest specific (Gas) genes, which have been shown to regulate gene expression, cell death and cell division. Although expression of PMP22 is highest in myelin-forming Schwann cells, the transcript is also detected in non-neural tissues, particularly at critical developmental time-points. Thus, PMP22 expression has been proposed to have two functions: a role in peripheral nerve myelination and a role in cell growth regulation in non-neural tissues. It is hypothesized that a similar dual expression of PMP22 is necessary for normal hearing.

The purpose of this study is to dissect the molecular pathology of deafness using the Trembler-J mouse as a model of PMP22-associated auditory dysfunction. Expression patterns of the murine PMP22 protein in the cochlear duct were evaluated in normal mice and mice with a mutant PMP22. Non-neural staining of PMP22 protein was observed primarily in the marginal and intermediary cells of the stria vascularis with weak, occasional localization of PMP22 protein in cells of spiral ligament and basilar membrane. A potential role for deafness genes expressed in the stria vascularis and spiral ligament may be in maintenance of the electrochemical potential through recycling of potassium ions. Concomitantly, differential display identified 74 transcripts differentially expressed relative to functional levels of PMP22, 85% of which are down-regulated in the Tr-J mouse. This study will provide insight to cellular functions and protein:protein interactions that involve PMP22 and help define the role of PMP22 in normal hearing.

1234/F

Characterization of SOX10 deletions in WS2 highlights the molecular complexity of Waardenburg syndrome. *L. Stanchina[1], F. Dastot-Le Moal[1, 2], N. Collot[1, 2], V. Baral[1], S. Marlin[3], A. Toutain[4], W. Reardon[5], M. Lackmy-Port-Lis[6], R. Touraine[7], T. Attie-Bitach[8], M. Goossens[1, 2], V. Pingault[1, 2], N. Bondurand[1].* 1) Genetic Department, INSERM U841, IMRB, Creteil, France; 2) AP-HP, Groupe Henri Mondor-Albert Chenevier, Service de biochimie et génétique, Créteil, France; 3) Service de Génétique, INSERM U587, Hôpital Armand Trousseau, APHP; 4) Centre Hospitalo-Universitaire, Service de Génétique, Tours, France; 5) Ladys Hospital for Sick Children, Dublin, Ireland; 6) Service de Pédiatrie, CHU de Pointe à Pitre, France; 7) CHU-Hôpital Nord Service de Génétique, Saint Etienne, France; 8) Département de Génétique et INSERM U781, Hôpital Necker, Paris, France.

Waardenburg syndrome (WS) is an auditory-pigmentary disorder that exhibits varying combinations of sensorineural hearing loss and abnormal pigmentation of the hair and skin. Depending on additional symptoms, WS is classified into four subtypes, WS1 to 4. Absence of additional features characterizes WS2 whereas association with Hirschsprung disease characterizes WS4, also called Waardenburg-Hirschsprung disease. Mutations within the genes encoding MITF and SNAI2 have been identified in WS2, whereas mutations of EDN3, EDNRB and SOX10 have been observed in WS4. However, not all cases are explained at the molecular level, raising the possibility that other genes are involved or that some mutations within the known genes are not detected by commonly used genotyping methods. The crucial role of SOX10 during melanocyte development and the phenotypic variability observed among patients with mutations of this gene prompted us to search for SOX10 mutations in 30 unexplained cases of WS2. In particular, we used semi-quantitative fluorescent multiplex PCR and Fluorescent in situ hybridization to search for SOX10 deletions. We identified 5 heterozygous deletions, making SOX10 a new gene of WS2. Full characterization of the deletions revealed different events ranging from the deletion of a single exon to that of up to 5Mb. This study further characterizes the molecular complexity and the close relationship that links the different subtypes of Waardenburg syndrome, and has direct consequences in terms of molecular diagnostic.

1235/F

Possible interaction between gap junction proteins Cx26 and Cx31 to cause non-syndromic deafness in Chinese patients. *D. Yan[1], P. Dai[2], X. Lin[3], Y. Yuan[2], W.X. Tang[3], X.M. Ouyang[1], H. Yuan[2], L.L. Du[1], X.Z. Liu[1].* 1) University of Miami, Miami, FL, USA; 2) PLA General Hospital, Beijing, China; 3) Emory University School of Medicine, Atlanta, GA, USA.

Congenital deafness in humans occurs in approximately 1 in 1000 live births, and at least 50% of these cases are hereditary. It is estimated that roughly 70% of all cases of hereditary hearing loss (HL) are non-syndromic, approximately 80% of which are inherited in an autosomal recessive fashion. Mutations in a single gene encoding connexin 26 (Cx26) or gap junction beta 2 gene (GJB2) are the leading cause of non-syndromic sensorineural HL (NSHL). GJB3 (Cx31) mutations have also been shown to cause deafness in the Chinese population. To determine if mutations at these two gap junction proteins can interact to cause HL, we have screened 108 Cx26 heterozygous Chinese patients for mutations in Cx31 by sequencing. A total of 3323 NSHL patients, consisting of 314 familial and 3009 sporadic cases, were initially analyzed for GJB2 mutations. In all cases the HL was congenital and severe to profound. After exclusion of the SLC26A4 (Pendred syndrome) caused HL and the A1555G mutation in the 12SrRNA gene, the full GJB3 coding region was analyzed. Two different mutations (N166S and A194T) occurring in compound heterozygosity with the 235delC and 299delAT of GJB2 were identified in 3 sporadic cases (235delC/N166S, 235delC/A194T and 299delAT/A194T). Western blots analysis identified both Cx26 and Cx31 in the mouse cochlea, which are known to be able to form heteromeric GJs. We are currently using an in vitro expression system to investigate functional consequences of compound Cx26 and Cx31 mutation on GJ functions. Acknowledgement: The work is supported by NIH DC 05575 *Cx26GJB2*.

1236/F

Myocilin interacting proteins: Screening of a human retina yeast two hybrid cDNA library. *M. Ohtsubo[1], K. Hosono[1], C.X. Wang[1,2], Y. Hotta[2], S. Minoshima[1].* 1) Photon Med Res Ctr,; 2) Dept Ophthalmol, Hamamatsu Univ Sch Med, Japan.

At present, 3 causative genes (myocilin, optineurin, WDR36) have been identified for primary open-angle glaucoma (POAG). However, these genes account for only small fraction of inherited glaucoma. At least 11 more chromosomal regions (GLC1B - D, F, H-N) have been described as candidate loci for POAG. We are attempting to identify new causative genes using "extended candidate gene (ECG) approach" which we designed. In this approach, novel proteins interacting with a known causative gene product are identified by yeast two-hybrid (Y2H) screening. If the gene of the novel protein is located to candidate chromosomal regions, it is extensively analyzed for mutation in patients.

MYOC (Myocilin) protein functions in the extracellular environment and mutations in the gene lead to a perturbed outflow of aqueous humor in the trabecular meshwork (TM). However, it was reported that myocilin protein is expressed also in retina. Thus, it is not clear whether myocilin functions intracellularly and what is the meaning of the expression in non-TM tissues. We chose MYOC for our ECG approach.

A Y2H cDNA library was constructed with total RNA from human retina by homologous recombination using prey vector pGADT7-Rec (invitrogen) containing GAL4 DNA activation domain. A full-length MYOC cDNA was cloned with pGBKT7 vector containing GAL4 DNA binding domain for a bait. As a result of Y2H screening, we have obtained tens of clones. Further confirmation for the interaction of isolated gene products with MYOC by co-immunoprecipitation and pull-down assay as well as characterization of each protein such as intraocular localization are in progress. These genes include 9 of them localized to candidate chromosomal regions (see above), cathepsin D, opticin, fibulin 5, lamin A/C, Fas-activated S/T kinase, transthyretin and myocilin itself. This study will help to understand the composition of protein functional complex surrounding MYOC and its normal function as well as to discover the novel glaucoma causative genes.

1237/F

Identification of interacting proteins for glaucoma-related optineurin (OPTN) by yeast two-hybrid system. *T. Rezaie[1], L. Huang[2], M. Walter[2], M. Sarfarazi[1].* 1) Molecular Ophthalmic Genetics, University of Connecticut Health Center, Farmington, CT; 2) Medical Genetics, University of Alberta, Edmonton, AB, Canada.

Our original study identified mutations in the *OPTN* gene in adult-onset primary open-angle glaucoma (Science 295, 2002). We hypothesized that altered protein-protein interaction caused by *OPTN* mutations may contribute to the glaucoma. This study aimed to identify novel OPTN interacting proteins (OPTN-IPs) and the pathways through which *OPTN* mutations lead to glaucoma. A cDNA library from human trabecular meshwork (HTM) cells was constructed by cloning of the cDNA in ProQuest prey vector containing GAL4 DNA activation domain. The *OPTN* full-length cDNA was cloned in pDEST32 containing GAL4 DNA binding domain as the bait. Approximately 2.4 X 10^5 colonies were screened from which 7 colonies activated the HIS3, URA3 and lacZ reporter genes. Bait and prey plasmids were recovered from yeast. Sequencing of bait plasmids confirmed the specificity and absence of undesired mutations. Four new potentially OPTN-IPs identified by sequencing of the prey colonies. Further characterization of these colonies with confirmation of interaction by co-immunoprecipitation and pull-down assay and full-length sequencing of prey plasmids is underway. The 4 new candidates consist of an interacting protein to TNF-α, an activator for RAB-like small GTPases, a subunit of RNA splicing factor and a kinase protein. The HTM cDNA library is a valuable tool for discovery of the genes related to ocular function. Association of the new OPTN-IPs with TNF-α and RAB pathways is in agreement with earlier findings that OPTN is inducible by TNF-α and is an interacting partner for RAB. The genes for these 4 new OPTN-IPs are potential candidates for mutation screening of glaucoma patients. Identification of OPTN interacting proteins and their disease-related pathways will provide new opportunities to study mechanism underlying the *OPTN*-linked glaucoma neuropathies. This study may further help to assign functions to uncharacterized proteins, to understand the composition of protein complexes and may lead to identification of novel therapeutic targets. Support: NIH Grant EY-014959 and Canadian Institutes of Health Research.

1238/F

Autophagy induction is a two-edged sword in the polyglutamine disease X-linked spinal and bulbar muscular atrophy (SBMA): evidence for a temporal disconnection between protection and toxicity. *J.E. Young[1], N. Gill[2], R.A. Martinez[1], G.A. Garden[2], A.R. La Spada[1,2].* 1) Dept Laboratory Medicine, Univ Washington, Seattle, WA; 2) Dept Neurology, Univ Washington, Seattle, WA.

Autophagy, a pathway that mediates intracellular degradation, has recently been studied in a variety of human disorders. Although autophagy induction reduces misfolded protein toxicity, persistent and excessive autophagy activation may be deleterious and contribute to neurodegeneration. In a YAC mouse model for the polyglutamine (polyQ) repeat disease SBMA, we noted significantly increased autophagosome formation in degenerating motor neurons in comparison to non-transgenic and control transgenic mice (26% vs. 5% and 2%; p <.05). To determine the role of autophagy in SBMA, we obtained transgenic mice expressing GFP tagged to LC3, a component of the autophagosome. In primary cortical neurons induced to undergo autophagy by rapamycin treatment or nutrient deprivation, we noted that GFP-LC3 undergoes an expected distribution change from diffuse to punctate, and that LC3 is processed, as expected, by Western blot analysis (p <.05; p <.01). We could block autophagy in neurons with 3-methyl-adenine (3-MA) (p <.01), or by shRNA knock-down of beclin-1 (p <.01). Using this model, we tested if polyQ androgen receptor (AR), the cause of SBMA, induces autophagy. Transfection of GFP-LC3 neurons with truncated AR (AR112Q vs AR16Q) induced autophagy in a polyQ length-dependent manner (p <.01). To determine if autophagy is beneficial or detrimental, we transfected neurons with AR112Q and treated with 3-MA for 4, 12, or 24 hrs. Neurons treated for 4 hrs displayed increased toxicity, while those treated for 12 or 24 hrs were protected. Co-transfection with beclin-1 shRNA for 24 hrs also yielded protection (p <.01). These data suggest that autophagy is activated in neurons in response to misfolded proteins. However, while autophagy may initially protect the neuron, prolonged autophagy activation may contribute to neurodegeneration. As autophagy inducers are being considered as a therapy for such diseases, our results indicate that the timing of autophagy induction may be a critical factor in their use.

1239/F

Protein-protein interactions and subcellular localisation of spartin (SPG20) in primary neuronal tissues. *R.D. Cook, H. Patel, A.H. Crosby.* Clinical Developmental Science, St George's University of London, Tooting, United Kingdom.

We have previously shown that mutation of spartin (SPG20) underlies an autosomal recessive complicated form of hereditary spastic paraplegia (HSP). Spartin is a protein of unknown function, but contains a MIT (contained within microtubule-interacting and trafficking molecules) domain found in proteins involved in membrane trafficking as well as in spastin, a gene commonly mutated in an autosomal dominant form of HSP. Our earlier work has shown that spartin has a ubiquitous and complex localisation in neuronal-like cells including dynamic nuclear and cytoplasmic localisations as well as a vesicular pattern along differentiated neurites. Here we define biochemical studies which indicate that synaptic associated proteins co-immunoprecipitate with spartin from synaptic enriched membrane fractions. Also, in developing primary neurons spartin, which contains a putative NLS sequence, has a dynamic nuclear localisation and is ubiquitously expressed in neuronal processes. Further work is underway to determine spartin's role in the early stages of neuron development as well as the significance of its presence in the nucleus.

1240/F

Changes in expression and chromatin structure of Jarid1c are associated with neural differentiation. *J. Xu[1], A.P. Arnold[2], C.M. Disteche[1].* 1) Department of Pathology, University of Washington, Seattle WA; 2) Department of Physiological Science and laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, CA.

The X chromosome encodes a disproportionately high number of genes essential for normal brain development, whose mutations cause various forms of X-linked mental retardation. The dosage difference in X-linked genes between XY males and XX females is largely compensated for by X-inactivation in females; however, some X-linked genes escape X inactivation and therefore would be expressed at a higher level in females. One of these genes, Jarid1c, encodes a histone H3K4 de-methylase and causes mental retardation when mutated in human. We have previously shown that Jarid1c is expressed more highly in female than in male mouse brains, whereas Jarid1d, the Y-linked paralog of Jarid1c, is expressed at a very low level and thus does not compensate for the X-linked gene. Our current in situ hybridization studies indicate that Jarid1c mRNA is abundant in specific brain regions including the olfactory bulb, piriform cortex, habenula, hypothalamic nuclei, hippocampus, cerebellum, triangular septal nucleus, and the interstitial nucleus of Cajal, indicating a role in the development of specific neural structures. We determined that Jarid1c was up-regulated following neural differentiation of plural potent P19 cells, confirming the role of Jarid1c in this developmental process. Histone modifications associated with active transcription, such as H3 and H4 acetylation and H3 di-methylation at lysine 4, were enhanced at the 5'end of Jarid1c gene in P19 neurons relative to stem cells. Conversely, no such modifications were detectable at the Y-linked Jarid1d sequence. Thus, specific epigenetic modifications target Jarid1c in the course of neural differentiation.

1241/F

Evaluation of CXorf2 as a Candidate Gene for X-Linked High Myopia. *R. Metlapally[1,2], A. Bulusu[2], M. Schwartz[3], T. Rosenberg[3], C.C. Kroner[2], S. Zuchner[2], Y.J. Li[2], T.L. Young[1,2].* 1) Duke Eye Center, Durham, NC; 2) Duke Center for Human Genetics, Durham, NC; 3) National Eye Clinic for the Visually Impaired, Denmark.

Purpose: X-linked high myopia with mild cone dysfunction has been mapped to chromosome Xq28. CXorf2 is a nested gene within the red and green opsin cone pigment gene tandem array on Xq28. We investigated whether CXorf2 gene alterations are associated with the X-linked myopia phenotype. Two pedigrees (with protanopia and deuteranopia respectively) that mapped to Xq28 were screened for genomic DNA mutations and copy number variations. **Methods:** All exons of the CXorf2 gene including intron/exon boundaries were amplified and sequenced using standard techniques. To examine the copy number variation, ultra-high resolution array-comparative genomic hybridization (aCGH, NimbleGen Inc) assays were performed comparing the patient genomic DNA with control samples (2 pairs from each pedigree). Quantitative real-time (ABI7900HT) gene expression assays (Assays-by-Design) targeted on opsin and CXorf2 genes were used to validate the aCGH findings. Data were analyzed using Comparative CT method to calculate the copy number present in each individual. **Results:** A 5' UTR SNP in CXorf2 was observed in all five affected males but not in unaffected males in the protanopic pedigree. Of the 183 external controls screened, 6.5% possessed the SNP. Logistic regression analysis, which takes into account family strata, revealed significant association ($p<0.05$) between the SNP and disease status. The aCGH findings in both pedigrees revealed predicted duplications in affected patients in the opsin array region. While only 3 copies of CXorf2 have been reported within the opsin array, quantitative real-time analysis of the CXorf2 gene targeted assay on affected individuals in both these pedigrees revealed 4 to 5 copies. **Conclusions:** The 5'UTR is thought to influence mRNA stability and translation efficiency, and 5' UTR SNPs have been associated with disease. Copy number variations play a role in disease inheritance and susceptibility as they affect gene dosage. These CXorf2 gene alterations may be responsible for this phenotype.

1242/F

A novel deletion variant of gamma D-crystallin with reduced solubility and nuclear relocalization leads to congenital cataract. *G.H.F. Yam, L.Y. Zhang, D.S.P. Fan, P.O.S. Tam, D.S.C. Lam, C.P. Pang.* Department of Ophthalmology & Visual Sciences, Chinese University of Hong Kong, Hong Kong China.

Purpose: To investigate the properties of a novel gamma D-crystallin (CRYGD) variant identified in a family with the lamellar type of autosomal dominant congenital cataract (ADCC). Methods: A Chinese family with 5 affected members diagnosed with lamellar cataract and 4 unaffected members were recruited for the mutational screening of 15 known ADCC candidate genes. Two-point linkage analysis with 39 single nucleotide polymorphisms and 22 microsatellite markers flanking these genes, together with direct sequencing was applied to identify the disease-causing mutation. Recombinant N-terminal FLAG-tagged wildtype and mutant CRYGD were expressed in COS-7 cells, respectively. The cellular expression, distribution and detergent solubility were analyzed by western blotting and confocal double immunofluorescence. Results: Linkage analysis located the candidate region in gamma C- and D-crystallin gene cluster. Direct sequencing identified c.494delG in CRYGD being co-segregated with the disease in all affected members. Neither unaffected family members nor 103 unrelated controls carried this deletion mutation, which causes a frameshift and results in early termination of the polypeptide to become Gly165AlafsX3. Significant reduced solubility was observed for this mutant protein. Unlike the wildtype protein which is cytoplasmic, Gly165AlafsX3 was predominantly located to the nuclear envelope and colocalized with lamin A/C. Conclusions: We have identified a novel mutation c.494delG in CRYGD associated with lamellar congenital cataract. This is the first characterized deletion mutation of CRYGD to be disease-causing for ADCC. The mutant protein with loss of detergent solubility and apparent impairment of stability is the first CRYGD variant found to localize in the nucleus.

1243/F

Novel *MMP20* mutation underlying autosomal recessive hypomaturation amelogenesis imperfecta. *S-K. Lee[1], F. Seymen[2], K. Gencay[2], B. Tuna[2], J-W. Kim[1,3].* 1) Department of Cell and Developmental Biology & Dental Research Institute, Seoul National University, Seoul, Korea; 2) Department of Pedodontics, Istanbul University, Istanbul, Turkey; 3) Department of Pediatric Dentistry & Dental Research Institute, Seoul National University, Seoul, Korea.

Autosomal recessive hypomaturation amelogenesis imperfecta can be caused by two genes (*KLK4* and *MMP20*). Both proteinases involved in enzymatic degradation of structural enamel matrix proteins. So far only 3 mutations have been identified (one in *KLK4* and two in *MMP20*). Here we report a novel *MMP20* mutation in a consanguineous kindred in Turkey. The identified mutation was g.18,742G>A, c.910G>A, p.A304T in the exon 6 (based on NT_033899.7, NM_004771.3, and O60882). Sequence analysis of 100 healthy normal controls did not reveal this sequence alteration, indicating that this mutation is not a common variation. The proband and his affected brother were both homozygous for this mutation, consistent with consanguinity. The parents and one unaffected sister were carriers of the mutation. The enamel thickness was normal. Lots of enamel of primary teeth and permanent first molar were lost due to hypomaturation combined with caries lesions. Newly erupted teeth had chalky white hypomaturation enamel with mild discoloration. Discoloration was getting dark with increasing ages. This study shows phenotypic variation according to patients' age and may lead us better understanding molecular basis of the disease. This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A060010) and the Korea Science and Engineering Foundation (KOSEF) through the Biotechnology R&D program (#2006-05229).

1244/F

Identification of the *DLX3* mutation (c.561-562delCT) in a new family and its phenotypic variation. *J-W. Kim[1,2], S-K. Lee[2].* 1) Department of Pediatric Dentistry & Dental Research Institute, Seoul National University, Seoul, Korea; 2) Department of Cell and Developmental Biology & Dental Research Institute, Seoul National University, Seoul, Korea.

The tricho-dento-osseous (TDO) syndrome is an autosomal dominant disease characterized by curly hair at birth, enamel hypoplasia, taurodontism, and thick cortical bone. Common 4 bp deletion (c.571-574delGGGG) in *DLX3* gene has been identified in multiple families with variable clinical phenotype. Recently another mutation (c.561-562delCT) in *DLX3* gene has been reported to cause autosomal dominant amelogenesis imperfecta with taurodontism. Here we report identification of c.561-562delCT mutation in *DLX3* gene in a new family and its clinical phenotype. The family was 3 generation Korean kindred. Enamel was hypomatured and slightly hypoplastic. Several teeth were suffered from excessive wear resulting spontaneous pulp exposures. The characteristic taurodontic feature was not identified in 3 affected individuals. Increased bone density or thickness could not be revealed by cephalometric and panoramic radiographs. Affected individuals reported that their fingernails and toenails were brittle. And also they had curly hair at birth. This study clearly showed that c.561-562delCT mutation had not only enamel defects, but also the other clinical phenotypes resembling TDO syndrome. This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A060010) and the Korea Science and Engineering Foundation (KOSEF) through the Biotechnology R&D program (#2006-05229).

1245/F

A balanced 2;7 translocation associated with hereditary gingival fibromatosis. *P.S. Hart[1], J.H. Guo[2], S.I. Jang[2], M.J. Pettenati[3], D. Pallos[4], T.C. Hart[2].* 1) Office of the Clinical Director, NHGRI, Bethesda MD; 2) Section of Human and Craniofacial Genetics, NIDCR, Bethesda MD; 3) Section on Medical Genetics, Wake Forest University, Winston-Salem NC; 4) Department of Periodontics, University of Taubate, Sao Paulo, Brazil.

Hereditary gingival fibromatosis (HGF) can occur as an isolated or syndromic trait. Genetic heterogeneity has been documented for the isolated, nonsyndromic forms, with at least 4 loci localized by linkage studies. Mutation of the *SOS1* gene on 2p22 is the only causative gene identified to date. The mutant SOS1 protein constitutively activates the MAP kinase signaling pathway, and is associated with increased fibroblast proliferation. We ascertained a father and son with isolated HGF who carry a balanced translocation: 46,XY,t(2;7)(p23.3;p13). Sequence analysis of *SOS1* revealed no mutations. Gingivectomy was performed on the son and excised tissue provided a source of RNA, DNA and gingival fibroblasts. A Nimblegen CGH array constructed to analyze small gains or losses of material in the translocated regions revealed no alterations. FISH analysis was subsequently undertaken to refine the breakpoints. The chromosome 2 breakpoint was localized to 777 kb, between BACs RP11-156M23 and -195B17. The chromosome 7 breakpoint localized to the last 386 kB of 7p13. Proliferation assays revealed higher growth rates in HGF fibroblasts compared to control fibroblasts, documenting the clinical phenotype of HGF. After 3 days of growth, BrdU incorporation in the HGF fibroblasts reached steady state and was 50% higher than in control fibroblasts. Increase in total cell numbers, monitored up to day 11, revealed the HGF growth rate was 3X higher than that of controls. Analysis of gene expression profiles in cultured fibroblasts using CodeLink Human Whole Genome Arrays revealed a pattern of expression similar to that seen in HGF due to *SOS1* mutation, including up-regulation of cyclins E1 and E2, DP1, E2F1 and E2F2, all of which function in the cell cycle progression from G1 to S phase. These findings indicate further genetic heterogeneity for HGF, but indicate common underlying alterations in gene expression and cell behavior.

1246/F

Twin complex rearrangements of Xq28 caused by distinct break-induced replication in haemophilia A. *C.R. Sheen[1], U.R. Jewell[2], C.M. Morris[2, 3], S.O. Brennan[1], C. Férec[4,5], P.M. George[1], M.P. Smith[6], J.M. Chen[4,5].* 1) Molecular Pathology, Canterbury Health Laboratories, Christchurch, Canterbury, New Zealand; 2) Cancer Genetics Research Group, Christchurch School of Medicine and Health Sciences, University of Otago, Christchurch, New Zealand; 3) Cytogenetics Unit, Canterbury Health Laboratories, Christchurch, New Zealand; 4) Institut National de la Santé et de la Recherche Médicale (INSERM), U613, 29220 Brest, FranceDistrict Health Board, Christchurch, New Zealand; 5) Etablissement Français du Sang-Bretagne, 29220 Brest, France; 6) Haematology Service, Canterbury District Health Board, Christchurch, New Zealand.

Rearrangements of the genome are a well-recognized cause of genetic disease and can form through a variety of mechanisms. We describe a complex rearrangement that causes severe haemophilia A, elucidated using a variety of PCR based methods and confirmed using array-CGH. The rearrangement consists of a 15.5 kb deletion/16 bp insertion that deletes exon 1 and the promoter of the Factor VIII gene, located 0.6 kb from a 28.1 kb deletion/263 kb insertion at Xq28. We propose that the rearrangement was formed by distinct cellular responses to double strand breakage. The latter insertion/deletion can be explained by break-induced replication, while we propose a novel model of break-induced serial replication slippage for the former. This may provide an alternative explanation to oligonucleotide capture for the frequent observation of short inserted sequences at deletion breakpoint junctions. The copy number of several genes is affected by this rearrangement, with deletion of part of the Factor VIII gene (causing haemophilia A) and the FUNDC2 gene, and duplication of the FAM11A, HSFX1, MAGEA9 and MAGEA11 genes. Given that the patient has manifested no detectable phenotype other than haemophilia, it appears the biological effects of the other genes involved are not strictly dosage dependent.

1247/F

UMD-predictor, a new prediction tool for missense mutation pathogenicity; application to the FBN1 gene. *C. Beroud[1,2,3], M. Frederic[1,2], M. Lalande[1], C. Boileau[4,5], D. Hamroun[3], M. Claustres[1,2,3], G. Collod-Beroud[1,2].* 1) INSERM, U827, Montpellier, F-34000 France; 2) Université MONTPELLIER1, UFR Médecine, Montpellier, F-34000 France; 3) CHU Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Génétique Moléculaire, Montpellier, F-34000 France; 4) INSERM, U383, Paris, F-75000 France; 5) AP-HP, Hôpital Ambroise Paré, Laboratoire de Génétique Moléculaire, Boulogne, F-92000 France.

Among the millions of nucleotide substitutions reported in the human genome, thousands are localized in the coding sequence of various genes and result in a synonymous or a non-synonymous change at the protein level. In parallel it has been shown that half of the gene lesions responsible for human inherited diseases are due to missense mutations. One of the biggest challenges in human genetics is therefore to distinguish neutral variations from disease causing mutations. We thus developed the UMD-predictor tool that allows the analysis of all substitutions. For each variation, the prediction is based on the combination of several arguments: its location at protein level, its conservation and biochemical properties (data from SIFT, BLOSUM62 and Biochemical data) and search for creation/suppression of potential splice sites or regulator splice sequences. Each variation is predicted to be a “pathogenous mutation”, a “probable pathogenous mutation”, a “probable polymorphism” or a “polymorphism”. To evaluate the performances of this new tool, we compared it with the different existing programs (SIFT, BLOSUM62, Biochemical Value and PolyPhen), using data from the UMD-FBN1 database that contains 1249 mutations among which 709 missense mutations corresponding to 528 mutational events. Our results show that the UMD-predictor algorithm is the most efficient tool to predict pathogenous mutations for the FBN1 gene with a positive predictive value of 99.4% (sensitivity of 95.6 % and specificity of 94.4 %). The UMD-predictor tool is available at http://www.umd.be/UMD-predictor and can be applied to all human genes. It can thus be useful to evaluate the pathogenous impact of all SNPs localized in the coding sequence as well as variations found in patients.

1248/F

Predicting Pathogenicity of *NF1* Missense Mutations. *R. Loda[1], D. Driscoll[2], M. Wallace[1,2].* 1) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) Department of Pediatric Genetics, University of Florida, Gainesville, FL.

Mutations in the neurofibromatosis 1 (*NF1*) gene cause this autosomal dominant disorder affecting 1 in 3,000 births, in which individuals are predisposed to multiple tumors. Hundreds of different *NF1* gene mutations are known, of all types and sizes. Most of these are clearly disruptive, predicted to result in reduced, absent, or truncated protein (neurofibromin), the cellular effects of which are not fully understood. Missense mutations are a diagnostic challenge because a priori they can be pathogenic or neutral polymorphisms. Further, most *NF1* missense mutations are nearly impossible to test functionally. In NF1, missense mutations account for 10-20% of germline lesions. Novel missense mutations identified in patients must first be tested to see if splicing errors instead of amino acid substitution occurs. If not, and there is no useful information from the family or literature, other methods must be used to predict the mutation’s pathogenicity. This is crucial, as it may determine if the patient is diagnosed with NF1. Therefore, we examine the fidelity of computational tools designed to predict the pathogenicity of missense mutations. Recently, new computational tools have been developed that exploit increasing genome sequence and structural data. Several comparisons of the fidelity of these programs are available. As neurofibromin is larger and more complex than proteins analyzed previously, it should be a robust test of the efficiency and accuracy of these programs. To establish accuracy, a data set including mutations known to be pathogenic, neutral, and those from functional studies of neurofibromin’s isolated Gap-related domain is used. An experimental set of several novel mutations of unknown pathogenicity is also analyzed. The *NF1* gene has proven a challenge for these programs, but through analysis we have gathered data about user interfaces, pitfalls, and nuances needed for their accurate application to predicting pathogenicity of *NF1* missense mutations. This data will be useful for diagnostic labs that need to provide predictive information on missense mutations.

1249/F

Ichthyosis, follicular atrophoderma, and hypotrichosis is associated with mutations in matriptase. *T. Alef[1], S. Kolberg[1], S. Torres[1], I. Hauβer[2], D. Metze[3], U. Türsen[4], G.G. Lestringant[5], H.C. Hennies[1].* 1) Univ. of Cologne, Cologne Center for Genomics, Div. of Dermatogenetics, Germany; 2) Univ. of Heidelberg, Dermatology, Germany; 3) Univ. of Münster, Dermatology, Germany; 4) Univ. of Mersin, Dermatology, Turkey; 5) Tawam Hospital, Al Ain, United Arab Emirates.

Autosomal recessive congenital ichthyosis encompasses a large, heterogeneous group of disorders of cornification. Isolated forms and ichthyosis associated with other signs of disease can be differentiated. We have recruited two consanguineous families with similar phenotypes from the United Arab Emirates and Turkey. Five sibs of the Emirati family, three girls and two boys, showed normal stature, diffuse congenital ichthyosis, patchy follicular atrophoderma, generalized and diffuse non-scarring hypotrichosis, and marked hypohidrosis. The affected girl of the Turkish family showed dispersed congenital ichthyosis, follicular atrophoderma, hypotrichosis, and woolly hair. Histopathologically, epidermis was of regular thickness, stratum granulosum thinned and stratum corneum orthohyperkeratotic. Hair follicle epithelium was thinned, hair infundibulum showed hyperkeratosis and a very thin stratum granulosum. EM analysis showed deposits of lamellar bodies in the lower parts of the stratum corneum. By genome wide linkage analysis we identified regions with LOD scores >3 on chromosomes 2 and 11. The interval on 11q24-q25 contained the suppression of tumorigenicity 14 gene (*ST14*), which has 19 exons and spans 50 kb of genomic DNA. Its gene product, matriptase, is a type II transmembrane serine protease expressed in most epithelia. We found a homozygous splice site mutation (c.2269+1G>A) in the Emirati patients and a 1-base deletion (c.2034delG) leading to a premature stop codon in the Turkish patient. The mutations confirm the role of matriptase as an essential enzyme in the process of epidermal differentiation. Western blot analysis showed reduced proteolytic activation of prostasin and processing of filaggrin. Since filaggrin monomers play a pivotal role in epidermal barrier formation, we suggest that matriptase acts upstream of prostasin in a zymogen activation cascade that regulates terminal epidermal differentiation.

1250/F

Facilitating DNA diagnostics by collecting human disease gene variation using an open source LSDB-in-a-Box platform - LOVD 2.0. *I.F.A.C. Fokkema, P.E.M. Taschner, G.J.B. van Ommen, J.T. den Dunnen.* Human and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland, http://www.DMD.nl/.

Locus-Specific mutation DataBases (LSDB) play an important role in DNA diagnostics facilitating proper evaluation of the variant, i.e. being pathogenic or not. For LSDBs to be of highest value, it is evident that all variants identified world-wide, pathogenic or not, should be collected and made available without delay. Although everybody realizes that only a complete and fully up-to-date LSDB is most helpful, daily practice shows that the reporting of mutations identified, outside scarce publication in scientific journals, is rather infrequent. With a local focus on genes involved in muscular dystrophies we have tried several approaches to improve the collection and curation of these variants. First, we have developed the Leiden Open Source Variation Database (LOVD[1]) software (http://www.LOVD.nl). The software is fully web-based, platform-independent, open source, built as an LSDB-in-a-Box and follows current HGVS guidelines. To promote initiation of LSDBs we offer free server space and installation on the Leiden server. LOVD currently stores 18,000 variants in 51 genes contributed by 150 submitters world-wide. In collaboration with UCSC, variants can be viewed in the genome browser using the Locus Variants track, linking directly back to the original data collected in LOVD. To improve consistency and reduce the numbers of errors we have implemented error-checking using the Mutalyzer mutation nomenclature checker module (http://www.LOVD.nl/mutalyzer). In the latest version of LOVD we have enhanced flexibility and e.g. included the possibility to store information about sequence variants in multiple genes per patient. LOVD 2 has a dynamic structure allowing curators to add per gene any column. These features allow the use of LOVD 2 for federated LSDBs as suggested in the recent recommendations of the Human Variome Project meeting[2].

1) Fokkema IFAC et al. (2005). Hum.Mutat. 26: 63-68. 2) Cotton RGH et al. (2007). Nat.-Genet. 39: 433-436.

1251/F

Biological Process of Aging: A Study on antioxidant enzymes, DNA damage, Smoking and Body mass index. *P.K.R. THAVANATI[1], K.R.R. KANALA[2], A. ESCOTO DE DIOS[3], J.M. CANTU GARZA[1].* 1) Instituto de Genetica, Departamento de Biologia Molecular y Genomica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, 950 Sierra Mojada, Col.Independencia, Guadalajara. Jal. 44340, Mexico; 2) Department of Anthropology, School of Biological & Earth Sciences, Sri Venkateswara University, Tirupati-517 502. Andhra Pradesh. India; 3) Genetica, Centro de Investigacion Biomedica de Occidente, Instituto Mexicano del Seguro Social, 900 Sierra Mojada, Col. Independencia, Guadalajara. Jal. 44340, Mexico.

Oxidative damage to DNA is shown to be extensive and could be a major cause of physiological changes associated with aging and degenerative diseases such as cancer, cardiovascular diseases, immune-system decline, diabetes mellitus etc.,. Antioxidants are believed to decrease the attacks on DNA by free radicals and thus, protect against mutations that cause disease status. It has also postulated that, variation in the life-style measures act as stimulants of free radical generation, DNA damage and reduced antioxigenic potential. Understanding how such damage contributes to age-related changes requires attention to explain how these different mechanisms relate and potentially interact with each other. The present study involves the findings on endogenous antioxidant enzyme levels and DNA damage which are critically examined in order to evaluate whether oxidants do contribute to the initiation and / or propagation of ageing for which 220 healthy male volunteer samples from the defined electoral area (suburbs of Tirupati, Andhra Pradesh, India) aged 20-80 years were studied for the evaluation of lymphocyte antioxidant enzymes i.e., glutathione S-transferase, superoxide dismutase, catalase and DNA damage in relation to smoking and BMI. A two fold increase of lymphocyte free radical generation and DNA damage in older than in younger age groups is observed. And, an increased free radical generation and reduced antioxidant potential form a link between cigarette smoking and oxidative stress represented by antioxidant imbalance. Body mass index had a positive relation with oxidative stress but, antioxidant levels didn't vary with.

1252/F

Genomic strategy identifies Stratifin (*SFN*) and WD-Repeat Domain 65 (*WDR65*) as candidate genes for cleft lip and palate. *N. Rorick[1], A. Kinoshita[1], M. Peyrard[2], S.L. Goudy[3], J. Weirather[1], R. Ferreira de Lima[4], D. Moretti-Ferreira[4], H. Koillinen[5], J. Kere[2], J.C. Murray[1], B.C. Schutte[1].* 1) Genetics PhD Program, Univ Iowa, Iowa City, IA; 2) Karolinska Institutet, Huddinge, Sweden; 3) Vanderbilt University, Nashville, TN; 4) UNESP, Botucatu, Brazil; 5) University of Helsinki, Helsinki, Finland.

Genetic variation in the transcription factor Interferon Regulatory Factor 6 (*IRF6*) causes Van der Woude (VWS) and popliteal pterygium syndromes (PPS), two autosomal dominant orofacial clefting disorders, and contributes risk for isolated cleft lip and palate (CLP). We hypothesized that genes regulated by IRF6 might also be involved in orofacial clefting disorders. We used five criteria to identify potential IRF6 target genes; differential gene expression in skin taken from Irf6 wild type and mutant mouse embryos, localization to the VWS2 critical region (1p36-1p32), overlapping expression with *Irf6*, presence of a conserved putative IRF binding site in the promoter region, and a mutant mouse phenotype that is similar to the *Irf6* mutant mouse. Microarray analysis showed altered expression for 573 genes, and 13 of these genes were located in the mouse region syntenic to the VWS2 locus. Two of these genes, *Wdr65* and *Sfn* (14-3-3 sigma), met four of the five possible criteria. *Wdr65* is a novel gene that encodes a predicted protein of 1250 amino acids with two WD domains. *Sfn* encodes a phosphobinding protein, and like *Irf6*, is required for skin, limb and craniofacial development in mice. As potential targets for Irf6 regulation, we hypothesized that disease-causing mutations will be found in both *WDR65* and *SFN* in clefting populations. No mutation was identified in either gene in the proband with VWS2. However, a potentially etiologic missense mutation was found in *WDR65* in a patient with VWS that does not have exonic mutations in IRF6, suggesting that *WDR65* could be a second causative gene for VWS. Additionally, a potentially etiologic mutation in *SFN* was found in a patient with isolated CLP. In sum, this genomic approach identified two new candidate genes for CLP, *WDR65* and *SFN*.

1253/F

Mutational Analysis of 58 patients with oculocutaneous albinism in Denmark. *J. Ek, K. Grønskov, A. Sand, T. Rosenberg, K. Brondum-Nielsen.* Kennedy Institute - National Eye Clinic, Glostrup, Denmark.

Oculocutaneous albinism (OCA) is a genetic heterogeneous disorder caused by hypopigmentation of the eyes, hair and skin. The hypopigmentation results from defects in melanin production. Lack of melanin in the eyes causes misrouting of the optic nerve fibers, resulting in nystagmus, foveal hypoplasia, strabismus, photophobia and greatly decreased visual acuity. Hypopigmentation of the skin results in enhanced disposition to skin cancers. Clinical diagnosis of subtypes of OCA is difficult due to phenotypic variation and overlap between the different types of OCA, and genetic analysis is often helpful in establishing the diagnosis, and essential for genetic counselling in relation to prenatal diagnosis. We investigated 58 patients with OCA for mutations in four genes known to cause OCA, namely TYR causing OCA1 (OCA1A and OCA1B), OCA2 causing OCA2, TYRP1 causing OCA3 and MATP causing OCA4. Overall, we found at least two mutations capable of explaining the OCA in 44 % of the patients (26 patients of 58), 29 % (17 of 58) had one mutation, and in the remaining 26 % (15 of 58) we did not find mutations in any of the four genes. Of the 26 patients with two mutations, 62 % (16 of 26) had two mutations in TYR, 31 % (8 of 26) had two mutations in OCA2 and 7 % (2 of 26) had two mutations in MATP. No mutations were found in TYRP1.

1254/F

Identity and Carrier Frequency of Cytochrome P450 1B1 Mutations in the U.S. Population: Implications for Primary Congenital Glaucoma Disease Incidence and Newborn Screening. *F.M. Hantash, D.M. Goos, W. Conlon, B. Anderson, S. Strom, W. Sun, C.M. Strom.* Molecular Genetics, Quest Diagnostics Nichols Institute. SJC, CA.

Several studies have associated the CYP1B1 gene with PCG. Untreated or late treated PCG accounts for an appreciable amount of adult blindness in the United States. Our DNA sequencing of CYP1B1 gene from a number of suspected PCG patients identified several mutations. However, there have been no comprehensive studies of carrier rate, mutation frequency, or PCG disease incidence in the U.S. population. We conducted comprehensive DNA sequencing analysis of the two coding exons 2 and 3 of CYP1B1 on anonymized DNA samples. Sixteen hundred forty five samples were analyzed. A total of seventy-eight DNA samples harbored one of twenty missense mutations, while one sample harbored a frame-shift mutation. Only eight mutations identified are predicted to be pathogenic based on prior published studies and/or consensus algorithm analyses. Results showed that the most common pathogenic mutations in the U.S. population to be R368H (N=12) and Y81N (N=9), with a carrier frequency of 0.67% and 0.55%, respectively. The R368H and Y81N mutations occurred on the same haplotypes as those reported previously. Interestingly, another predicted pathogenic mutation affecting codon 368 was identified in one sample. The combined carrier rate of different pathogenic mutations identified is 1.52% (~1:66). Since various studies showed delayed or reduced penetrance of homozygotes or compound heterozygotes for deleterious CYP1B1 mutation alleles in PCG, our data would predict an incidence of PCG in the U.S. population to be 1:17313-1:34626, a range of the same order of magnitude for many inherited disorders in established newborn screening programs. Since PCG can be confirmed by measuring intraocular pressures and visual impairment can be prevented or minimized with timely surgery, PCG is an excellent candidate for newborn screening. We designed a rapid carrier mutation assay for 10 pathogenic CYP1B1 mutations and showed the assay to work on DNA from blood spot cards, showing feasibility of genetic newborn screening test.

1255/F

Heterogenous and differential spectrum of mutations in CFTR gene in Indian Cystic fibrosis and obstructive azoospermia population. *R. Prasad[1], N. Sharma[2], G. Kaur[3], S. Singh[4], N. Acharya[5], M. Singh[6].* 1) Biochemistry, PGIMER, Chandigarh, India; 2) 1; 3) Physiology, GMCH, Chandigarh, India; 4) Urology, 1; 5) 4; 6) Pediatrics,1.

Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) gene. We conducted a study to characterize mutations in the CFTR gene in Indian CF and obstructive azoospermia population. Fifty CF and 25 obstructive azoospermia subjects were included. Obstructive azoospermia patients had no clinical symptoms of CF, except unilateral/bilateral absence of vas deferens. Age at the time of diagnosis was significantly higher in CF patients. Sweat chloride was raised in 47 CF and 14 azoospermia subjects. Mutation analysis was performed on genomic DNA isolated from peripheral blood of CF patients. Sixteen substitution, 3 deletion, 3 insertion, 1 splice site, 2 nonsense and 2 intronic mutations were detected. Delta F508, the most common mutation, had a frequency of 24.0% in Indian CF population. However, increased number of patients (n=18) were found heterozygous for deltaF508 mutation in obstructive azoospermia population. Other mutations like G551D, R553X, N1303K, R117H and 3849+10kbC-T had a frequency less than 2%. On SSCP and direct sequencing, mutations detected were 1161 del C, 3986 delC, 1580 Ins A, 1792 Ins A, 4333InsG, L69H, F87I, G126S, F157C, S158N, Q493L, Y517C, V520F, I530L, S549N, E1329Q, Y1381H, 3120+1G-A, L218X, R553X, 876-4delACAG. Nine mutations were found novel. Genotype-phenotype correlation indicated that Class I, II and III mutations were severe. Haplotyping revealed that all analysed delta F508 chromosomes carried the same KM19-GATT-M470V-T854T-TUB20 haplotype. We report a heterogenous and differential spectrum of mutations in CFTR gene in CF and obstructive azoospermia cases. We confirm the existence of CF in Indian population and it is proposed that decreased incidence of delta F508 mutation among CF patients is due to under diagnosis, environmental differences, dietary factors and the role of modifier genes.

1256/F

Validation of GNE:pM712T Identification by Melting Curve Analysis. *Y. Valles-Ayoub[1], C. Saechao[1], A. Haghighatgoo[1], M.S. Neshat[1], M. Pietruszka[1], D. Darvish[1, 2].* 1) HIBM Research Group, Encino, CA; 2) VA Greater Los Angeles (VA-GLA/UCLA), Los Angeles, CA.

HIBM/DMRV is an adult onset autosomal recessive muscle wasting disease common in people of Iranian-Jewish descent, due to the founder allelic variant GNE:p.M712T. High correlation of disease susceptibility with GNE:p.M712T allows its use as a molecular marker for diagnosis. In this study, we applied and validated the use of Melting Curve Analysis using SimpleProbe® technology for detection of this mutation using specimens obtained by mouthwash, buccal swab, and whole blood. The assay was then applied to 43 clinical specimens and results were validated by additional methods. A probe spanning this mutation in exon twelve accurately discerns two Tm corresponding to its hybridization to wild type and M712T derived amplicons. A 10ºC divergence in Tm allowed rapid single tube genotyping of reference and patient samples with 100% accuracy. Distal myopathy constitutes a large heterogeneous group of pathologies with similar physiological manifestations and little molecular markers for distinguishing subtypes. Application of Simple Probes® for detection of GNE:p.M712T on genomic DNA obtained from buccal epithelial cells allows accurate, rapid and cost effective identification of this allele in individuals at risk. This procedure is amenable to automated high throughput applications and can be extended to both clinical and research applications.

1257/F

Novel mutations of DNA polymerase γ *(POLG1).* *Q. Zhang, E.S. Schmitt, N. Brunetti-Pierri, P.C. Chou, C. Truong, J. Wang, W.J. Craigen, L-J. Wong.* Department of Molecular and Human Genetics, Baylor Colloge of Medicine , Houston, TX 77030.

Human mitochondrial DNA is replicated by the nuclear-encoded DNA polymerase γ *(POLG1).* Mutations of *POLG1* are responsible for a variety of mitochondrial diseases including dominant and recessive forms of progressive external ophthalmoplegia (PEO), Alpers syndrome, Parkinsonism, juvenile spinocerebellar ataxia-epilepsy syndrome (SCAE), as well as sensory ataxia, neuropathy, dysarthria and ophthalmoparesis (SANDO). In order to understand the importance of this gene in the molecular etiology of patients with these mitochondrial disorders, we sequenced the coding exons of *POLG1* in approximately 370 patients. A total of 37 different *POLG1* mutations were identified in 44 patients. 27 patients carried two mutated alleles, 17 had only one identified mutation, two patients were homozygous. The mutations include 71 missense (32 unique), 1 nonsense, 2 insertion/deletion frameshift (both unique), and 2 splice site mutations (both unique). Large deletions or duplications were not found. Approximately 54% (20 out of 37 different ones) of molecular alterations were located in the polymerase domain, the remaining were in the exonuclease domain or linker region. A467T is the most common mutation accounting for 20% of mutations (18 alleles in the 44 positive patients). G848S was the second most common mutation (observed 8 times), which resided in the polymerase domain. Significantly, 22 novel mutations were identified in either heterozygous or compound heterozygous type. They are: G11D, Q68X, L83P, H110Y, S305R, L392V, R853Q, V855A, L886P, G888S, R943C, R946C, I1079L, S1095R, R1138C, K1191R, D1196N, G1205A, c.1270delCT, c.2157+5 G>A, c.2480+1G>A, and c.2544_2545insGC. Each novel *POLG1* mutation was specific to an individual family. These findings indicated that *POLG1* is a major nuclear gene responsible for mitochondrial disorders.

1258/F

A recurrent mutation in the ARS gene in a Tunisian family with Mal de Meleda and congenital cataract. *M. BCHETNIA[1,2], C. CHARFEDDINE[1], S. KASSAR[3], M. MOKNI[2, 4], S. BOUBAKER[3], S. GHEDAMSI[4], A. DHAHRI-BEN OSMAN4[4], S. ABDELHAK[1].* 1) Molecular Investigation of Genetic Orphan Diseases Research Unit (MIGOD) UR 26/04, Institut Pasteur de Tunis, Tunisia; 2) Study of Hereditary Keratinization Disorders Research Unit (THK) UR 24/04, La Rabta Hospital, Tunis, Tunisia; 3) Anatomo-Pathology Department, Institut Pasteur de Tunis, Tunisia,; 4) Dermatology Department, La Rabta Hospital, Tunis, Tunisia.

Mal de Meleda (MDM) also referred to as keratosis palmoplantaris transgrediens of Siemens, is a rare autosomal recessive skin disorder with a prevalence in the general population of 1 in 100 000. The disease locus of MDM has been mapped to chromosome 8qter and in recent studies; homozygous mutations in the ARS (component B) gene have been identified in families with this disorder. The ARS gene encodes a secreted Ly-6/uPAR (lymphocyte antigen 6/urokinase-type plasminogen activator receptor) related protein 1 (SLURP-1). In this report, we performed mutational analysis of the ARS gene by direct sequencing in a large consanguineous family of Tunisian origin with MDM and hereditary congenital cataract. The mutation C99Y previously reported exclusively in Tunisian families was identified. This finding suggests that the ARS gene is likely to be responsible for MDM in this family and shows evidence of a founder effect in the Tunisian families sharing a common ancestral allele. The molecular exploration of the congenital cataract and its association with this type of palmoplantar keratoderma needs further investigations.

1259/F

Jagged1 (JAG1) mutation metection in a Brazilian Alagille Syndrome population. *I.K. Miura[1], F.E. Arimura[1], M.S. Floriano[2], A.C. Pereira[2], G. Porta[1].* 1) Department of Pediatrics, University of São Paulo Medical School, São Paulo, Sao Paulo, Brazil; 2) Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil.

Alagille syndrome (AGS) is a dominantly inherited disorder characterized by liver disease in combination with heart, skeletal, ocular, facial, renal, and pancreatic abnormalities. The human Jagged1 gene (JAG1) on chromosome 20p12 was identified as the AGS disease gene. JAG1 encodes a ligand in the Notch intercellular signaling pathway. Mutations in JAG1 have been found to result in the AGS phenotype and both protein truncating mutations and missense mutations have been identified. Through sequencing, we are screening 28 AGS affected individuals from 27 families for mutations within Jagged 1. So far, six distinct mutations were identified in 7 (25%) AGS cases. The mutations include three small deletions (43%) and four missense mutations (57%). Thirteen polymorphisms were found. These mutations as well as several polymorphisms are spread across the entire coding sequence of the gene. There are no phenotypic differences between patients. As shown in other studies from different cohorts, this study did not find genotype-phenotype correlation. The results of this study are consistent with the proposal that haploinsufficiency for wild type Jagged 1, in missense mutations, may result in AGS phenotype. Further studies like family screening are needed to determine the rate of de novo mutations as well as microsatellite analysis to determine deletions and translocations.

1260/F

Genotype-phenotype correlation in a group of cystic fibrosis mexican patients. *E. Yokoyama[1], M. Chávez[1], C. Villarroel[1], F. Cuevas[2], A. Carnevale[3], J.L. Lezana[4], S. Frías[1], B. Molina[1], L. Orozco[5].* 1) Departamento de Investigación en Genética Humana, Instituto Nacional de Pediatría; 2) Servicio de Neumología, Instituto Nacional de Pediatría; 3) Coordinación Nacional de Medicina Genómica, ISSSTE; 4) Departamento de Neumología, Hospital Infantil de México; 5) Laboratorio de Genómica de Enfermedades Multifactoriales, INMEGEN.

INTRODUCTION: Cystic fibrosis (CF) is the most common autosomal recessive disorder. More than 1,400 mutations have been described in the cystic fibrosis transmembrane conductance regulator (*CFTR*) and the most common in Caucasian population is the ΔF508 mutation. The classification of these mutations, as "severe" and "mild", is according to their effect on the protein. There is a strong genotype-phenotype correlation for the pancreatic sufficiency; however this correlation is not clear for the other clinical manifestations as pulmonary disease. **OBJECTIVE**: This study aimed to correlate *CFTR* genotype with the clinical manifestations in a group of Mexican patients with CF. **MATERIAL AND METHODS**: Sixty Mexican patients with CF were included. They were divided in three groups; GROUP 1: homozygous for ΔF508; GROUP 2: both alleles with severe mutations non ΔF508; GROUP 3: at least one allele with mild mutation. Statistical analysis were done with the SPSS® 11.0 version and an alpha level of 0.05 was considered to indicate statistical significance. **RESULTS AND DISCUSION**: Groups 1 and 2 had pancreatic insufficiency. In these groups the age of CF diagnosis and the first infection by *Pseudomona aeruginosa* were earlier than in group 3, making the phenotype more severe in patients with pancreatic insufficiency. At the same time, patients of the groups with severe mutations showed high levels of sweat chloride test. The presence of meconium ileus as well as the pulmonary phenotype measured by spirometry and expressed as a percentage of the predicted value for sex, age and height, did not correlate with *CFTR* genotype, probably because of the sample size or because there are other modifier genes involved.

1261/F

Fragile X Related Protein 2 (FXR2P) interacts with non-POU domain containing, octamer binding protein (NonO). *S.S. Pataskar, D.L. Nelson.* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX. USA.

Fragile X syndrome is a common form of mental retardation caused by the absence of the FMR1 protein, FMRP (Fragile X Mental Retardation Protein).Two paralogs of FMRP have been identified, FXR1P and FXR2 (Fragile X Related Protein). All three proteins share highly conserved RNA binding domains, high sequence similarity and show overlap in their tissue distribution. FXR2P shows high expression in brain and testes similar to FMR1P. NonO (also called p54nrb)is an unusual nucleic acid binding protein expressed ubiquitously. It not only binds both DNA and RNA but also interacts with other nucleic acid binding proteins. NonO enhances association of many DNA binding proteins to their targets. NonO interacts with the Parkinson's disease associated DJ-1 protein. NonO is also involved in splicing of mRNAs. NonO modulates the transcriptional activity of Androgen Receptor. NonO has also been shown to be associated with circadian clock protein, PERIOD 1 and modulates activity of PER proteins antagonistically. FXR2P has also been shown to modulate clock activity, and *Fxr2* knockout mice showed irregularities in their circadian cycles.We investigated interaction between NonO and FXR2P. Our results indicate that endogenous FXR2P coimmunoprecipitates endogenous NonO in cell lysates prepared from mouse tissues and cell lines. Both FXR2P and NonO are RNA binding proteins and play a role in regulating clock proteins. Direct interaction between FXR2P and NonO suggests a potential mechanism for clock abnormalities in *Fxr2* knockout mice and adds to our understanding of the various functions carried out by members of the Fragile X gene family.

1262/F

Utilization of Model Systems to Characterize Mutant BBS3/ARL6 Function. *P.P. Pretorius[1,2,3], R.F. Mullins[4], C.C. Searby[1,3], E.M. Berg[1,3], D.Y. Nishimura[1], M.P. Andrews[1,3], E.M. Stone[3,4], D.C. Slusarski[5], V.C. Sheffield[1,3].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Genetics Ph.D. program, Univ Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute; 4) Dept Ophthalmology, Univ Iowa, Iowa City, IA; 5) Dept of Biology, Univ Iowa, Iowa City, IA.

Bardet-Biedl Syndrome (BBS) is a pleiotropic disorder characterized by obesity, retinitis pigmentosa, polydactyly, renal abnormalities, hypogenitalism and cognitive impairment. To date, twelve BBS genes have been identified. BBS3 accounts for approximately 1% of all BBS cases. A member of the Ras family of small GTP-binding proteins, *BBS3* contains a GTP-binding site motif and is postulated to play a role in vesicular transport. In order to characterize known human *BBS3* mutations, a variety of model systems were utilized. Immunohistochemical analysis of human and mouse retina reveals BBS3 expression in both the ganglion and photoreceptor cell layers. Subcellular localization in 293T cells indicates that BBS3 is localized to the plasma membrane in the GTP-bound state. BBS3 mutations that have been identified in patients appear to impair BBS3 protein expression as well as diminish localization to the plasma membrane in 293T cells. To further evaluate the effects of *BBS3* deficiency, a morpholino antisense oligonucleotide approach was utilized to transiently knock down *bbs3* gene expression in zebrafish. Morphant embryos show defects to the ciliated Kupffer's Vesicle as well as a delay in retrograde transport. Both defects exhibited a dose dependent response to knock down and are typical of BBS phenotypes in zebrafish. Additional studies are underway to examine the BBS3 mutations in zebrafish. These studies establish an initial functional characterization of wild type and mutant BBS3 proteins.

1263/F

Towards saturation mutagenesis of human *NIPBL* to evaluate its function in Cornelia de Lange Syndrome and sister chromatid cohesion. *M.A. Deardorff[1], M. Kaur[1], D. Yaeger[1], L.G. Jackson[2], I.D. Krantz[1].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Drexel University School of Medicine, Philadelphia.

Cornelia de Lange Syndrome (CdLS; OMIM 122470) is a multisystem developmental disorder characterized by facial dysmorphia, hirsutism, growth and cognitive retardation, gastrointestinal abnormalities and limb deficiencies. In 2004, we demonstrated that mutations in human *NIPBL* cause CdLS. NIPBL orthologs in yeast (Scc2/Mis4) and Drosophila (Nipped-B) are required for sister chromatid cohesion and long-range transcriptional regulation. It is believed that NIPBL is required for the attachment of Cohesin to chromosomes. Subsequently mutations in other Cohesin complex members *SMC1* and *SMC3* have been identified in CdLS. Unlike data on SMC1A and SMC3, much less is understood about the function of NIPBL orthologs. Furthermore, despite several screens, few mutations have been reported in yeast and Drosophila NIPBL orthologs that serve to dissect functional units of this protein. We have collected a cohort of over 400 probands with a clinical suspicion of CdLS and have performed extensive screening of *NIPBL*, including sequencing of exons and adjacent intronic sequence, sequencing of evolutionarily conserved elements, and analysis for microdeletions. To date, we have found *NIPBL* mutations in 172 individuals comprising approximately 60% of patients with CdLS. In addition, 55 NIPBL mutations have been reported by others. Together, this includes more than 60 missense mutations, which represent key amino acids in understanding functional domains of this novel protein. Most of these mutations are unique, however several regions of human NIPBL appear to demonstrate clustering of mutations, particularly, the C-terminal HEAT repeat. Most of these missense mutations result in mild or moderate features of CdLS, however several are seen in patients with more severe disease, suggesting relative importance of these residues. This work is the most complete to date that serves to add insight to delineating the functional domains of NIPBL and the roles that each may play in causing CdLS or other Cohesinopathies.

1264/F

SIX3 mutations in holoprosencephaly (HPE) are loss-of-function alleles. *S. Domene[1], K.B. El-Jaick[2], E. Roessler[1], F. Lacbawan[1], B. Feldman[1], M. Muenke[1].* 1) NHGRI/NIH, Bethesda, MD; 2) Laboratorio de Genetica Molecular, Brazil.

Holoprosencephaly (HPE) is the most common structural anomaly of human forebrain development, with a prevalence of ~1 in 250 conceptuses and ~1 in 16,000 at birth. Mutations in at least eight different genes have been identified in human HPE patients. We have previously shown that SIX3, a transcription factor known to be involved in midline forebrain and eye formation during early development in the mouse, is associated with HPE in humans. No functional studies have been performed to date. It consists of two highly conserved domains: a SIX domain needed for interaction with other proteins and a DNA-binding homeodomain. SIX3 interacts with groucho corepressor proteins through two eh1-like motifs located within the SIX domain. This interaction is required both for the autorepression of six3 itself and for the regulation of other early developmental genes.

In addition to 18 previously reported SIX3 mutations we describe here 29 novel mutations. The total of 47 mutations are located throughout the entire SIX3 gene and include 33 missense, 5 nonsense, 8 frameshift mutations and 1 in frame deletion. To demonstrate the function of these mutations we established several complementary approaches using the zebrafish as a model system: 1) overexpression of SIX3, 2) morpholino (MO) knockdown and rescue assay and 3) detection of marker changes using in situ hybridization. With these assays we have functionally characterized these SIX3 mutations for the first time as significant loss-of-function alleles. For example, single point mutations in the eh1-like motif result in loss of function suggesting that interaction with groucho is essential for SIX3 activity. In addition, several nonsense mutations located in the SIX domain and homeodomain which result in early termination of the protein result in loss of function. Our data elucidate how SIX3 functions during development and increase our understanding of its role in the pathogenesis of HPE. Furthermore, these results are crucial for genetic counselling of families with children with HPE.

in situ.

1265/F

Mutations in FHL1 cause a novel X-linked myopathy with specific/unique clinical features. *C. Windpassinger[1,2], B. Schoser[3], S. Hochmeister[4], A. Noor[1], B. Lohberger[4], N. Farra[1], E. Petek[2], T. Schwarzbraun[2], L. Ofner[2], W. Löscher[5], K. Wagner[2], H. Lochmüller[3], J.B. Vincent[1], S. Quasthoff[4].* 1) Neurogenetics Section, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario, M5T 1R8, Canada; 2) Institute of Human Genetics, Medical University of Graz, Graz, Austria; 3) Friedrich-Baur Institute, Department of Neurology, Ludwig Maximilians University Munich, Munich, Germany; 4) Department of Neurology, Medical University of Graz, Graz, Austria; 5) Clinical Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria.

We have identified a large multigenerational Austrian family displaying a novel form of X-linked recessive myopathy. Affected individuals develop a late-onset scapulo-axio-peroneal myopathy with bent spine syndrome characterized by specific atrophy of postural muscles along with pseudo-athleticism/hypertrophy, and cardiac involvement. Known X-linked myopathies were excluded by microsatellite analysis and direct gene sequencing. Marker analysis revealed significant linkage at Xq26-q27. Haplotype analysis based on SNP microarray data from selected family members confirmed this linkage region on the distal arm of the X-chromosome (Xq25-q27.1), narrowing down the critical interval to 10 Mb. Sequencing of functional candidate genes led to the identification of a missense mutation within the four-and-a-half LIM domain 1 gene (FHL1), which putatively disrupts the 4th LIM domain. FHL1 on Xq26.3, is highly expressed in skeletal muscle and oxidative fibers in particular, as well as cardiac muscles. Thus, we have characterized a new form of X-linked recessive myopathy, and identified FHL1 as the causative gene.

1266/F

The fibroblast growth factor gene FGF19 is regulated by both FOXC1 and FOXC2. *L. Huang[1], Y. Tamimi[2], M.A. Walter[1].* 1) Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Oncology, University of Alberta.

FOXC1 and FOXC2 are related members of the Forkhead Box transcription factor family. Mutations in *FOXC1* cause Axenfeld-Rieger malformations. Mutations in *FOXC2* cause hereditary lymphedema with distichiasis. During eye development, *Foxc1* and *Foxc2* have overlapping expression patterns, and *Foxc2*+/- mice and *Foxc1*+/- mice display very similar eye phenotypes. *Foxc1*+/- and *Foxc2*+/- double heterozygous mice present more severe ocular defects than in either single heterozygote, suggesting overlapping function of FOXC1 and FOXC2 in the developing eye. We hypothesize that FOXC1 and FOXC2 co-regulate some downstream target genes. Recent work revealed that FOXC1 directly regulates *FGF19* expression in cell culture and zebrafish embryos. This study aimed to test if FOXC2 also regulates the expression of *FGF19*.

Luciferase assays were used to test if FOXC2 can activate transcription from *FGF19* regulatory elements in non-pigmented ciliary epithelium cells (NPCEs). A 354bp amplicon (FGF19RE) of the FGF19-5'UPE containing FOX binding site was cloned into the pGL3TK luciferase vector. Luciferase activities were monitored after cotransfecting NPCEs with FGF19RE-pGL3TK luciferase reporter vectors, and FOXC2 or FOXC1 expression vectors. Both FOXC2 and FOXC1 significantly activated FGF19RE luciferase reporter. Consistent with this result, chromatin-immunoprecipitation experiments in NPCEs revealed that both endogenous FOXC2 and FOXC1 bind to the *FGF19* promoter *in vivo*. The fragment of the *FGF19* promoter sequence containing a FOX binding site was PCR amplified from sonicated chromatin purified by antibodies to FOXC1, or to FOXC2. These results indicate that *FGF19* is a shared downstream target gene of both FOXC1 and FOXC2, supporting the hypothesis that both of these transcription factors are key regulators in overlapping ocular genetic pathways. Our finding that FOXC1 and FOXC2 independently regulate *FGF19* expression provides an explanation for the similar phenotypes of heterozygous *Foxc2*+/- mice and *Foxc1*+/- mice.

1267/F

Missense mutations in the forkhead domain of the transcription factor FOXL2 lead to subcellular mislocalization and protein aggregation. *E. De Baere[1], L. Moumne[2], H. Peters[3], B.P. Leroy[1,4], A. De Paepe[1], R.A. Veitia[2], D. Beysen[1].* 1) Center for Medical Genetics, Ghent Univ Hosp, Belgium; 2) INSERM U709, Paris, France; 3) Inst of Medical Genetics, Charité, Berlin, Germany; 4) Dept of Ophthalmology, Ghent Univ Hosp, Belgium.

The FOX family of transcription factors is characterized by a conserved forkhead domain (FHD). To date, disease-causing mutations, many of which are missense mutations in the FHD, have been identified in 8 human *FOX* genes. Mutations of *FOXL2* have been shown to cause the blepharophimosis syndrome (BPES), characterized by an eyelid malformation associated or not with premature ovarian failure (POF).

Here, we report on the subcellular localization and distribution in COS-7 cells of 18 unique naturally occurring missense mutations in *FOXL2*. Their subcellular localization and distribution could be subdivided in four groups: those with (1) a normal nuclear distribution, (2) nuclear aggregation, (3) nuclear aggregation with strong cytoplasmic aggregation and (4) isolated cytoplasmic aggregation. These data enlarge the spectrum of *FOXL2* mutations inducing protein aggregation. In addition, we showed that the N- and C-terminal nuclear localization signals (NLSs) are not the only mechanisms for nuclear targeting of FOXL2. Interestingly, our data suggest that missense mutations leading to nuclear and cytoplasmic aggregation might cause a more severe ovarian phenotype than those that do not alter nuclear localization. Moreover, missense mutations located outside the FHD of FOXL2, leading to normal subcellular localization, give rise to a mild ocular phenotype associated with pituitary deficiency, at least for one missense mutation (S217F). In conclusion, our study is the first to demonstrate that missense mutations in the FHD of FOXL2 lead to mislocalization and nuclear and cytoplasmic aggregation of the mutant protein. These data suggest that this is one of the pathogenetic mechanisms of a major proportion of missense mutations in *FOXL2*.

1268/F

Detection of known and novel protein interactions with γ- and β-actin using a yeast 2-hybrid screen. *M.C. Drummond[1], T.N. Turner[1], E.T. Boger[2], M. Zhu[1], K.H. Friderici[1].* 1) Microbiology and Molecular Genetics, Michigan State Univ, East Lansing, MI; 2) Section on Human Genetics, National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

Mutations in γ-actin are known to cause non-syndromic sensorineural hearing loss. In an attempt to identify known and novel actin:protein interactions specific to the γ isoform of actin, two independent yeast 2-hybrid experiments were performed. In the first experiment, human γ-actin was used as the bait to screen a prey library constructed from cDNA from the inner ear of a P1 mouse. Over 1.2 million clones were screened and 369 positive interactions identified. The interacting prey were identified as 252 γ-actin, 76 β-actin, 12 ubiquitin E2 conjugating enzyme, 4 cofilin-1, 3 cofilin-2, and 11 different single copy transcripts. The ratio of γ-actin to β-actin prey identified was 3.3:1; a value higher than the anticipated 2:1 based on previous data. In the second experiment, β-actin was used as the bait protein to screen the inner ear prey library. While this experiment is still underway, thus far 324,000 clones have been screened and 176 positive interactions identified. Of the 176 positive prey 115 were γ-actin, 48 β-actin, and 13 undetermined. This yields a γ-actin:β-actin ratio of 2.4:1. These results suggest that either the ratio of γ-actin to β-actin in the inner ear is higher than previously reported or that γ-actin has a higher affinity for itself than β-actin. Research plans to address this are currently underway.

1269/F

Novel ciliary function for TOPORS (RP31 gene) associated with autosomal dominant retinitis pigmentosa. *A.Z. Shah[1], C. Chakarova[1], H. Khanna[2], S. Parapuram[2], P. Munro[1], M. Cheetham[1], K. Matter[1], R. Koenekoop[3], A. Swaroop[2], S.S. Bhattacharya[1].* 1) Institute of Ophthalmology, London, United Kingdom; 2) The University of Michigan, Kellogg Eye Center, Ann Arbor, Michigan, USA; 3) The McGill Ocular Genetics Laboratory, McGill University Health Centre, Montreal, Canada.

Purpose: We have recently identified mutations in *TOPORS* (RP31 gene) responsible for autosomal dominant retinitis pigmentosa (unpublished data). *TOPORS* is a ubiquitously expressed gene; a protein showing polyfunctional character. The purpose of our work is to characterise the role TOPORS plays in the photoreceptors, which may explain the retinal degeneration seen with mutations in this gene. Methods: *TOPORS* was cloned from human retinal cDNA into FLAG-tagged vector pCATCH, and identified mutations were introduced by site-directed mutagenesis. The WT and mutants were transfected into cell lines and imaged using fluorescent microscopy. A commercially available antibody for TOPORS was acquired and used to localise endogenous TOPORS in cell lines, and for immunoblot analysis. Mouse cryo-sections were used to find the specific protein localization in the retina. Results: Both mutations result in frameshift, prematurely terminating the TOPORS protein. To identify the functional consequences of the mutations we created mutant constructs with an N-terminal tag and transfected MDCK and 661W cells. The WT showed the expected pattern of expression within the nucleus in MDCK cells, but an increased cytoplasmic presence in 661W cells. Localisation studies in WT mouse retinal cryo-sections show a discreet signal from the inner-outer segment boundary (connecting cilium) of the photoreceptor layer. TOPORS also localises to the primary cilia of MDCK cells. Immunoblot analysis confirmed the expression of TOPORS as a 150 kDa band in cell lines and retinal tissue extracts. Conclusion: Given the specific signal from the connecting cilium of the retina, identification of mutations in *TOPORS*, an otherwise predominantly nuclear protein, suggests a new function within photoreceptor cells. Mutation carriers only manifest retinal degeneration with no other symptoms therefore suggesting a novel mechanism for photoreceptor degeneration.

1270/F

BBS7 is involved in BBSome formation and loss of BBS7 in mice results in Bardet Biedl Syndrome phenotypes. *Q. Zhang[1], M. Nachury[2], A. Loktev[2], P. Jackson[2], E. Stone[3], V. Sheffield[1].* 1) Department of Pediatrics, HHMI, University of Iowa, Iowa City, IA; 2) Department of Tumor Biology and Angiogenesis, Genentech, CA; 3) Department of Ophthalmology, HHMI, University of Iowa, Iowa City, IA.

Bardet Biedl Syndrome (BBS) is a phenotypically pleiotrophic and genetically heterozygous disorder. Through linkage analysis, homozygotic mapping, positional cloning, and mutation analysis of candidate genes, twelve BBS genes have been identified. These twelve BBS genes account for approximately 70% of the patients. Recently, seven highly conserved BBS proteins have been shown to form a complex known as the BBSome. The BBSome is involved in ciliary membrane biogenesis. In order to learn more about interactions between BBSome components, we performed pairwise co-immunoprecipitation assays in cultured cells. BBS9 was shown to strongly interact with BBS2 and BBS8, BBS9 was also shown to interact with BBS1 and BBS5. These data indicate that BBS9 is a central component of the BBSome. BBS7 was shown to be part of the BBSome through interaction with BBS2. Neither the N-terminus nor the C-terminus of BBS7 is required for the interaction. The interaction domain localizes to the center of the protein, which has homology to BBS2. To further study BBS7, we generated Bbs7 knockout mice. Bbs7-/- mice show similar phenotypes to other BBS gene knockout mice, including retinal degeneration, hyperphagia, obesity, hydrocephalus, and male infertility. Using tissues from Bbs7-/- mice, we showed that BBS7 and BBS2 depend on each other for stability. Using BBS6 knockout mice, we also demonstrate that BBS2 and BBS7 protein stability requires BBS6. Together our results suggest that BBS7 protein plays a role in BBSome formation and that BBS6 plays a role in BBSome formation by affecting BBS2 and BBS7 stability.

1271/F

Contribution of Chaperonin-like BBS Genes (BBS6, BBS10, BBS12) in a Multiethnic Canadian Population Affected with Bardet-Biedl Sydrome. *G. Billingsley[1], L. Deda[1], S. Herd[1], D. Chitayat[2,3], K.J. Fieggen[4], J.L. Duncan[5], G.A. Fishman[6], E. Héon[1,7].* 1) Dept Genetics & Genome Biology, Hosp Sick Children, Toronto, ON, Canada; 2) Prenatal Diagnosis and Medical Genetics Program, Mt Sinai Hosp, Toronto; 3) Dept of Clinical and Metabolic Genetics, Hosp Sick Children, Toronto; 4) Division of Human Genetics, University of Cape Town, Cape Town, S Africa; 5) Dept of Ophthalmology, UCSF, San Francisco, CA, US; 6) Dept of Ophthalmology & Visual Sciences, University of Illinois at Chicago, Chicago, IL; 7) Dept of Ophthalmology and Vision Sciences, Hosp Sick Children, University of Toronto.

Bardet-Biedl syndrome (BBS: OMIM 209900) is a genetically heterogeneous disorder characterized by the primary features of progressive retinal dystrophy, obesity, polydactyly, renal malformations, cognitive impairment and genital abnormalities. 12 BBS genes have been identified to date. BBS6 (NM_018848), BBS10 (NM_024685), and BBS12 (NM_152618) define a novel branch of the type II chaperonin superfamily and together are reported to account for about one-third of the mutational load in BBS patients (Stoetzel et. al. AJHG 80: 1-11, 2007). Mutational analysis of these 3 genes was performed on a Canadian BBS patient cohort (n=62) of mixed ethnicity. Family segregation and control screening (n=150) confirmed the pathogenicity of novel sequence changes. Three of 33 probands were found to have 4 mutations (1 novel) in BBS6. BBS10 was found to be a major contributor to BBS in our patient cohort, accounting for 26% of the mutational load. Sixteen different BBS10 mutations, 10 of which are novel, were observed. In addition, 10 (9 novel) pathogenic changes in BBS12 were found to contribute to 9.7% of the patient mutational load. Together these 3 genes account for ~45% of the mutational load in our patient cohort. Three patients have at least 3 pathogenic changes in 2 of the chaperonin-like genes. One of these, a young female diagnosed with MKKS (fundus appears normal at ~3 years of age), has a heterozygous BBS6 mutation as well as compound heterozygous BBS12 changes. Our results support the major importance of these three chaperonin-like proteins in BBS, together accounting for ~45% of our BBS cases.

1272/F

Novel *CHMP4B* mutations underlie autosomal dominant cataracts linked to chromosome 20q. *A. Shiels[1], T.M. Bennett[1], H.L.S. Knopf[1], K. Yamada[2], K-i. Yoshiura[3], N. Niikawa[3], S. Shim[4], P.I. Hanson[4].* 1) Ophthalmology/Visual Sci, Washington Univ Sch Medicine, St Louis, MO; 2) Ophthalmology/Visual Sci, Nagasaki Univ Grad Sch Biomedical Sciences, Nagasaki, Japan; 3) Human Genetics, Nagasaki Univ Grad Sch Biomedical Sciences, Nagasaki, Japan; 4) Cell Biology/Physiology, Washington Univ Sch Medicine, St. Louis, MO.

Cataracts are a clinically and genetically heterogeneous disorder of the ocular lens, and a leading cause of visual impairment. Here we report linkage of autosomal dominant "progressive childhood posterior sub-capsular" cataracts segregating in a 6-generation Caucasian-American family to STR markers D20S847 ($Z = 5.50$, $\theta = 0.0$) and D20S195 ($Z = 3.65$, $\theta = 0.0$) on 20q. Haplotyping with SNP markers refined the cataract locus to the physical interval rs2057262-[3.8 Mb]-rs1291139. Sequencing of positional-candidate genes detected a heterozygous A>T transversion in exon-3 of the gene for charged multi-vesicular body protein-4B (*CHMP4B*) that co-segregated with affected status. Similarly, we detected a heterozygous G>A transition in exon-3 of *CHMP4B* co-segregating with autosomal dominant posterior polar cataracts in a Japanese family. Neither coding change was present in 192 controls or the SNP database. The A>T transversion was predicted to result in the missense substitution of valine (V) for a phylogenetically conserved aspartic acid residue (D), whereas, the G>A transition resulted in the substitution of lysine (K) for a conserved glutamic acid residue (E). Transfection studies of cultured cells revealed that a truncated form of the recombinant D>V-mutant protein had a different sub-cellular distribution than wild type and an increased capacity to inhibit release of virus-like particles from the cell surface, consistent with deleterious gain-of-function effects. Our data provide the first evidence that *CHMP4B*, which encodes a key component of the endosome sorting complex required for transport-III (ESCRT-III) system of mammalian cells, plays a vital role in the maintenance of lens transparency.

1273/F

TBX22 missense mutations found in X-linked cleft palate (CPX) patients affect DNA binding, transcriptional repression and sumoylation. *E. Pauws[1], A.M. Andreou[1], M.C. Jones[2], G.E. Moore[1], J.J. Brosens[2], P. Stanier[1].* 1) Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom; 2) IRDB, Imperial College London, Du Cane Road, London W12 0NN, United Kingdom.

The T-box transcription factor TBX22 is essential for normal craniofacial development as demonstrated by the finding of nonsense, frame shift, splice site or missense mutations in patients with X-linked cleft palate and ankyloglossia (CPX). To better understand the function of TBX22, here we studied 9 different naturally occurring missense mutations that are phenotypically equivalent to loss-of-function alleles. Since all missense mutations are located in the DNA-binding T-box domain we investigated their effect on DNA binding in an EMSA assay using a TBX22 specific binding site. We find that all mutants exhibit compromised DNA-protein interactions, with the strongest effects seen with missense mutations at or near predicted contact points with the DNA backbone. The transcriptional function of TBX22 was investigated using a luciferase reporter assay which demonstrated that TBX22 functions as a transcriptional repressor. In this assay all missense mutations showed impaired repression activity. We demonstrate that TBX22 is a target for the small ubiquitin like modifier SUMO-1 and that this modification is a requirement for the observed repressor activity. Although the site of SUMO attachment is located at K63, upstream of the T-box domain, we find that all pathogenic CPX missense mutations attenuate SUMO-1 modification. This suggests that the loss of TBX22 function associated with missense mutations may be linked by a general mechanism affecting SUMO conjugation as well as DNA binding. Orofacial clefts are well known for their complex etiology and variable penetrance, involving both genetic and environmental risk factors. The sumoylation process is also subject to and profoundly affected by similar environmental stress. Our data supports recent evidence that suggests that SUMO modification represents a common mechanism involved in the regulation of normal craniofacial development and is involved in the pathogenesis of both Mendelian and idiopathic forms of orofacial clefting.

1274/W

Evolution of Metabolic Networks. *H. Dong[1], YH. Xiao[2], L. Jin[1], M. Xiong[1,3].* 1) Dept Genetics, Fudan Univ, Shanghai, China; 2) Dept of Computer Science, Fudan University, Shanghai, China; 3) Human Genetics Center, University of Texas, School of Public Health.

Recently, there has been increasing interests in application of general theory of complex network to evolution study of metabolic networks. Although general theory of complex networks can explain many features of topology of metabolic networks, this approach rarely compare the structure patterns of networks and investigate their different forms within and between species. Therefore, the structure variations of networks within and between species will be difficult to discover by pure application of general theory of complex networks to metabolic networks. To overcome these limitations, we first develop new methods for network alignment. Then, we explore basic idea of DNA sequence evolution theory for developing a novel paradigm for evolution of metabolic networks. We propose a novel concept of distance between the metabolic networks to measure difference in the structure of the networks. We use algebra topology to identify the symmetry structure and find the equilibrium of evolution of the metabolic networks for each specifies. Based on the distance between the metabolic networks and the equilibrium of the evolution of the metabolic networks, we construct the evolutionary tree of the metabolic networks. Finally, the proposed algorithms and methods were applied to evolution of more than one hundred of metabolic networks. We compare the evolutionary trees of the species based on evolution of metabolic networks with that based on DNA sequences.

1275/W

Acatalasemia: molecular evolutionary inferences into the nature of the mutation. *A.M. Smits, S.E. Braik, L.A. Tollini, B.J. Carr, E.S. Tignor, K.A. Eskay, N.J. Schisler.* Biology Department, Furman Universiity, Greenville, SC.

Acatalesemia, a deficiency in catalase (CAT) activity, has been described in many human populations; similar phenotypes exist in the guinea pig, dog, domestic fowl, and mouse. Shaffer and Preston (1990) showed that the acatalesemic mouse harbored a CAG-to-CAT transversion in codon 11, but this lesion cannot fully explain the complex tissue -specific phenotype associated with murine acatalesemia. Mouse CAT activity levels have been shown to be variable among different strains, tissues, and developmental stages (Schisler and Singh, 1991) and may be affected by uncharacterized loci such as Ce1 (affects liver CAT), and Ce2 (affects kidney CAT). The 3' untranslated region (UTR) of the mouse CAT gene also binds distinct cytoplasmic proteins that could also regulate CAT activity (Reimer and Singh, 1996). To further assess the nature of the mouse acatalesemic phenotype, we have applied bioinformatic and comparative genomic methods to study CAT gene (approx. 30 kb consisting of 13 exons that produces a mature transcript of 2613 bp) sequences from several inbred mouse strains including C3Ga.Cg-Catb/J (acatalesemic mutant), BALB/cJ (high level CAT activity), C57Bl/6J (hypocatalasemic), and mouse strains 129P1/ReJ, C3H/HeSnJ, and DBA, as well as several other mammalian species. Comparative mouse data would be valuable to assess regions of the gene that change rapidly (i.e. introns) whereas other mammalian species could be used to identify highly conserved regions. Analyses indicated intron positions among the various CAT genes were highly conserved but intron lengths and sequence had varying levels of conservation depending on the size of the intron and relative location within the gene. Many introns in the 5' end of the CAT gene had numerous repeated motifs as well as putative transcription factor binding domains. Intron 7 in some mouse strains shared 60.7% identity with its human counterpart. This approach of using phylogenetically close and distant strains/species to determine relative mutatbility/conservation of gene sequences could assist with the elucidation of the nature of disease-associated genes in humans and other species.

1276/W

Does genotyping multiple controls help proving causal effect of a mutation? *S. Sunyaev[1,2], G.V. Kryukov[1,2].* 1) Division of Genetics, Department of Medicine, Brigham & Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

Historically, genotyping a hundred of controls has been the most common approach to discriminate between causative mutations and neutral polymorphisms. However, due to observed abundance of low frequency allelic variation in human genes, the reliability of this approach has been questioned. It was also suggested that the number of genotyped controls should be increased to at least two or three hundreds. New systematic re-sequencing datasets help quantifying levels of rare polymorphism in the human population and ultimately resolve whether a missense mutation present in a patient and absent in hundreds of controls should be considered functional at a stringent level of statistical significance. Here we attempt to answer this question by means of computer simulations with parameters of demographic history and strength of natural selection estimated from large systematic re-sequencing datasets. The model very well reproduces site frequency spectrum observed in human re-sequencing data. We consider several scenarios frequently arising in human genetics research and in practice of genetic diagnostics. Our results suggest that absence of a mutation in hundreds of control subjects cannot be considered a reliable indication of the functional significance even for fully penetrant mutations.

1277/W

Nonsense polymorphisms in Japanese population. *Y. Yamaguchi-Kabata[1], N. Kamatani[1,2].* 1) Lab for Statistical Analysis, SNP Research Center, Minato-ku, Tokyo, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo.

Genetic variations in the human genome are maintained by the balance of mutation, selection and random genetic drift. Nonsense mutations, which cause premature stop codons in protein-coding regions, result in truncated proteins or absence of gene product. From the standpoint of evolution, a nonsense mutation can be a cause of pseudogene if it is fixed in the population. How nonsense polymorphisms, even with profound effects on phenotypes, are maintained in human populations is little understood. In this study, we intended to clarify how many nonsense mutations exist on the genome, focusing on the Japanese population. We conducted data analysis using bioinformatics resources such as dbSNP, JSNP, and H-InvDB to retrieve data of possible nonsense SNPs. The number of nonsense SNP candidates was more than 1200 by selection of SNPs from the bioinformatics resources. Then, we selected SNPs that are polymorphic in the Japanese population with allele frequency data from JSNP and HapMap. For more than 200 nonsense SNP candidates for Japanese, we checked whether classification of SNP is appropriate. The results show that there are at least 90 nonsense SNPs in the Japanese population. Frequency distribution of the nonsense alleles were much biased toward lower frequencies. However, some of the nonsense SNPs are very common and also found in other ethnic groups. We also examined the positions of the nonsense polymorphisms in gene structure to see whether the premature stop codon cause nonsense-mediated decay. By these analyses, we estimate the average number of null mutations by nonsense mutations for the typical person.

1278/W

Dissecting the origins of (ATTCT)n expanded chromosomes in Brazilian SCA10 families through a haplotype study. *T. Almeida[1], I. Alonso[1], L. Saraiva-Pereira[2], L. Jardim[2], P. Magalhães[3], S. Martins[1,4], J. Sequeiros[1,5], I. Silveira[1].* 1) Unigene, IBMC, Univ. Porto, Portugal; 2) Hosp. Clínicas de Porto Alegre, Brasil; 3) CCGen, IBMC, Univ. Porto, Portugal; 4) IPATIMUP, Univ. Porto, Portugal; 5) ICBAS, Univ. Porto, Portugal.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant neurodegenerative disorder caused by a dynamic mutation in intron 9 of the *ATXN10* gene, at chromosome 22q13.3. A large expansion of 400 to 4500 ATTCT repeats is found in SCA10 patients, whereas normal alleles vary usually from 10 to 29 repeats. To date, the mutation has only been found in families of Mexican or Brazilian origin. We found three SCA10 Brazilian families with patients carrying expanded SCA10 alleles. These families have a mixed Portuguese and Amerindian ancestry. In this study, we aim to determine the origin of these expanded alleles. The identification of new polymorphisms within a region of 800 bp flanking the repeat was performed in 50 Brazilian individuals, by PCR followed by DHPLC. Three new SNPs were identified in this region and haplotypes were reconstructed in our SCA10 Brazilian families. G-G-C was the most common haplotype found in the Brazilian population and was also shared by all expanded chromosomes. Our preliminary results suggest that the SCA10 expansion in these Brazilian families may have arisen in a common ancestral haplotype, but the study of additional polymorphisms in the region is still needed to assess the origin of expanded chromosomes.

1279/W

Mammal-specific domain in BRN-2 associated with maternal behavior. *M. Nasu[1], Y. Kataoka[2], M. Sato[2], H. Ichise[2], N. Yoshida[2], S. Ueda[1].* 1) Department of Biological Sciences, The University of Tokyo, Tokyo, JAPAN; 2) The Institute of Medical Science, The University of Tokyo, Tokyo, JAPAN.

Brn-2 is a neuronal transcription factor, expressed in the neocortex, the hypothalamus, the cerebellum, etc. and known to regulate the expression of some neuronal factors and proliferation, differentiation and migration of neuronal cells. BRN-2 protein has three stretches of homopolymeric amino acids, polyG, polyQ and polyP, in its transactivation domain. These are conserved among mammals but lacked in fishes and amphibians, meaning that these are mammal-specific sequences. Mammal-specific domain could be associated with mammal-specific function, but there is no verification of that. To investigate the function of mammal-specific domain of BRN-2, we generated mutant mice lacked all of three stretches of homopolymeric amino acids in BRN-2, namely BRN-2ΔGQP.

We found that mutant mice appeared normally to develop, grow and mate, but they were more prone to fail to nurture their pups. Not all but larger number of pups delivered from mutant dams could not survive to be weaned. The decline of pups' viability depended not on the genotype of pups but on that of dams. These results suggest that BRN-2ΔGQP retains basal functions for life in spite of the lack of the mammal-specific domain, but BRN-2ΔGQP fails to motivate particular functions. The mammal-specific domain in BRN-2, namely three stretches of homopolymeric amino acids, might contribute to establish maternal behavior including nursing, which was characteristic of and essential to mammals.

1280/W

A recurrent frame-shift mutation of *PMS2* occurs within a short common haplotype from ostensibly unrelated individuals and is suggestive of an early founding event. *M. Clendenning[1], L. Senter[1], H. Hampel[1], A. Lindblom[2], K. Lagerstedt Robinson[2], M. Nilbert[3], J. Green[4], J.D. Potter[5], A. de la Chapelle[1].* 1) Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio; 2) Karolinska Institute, Department of Molecular Medicine, Stockholm, Sweden; 3) Department of Oncology, Clinical Sciences, Lund University, Sweden; 4) Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, Canada; 5) Fred Hutchinson Cancer Research Center, Seattle, Washington.

When compared to the other mismatch repair genes involved in Lynch syndrome, the identification of mutations within *PMS2* has been limited (<2% of all identified mutations), yet the immunohistochemical analysis of tumor samples indicates that approximately 5% of Lynch syndrome cases are caused by PMS2. The primary reason for this disparity is due to complications in the study of this gene caused by interference from pseudogene sequences. We recently developed a method for detecting mutations in the *PMS2* region and have routinely screened selected patients for *PMS2* variants. We have identified a frequently occurring frame-shift mutation (c.736_741del6ins11) within a region not affected by pseudogene sequences. To date, we have detected this mutation in 10 ostensibly unrelated Lynch syndrome patients (~30% of patients we have identified with a deleterious mutation in *PMS2*, n = 33) who all carry the rare allele (population frequency = 0.033) at a SNP (c.1531A>G) in exon 11. Six of these individuals have been studied in more detail and have been shown to possess a very short common haplotype; however the characterization of a larger haplotype has been made difficult due to extensive homologous sequences which flank the 3' end of the gene. Ancestral analysis of the affected individuals indicates that this mutation might be enriched in the British Isles and Scandinavia. The identification of both the mutation and the common haplotype in one Swedish control sample (n = 225), along with evidence that Lynch syndrome associated cancers are rarer than expected in the probands' families would suggest that this is a prevalent mutation with low penetrance.

1281/W

Characterization of junction sites of deletion type variants detected by Array-CGH in Japanese populations. *Y. Satoh[1], N. Tsuyama[1, 2], K. Sasaki[1], M. Kodaira[1], H. Omine[1], Y. Shimoichi[1], H. Katayama[3], N. Takahashi[1].* 1) Dept Genetics, RERF, Hiroshima, Japan; 2) Dept Anal Mol Med Dev, Hiroshima Univ Grad Sch Biomed Sci, Hiroshima, Japan; 3) Dept Info Tech, RERF, Hiroshima, Japan.

We have investigated the effect of atomic bomb radiation to human germ cells at the DNA level, that is, whether mutation rate of atomic bomb survivor's offspring increase significantly more than control group. In order to examine whole human genome, we introduced array CGH method that a comparative genomic hybridization was performed on a microarray system. A population study was performed using genomic DNA from 40 offspring of atomic bomb survivors and 40 offspring of control group. A total of 251 variations was detected using arrays on which about 2,300 human BAC clones were printed. 14 of these variation were "rare variant", which were identified in only one offspring among the population. 8 variation was amplification type and 6 variation was deletion type. We report the characterization of deletion type variation. The characterizations of variations were done as follows: (1) Estimations of fragment length of deletion variations by pulse field gel electrophoresis, (2) Narrowing down of junction site by quantitative PCR, (3) Determination of sequence of which junction site was amplified by PCR. Junction site sequences of five kinds of deletion type variation were determined. Average length of deletion region was about 134 kb, and shortest one was 84 kb and longest was 239 kb. Alu- family like sequences were existed in two breaking points in the junction sites and LINE sequence was existed in one breaking point, and the others showed unique sequences at the breaking points. Some variations had a insertion or a inversion inside the deletion region. We report conceivable mechanisms that may produce these deletion variants.

1282/W

Copy number variation analysis in the Mexican population. *L. Uribe, A. Hidalgo, L. Del Bosque, R. Goya, J.C. Fernandez, I. Silva-Zolezzi, G. Ramos, A. San Juan, J. Cruz, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Copy number variation (CNV) is an important source of genomic diversity. They can vary in frequency between populations and some have been associated with susceptibility to human disease. We conducted a systematic analysis of CNV in Mexican Populations, using the Affymetrix 500K SNP array. We genotyped 300 Mestizo individuals from six geographically distinct regions of Mexico (50 samples from each region: 25 males and 25 females) and 26 Mazatecan amerindians from Oaxaca. All samples were compared to a randomly selected reference sub-set of 30 females from the same studied population. We used CNAT 4.0 performing quantile normalization with genomic smoothing of 0.1 Mb. The value for the Hidden Markov ploidy priors was set to 0.2 with a 10 Mb transition decay. Our analysis showed a total of 1,682 changes in copy number in the Mexican mestizos. From these alterations, 592 regions (35.2%) showed less than the 2 copies expected for a normal diploid (n=2), and 1,090 (64.8%) showed an increased copy number in the range of 3-5. A total of 35 regions showed CNV in > 5% of the samples. The largest region was1.55 Mb (9q12) while the shortest was 6.7Kb (1q12). In the Mazatecan population a total of 238 alterations were found in > 5% of the samples, with an average of 9.15 CNVs per sample. From the CNVs detected, 65 (27.3%) were amplifications and 173 (72.7%) had less than the 2 normal copies for a diploid individual. The largest region detected (3.2 Mb) mapped to 9p12-p11.2, while the shortest region (21.7 kb) mapped to 8p23.1. 13% of the CNVs detected in the Mazatecans have not been previously reported in the genomic variants databases. We observed a greater amount of gene containing regions with CNVs in the Mexican mestizos compared to the Mexican Mazatecs. Characterization of these variants in different populations will contribute to a better understanding of their contribution to human disease, and may help define sub-population specific variations.

1283/W

Mapping complex traits in the domestic dog. *E.A. Ostrander[1], H.G. Parker[1], B. Hoopes[1], K. Bryc[3], B. vonHoldt[4], N.B. Sutter[1], K. Chase[2], K.G. Lark[2], P. Quignon[1], D.S. Mosher[1], C. Bustamante[4], R.K. Wayne[3].* 1) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Dept. of Biology, University of Utah, SLC, UT; 3) Dept. Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 4) Dept. of Ecology and Evolution, UCLA, Los Angeles, CA.

The availability of a high quality draft sequence of the dog genome has changed the way geneticists studying companion animals are tackling the problem of finding genes that control complex traits. Of particular interest are genes controlling the morphologic differences which define different domestic dog breeds, genes regulating behavior, and those that increase disease susceptibility. Central to our ability to use the newly available resources is an understanding of dog breed structure and we herein present a detailed discussion of a new cluster analysis involving 135 U.S. breeds. Also important is an understanding of the strengths and limitations of the current molecular resources, and consideration of the traits which are likely to lend themselves to mapping using available approaches and resources. We describe our recent efforts to localize genes important in controlling body size. Our initial studies suggest a primary role for the IGF-1 gene in making small dogs small. But studies with Portuguese Water Dogs strongly suggest the existence of other loci in controlling overall body size in the dog. Building upon those findings, and using a large number of samples collected from small and large dog breeds we describe other genes and loci which potentially play a role in regulating canine morphology, particularly body size and leg length. Finally we discuss the problem of breed substructure in the context of candidate gene approaches. By way of example we discuss efforts to find genes for behavior traits in the dog, including racing speed among whippet dogs. Extending from our most recent work, we demonstrate that candidate gene analysis can work if special consideration is paid the likely occurrence of population substructure.

1284/W

Further studies on Leber's hereditary optic neuropathy (LHON) in Russia/Siberia. *N. Volodko, E. Starikovskaya, P. Naidenko, N. Eltsov, R. Sukernik.* Laboratory of Human Molecular Genetics, Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Branch, Novosibirsk, Russia.

LHON (MIM535000) is a form of maternally transmitted visual failure associated with mtDNA mutations affecting genes that contribute polypeptides to the ND (NADH dehydrogenase) subunits of the mitochondrial respiratory complex I. To further clarify the role of the basal polymorphisms of mtDNA Eurasian phylogeny in the expression of pathogenic mtDNA mutations, previously identified in 13 Slavic and 3 aboriginal LHON families from Southwestern Siberia (Volodko et. al. 2006), we filled the gaps existing in the complete sequencing the mtDNAs from the probands of either family. Similar strategy was applied to 7 new LHON families of Slavic-European origin revealed recently, resulting in comprehensive haplotype analysis of 23 LHON families. The phylogeny encompassing complete mtDNAs with three "classical" LHON mutations (G1178A/ND4, T14484C/ND6 and G3460A/ND1) and two rare (T10663C/ND4L, G3635A/ND1) shows a varying degree of association between haplogroup background and phenotypic expression of these mutations. For example, of 13 pedigrees with G11778A, 9 (70%) were associated with different sublineages of the TJ haplogroup (T2, J2b, J1c), and 4 four (30%) were found to be scattered within the pre-HV cluster. The G3460A mutation detected in 4 families, of whom 3 belonged to native Siberians, were found in association with different derivatives of haplogroup M (C3, D4 and D5a), whereas one of German ancestry with haplogroup R (H*). Contrary to our previous persuasions, the roles of haplogroup J specific or associated variants in the expression of the T14484C remains unclear; of four LHON families with T14484C, only one exhibited association with TJ cluster, while two were distributed among the branches of HV, and one belonged to eastern Eurasian M9.

1285/W

Promoter regions of many neural- and nutrition-related genes have experienced positive selection during human evolution. *O. Fedrigo[1,2,3], R. Haygood[1,3], C.C. Babbitt[2,3], T. Severson[2], S. Morrow[2], G.A. Wray[1,2].* 1) Biology Department, Duke University, Durham, NC; 2) Institute for Genome Sciences and Policy, Duke University, Durham, NC; 3) These authors contributed equally to this work.

Surveys of protein-coding sequences for signatures of positive selection in humans and chimpanzees resulted only in a few genes involved in neural or nutritional processes, despite the pronounced differences between humans and chimpanzees in behavior, cognition, and diet. It is likely that most phenotypic differences between human and other primates are due to gene regulation rather than protein structure. We performed a genome-wide scan for signatures of positive selection on promoter (5'-flanking) regions unique to the human lineage compared to macaque and chimpanzee. We adapted and used a lineage specific Random Effect Likelihood model comparison to test for faster evolution along the human lineage in a promoter region relative to a putative neutral region picked from nearby intronic sequences. A Bayes Empirical Bayes method has been applied to detect particular sites under lineage specific selection. We were able to analyze 6,280 promoter regions from which 46 showed a signal of positive selection after correction for multiple comparisons. We examined the biological trend of the top scoring genes using PANTHER and GO ontology classifications. Our results indicate that positive selection has targeted the regulation of many genes known to be involved in neural development and function, both in the brain and elsewhere in the nervous system, and in nutrition, particularly glucose metabolism. We empirically validated select top scoring genes by performing in-vitro reporter assays showing significant difference in transcriptional inducibility.

1286/W

Identification and analysis of positive selection in the FoxC subfamily of forkhead transcription factors. *C.D. Fetterman, M.A. Walter.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Forkhead gene family members are defined by a DNA binding domain termed 'forkhead' and act as transcription factors in processes such as development and metabolism. Subfamilies, which are delineated by the letters A-R, have been defined using phylogenetic methods. The FoxC subfamily contains two human genes, *FOXC1* and *FOXC2*, both of which function as transcription activators and contain self-regulating inhibitory domains. Mutations in *FOXC1* lead to Axenfeld-Rieger syndrome, a disorder with anterior segment of the eye, craniofacial and dental anomalies, while mutations in *FOXC2* lead to lymphedema-distichiasis. We have utilized *in silico* methods to identify selection pressures on codons in FoxC subfamily members and *in vitro* methods to characterize a positively selected site that was identified. The *in silico* analyses included 13 FoxC sequences from six different species. An alignment and phylogeny were created and input into the codeml program, contained in the PAML package, to identify selection pressures on individual amino acid sites. One site, within the inhibitory domain of FOXC1, was under positive selection and all other sites were under negative selection. We are utilizing *in vitro* methods to study the hypothesis that positive selection of an amino acid indicates that the site is functionally important and that changes at a positively selected site will result in improper protein function. The positively selected site was changed from the wild type amino acid Ala to Gly, Pro, Phe, Glu and Arg, as well as deleted from FOXC1. Expression of the altered FOXC1 proteins indicated that these amino acid changes do not abolish protein production or alter FOXC1 localization within the cell. However, transactivation assays have shown that amino acid changes may reduce the transactivation capacity of FOXC1. The positively selected site may therefore be important for control of negative regulation of FOXC1 activity by the inhibitory domain. The reduction in transactivation supports the hypothesis that a positively selected site is important for proper function and that changes at a positively selected site may result in abnormal protein function.

1287/W

Distinctive LD and haplotype frequencies in human populations extend beyond ALDH2. *H. Ota[1], A.J. Pakstis[2], M.-Y. Lee[2], J.R. Kidd[2], K.K. Kidd[2].* 1) Dept Integrated Biosciences, U. Tokyo, Kashiwa, Japan; 2) Dept Genetics, Yale U. Sch Med, New Haven, CT USA.

The ALDH2 gene codes for acetaldehyde dehydrogenase 2, a key enzyme in ethanol metabolism. We previously published on normal variation in LD and multi-site haplotype frequencies for 6 SNPs and 1 STRP in 38 human populations. We have expanded our study to include more populations (45-50) and polymorphisms (41 SNPs) at higher density within ALDH2 and extended to include flanking ACAD10 and MAPKAPK5 genes (~193 KB total). Except for a functional site varying only in E.Asia, the avg heterozygosity across 45 population samples for 40 SNPs ranges from .18 to .37. In general, very strong LD (R^2 >.65) prevails in the 193 kb region but LD weakens considerably in several intervals for the African samples and to a lesser extent in E.Asia, Pacific, and the Americas; LD is very strong and more continuous for the European and some SW Asian samples. The unusual pattern of alternating intervals of very high (mostly .23 to .39) and low Fst (.05 to .09) values (compared to a reference distribution where avg Fst[45pops]=~.14) reported in our earlier work is also present in the genes flanking ALDH2. More high Fst values are concentrated in ACAD10 and ALDH2 (including promoter) than in MAPKAPK5 or the intergenic regions. In each of two 12-SNP and one 17-SNP haplotypes constructed (EM algorithm) there are 14-17 haplotypes at common frequencies. Consistent with the strong LD, this is a relatively small number of haplotypes at common frequencies in the 193KB region but this is more complex than the core 31kb of ALDH2 where 2 haplotypes account for 98% of chromosomes with strikingly similar frequencies in all populations studied suggesting balancing selection occurred. By continent, the haplotype frequencies display distinctive patterns across the 193 kb region. African samples show the most haplotype diversity; each continental grouping of populations has one or more haplotypes at distinctively higher frequencies compared to other world regions or has a haplotype essentially unique to the region, e.g. E.Asian haplotype carrying the functional variant. Supported in part by NIH AA09379 to KKK.

1288/W

Evolutionary analysis of pre-synaptic genes. *L.M Pardo[1], Z. Bochdanovits[1], R. Toonen[2], M. Verhage[2], P. Heutink[1].* 1) Section Medical Genomics, Department of Clinical Genetics, VU University Medical Center, 1081 BT Amsterdam, The Netherlands; 2) Department of Functional Genomics, Center for Neurogenomics and Cognitive Research (CNCR), Vrije Universiteit (VU) Amsterdam and VU University Medical Center, 1081 HV Amsterdam, The Netherlands.

Single Nucleotide Polymorphisms (SNPs) are the most common source of genetic variation in humans, but only a fraction of these has effects on human traits. From an evolutionary perspective, gene variants with an effect on the fitness of individuals will deviate from a pattern of neutral evolution. Natural selection is one of the forces behind the departure from neutral molecular evolution of gene variants, and has been shown to be relevant in humans. A robust approach to measure the effect of natural selection is to estimate the ratio of non-synonymous to synonymous substitutions (denoted as w) in a protein-coding gene across different species. A ratio significantly different from 1 indicates that selective pressures operate on the gene or at specific amino acid residues. This approach may be used to choose candidate SNPs to test in association with specific human traits. We are analyzing the protein coding regions of more than 200 presynaptic genes to estimate the selective pressure at individual codons. We chose 279 human presynaptic proteins to study in relation to human behaviour-like traits. These protein sequences were used to BLAST the RefSeq protein database to retrieve putative vertebrate orthologs. The orthologous coding sequences were aligned using CLUSTALW and MUSCLE. To estimate w we used codon-based maximum likelihood methods implemented in PAML that allows the estimation of w under different models of evolution (negative, neutral and positive selection). Our analysis showed that although several genes are under strong selective constraint, w is not constant across sites. In addition, for other genes we observed a few residues that were subjected to positive selection. Our results suggest that this approach may be used to identify a priori candidate functional SNPs related to common diseases for association studies.

1289/W

Evolutions of Dynamic Metabolic Networks. *Q. Zhou[1], L. Jin[1], M. Xiong[1,2].* 1) Dept Genetics, Fudan Univ, Shanghai, China; 2) Human Genetics Center, University of Texas, School of Public Health.

In the past, molecular evolution and population genetics have focused on using DNA sequences as tools for studying evolution. Recently, some researchers begin to study evolution of the biological networks such as metabolic, signal transduction, gene regulation and protein-protein interaction networks. However, all these researches mainly study evolution of the network structures and individual enzymes and proteins. Evolution of dynamic properties of the biological networks have never been studied. It is important to know how the evolution of the network structure affects the dynamic behavior of the biological networks. In this report, we treat a biological network as a dynamic system. We study the evolution of glycolysis. Based on our recent development of network alignment algorithms and permutation group graph theory, we identify the typical structure of the metabolic network for each species. Then, we study the evolution of the structure of glycolysis for more than 200 species. For each structure, we derive the kinetic models of glycolysis. We take the yields of ethanol as an objective function and apply the constrained nonlinear control theory to these evolved kinetic models from which we calculate the optimal yields of ethanol For each species, we investigate how the structure of the network affects the yields of the ethanol and other dynamic properties of the networks. These analyses allow us to link the structure of the metabolic network with function of the cell. Finally, we identify the optimal structures of glycolysis pathway which produce the largest yields of ethanol.

1290/W

Phylogenetic analysis in 14 primates reveals positive selection in the C-terminal domain of the cholesterol metabolism gene *PCSK9*. *K. Ding, S.J. McDonough, I.J. Kullo.* Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN.

Cholesterol homeostasis is maintained through finely tuned mechanisms regulating dietary uptake, hepatic biosynthesis and secretion as well as plasma clearance. The low-density lipoprotein (LDL) receptor gene (LDLR) plays a key role in cholesterol homeostasis by receptor-mediated endocytosis of LDL cholesterol. *PCSK9* (proprotein convertase subtilisin/kexin type 9), is a newly discovered regulator of LDLR that encodes a secreted enzyme of the serine protease family and reduces cellular uptake of plasma LDL cholesterol by promoting LDLR degradation. An evolutionary analysis of *PCSK9* may aid in the identification of conserved elements with functional importance. In addition, non-conserved regions involved in the generation of novel biological functions may also be detected. We compiled the sequences of the coding regions of *PCSK9* from 14 primates in the clade of Hominids, Old World monkeys and New World monkeys (sequences for six species were downloaded, and eight species were resequenced in our laboratory). A comparative analysis describing the evolutionary relationships across the primate *PCSK9* gene was carried out. The ratios of nonsynonymous/synonymous substitution rate (d_N/d_S) under different evolutionary models (e.g., one-ratio model and free-ratio model) were calculated across the phylogeny of *PCSK9* to detect selective pressure at the protein level. Maximum likelihood analyses of d_N/d_S ratios for the aligned coding regions sequences among 14 primate species suggested that *PCSK9* was subject to a strong functional constraint (i.e., purifying selection). However, a relaxed selective constraint or positive selection was noted in the functional carboxyl-terminal (C-terminal) domain of *PCSK9* across the phylogeny, especially in the lineage leading to the orangutan. Further, at least five positively selected amino acids were detected using the branch-site model A (i.e., the Bayes Empirical Bayes probability > 0.95) in the lineage leading to the orangutan. We speculate that differential selective pressure among primates has shaped evolutionary patterns in different functional domains of *PCSK9*.

1291/W

A comparison of human and chimpanzee recombination landscapes in the pseudo-autosomal regions. *A. Fledel-Alon[1], D. Serre[2], M. Przeworski[1].* 1) Department of Human Genetics, University of Chicago, 920 East 58th Street, Chicago, Illinois 60637, USA; 2) McGill University and Genome Quebec Innovation Center, Montreal, Quebec H3A 1A4, Canada.

Recent studies have revealed a rapid evolution of recombination hotspot locations between humans and their closest living evolutionary relative, chimpanzees. Over larger genetic distance, however, genetic maps estimated in extant humans and historical rates estimated from human patterns of linkage disequilibrium are highly concordant. These observations led to the suggestion that while fine scales evolve rapidly, broader scale rates are conserved, either because of constraint on recombination rates or competition among hotspots. We tested this model by comparing rates of recombination in humans and chimpanzees in the pseudo-autosomal regions (PARs): PAR1, a 2.7 Mb region experiences obligate crossing-over in males and which therefore serves as a miniature model of a chromosome, and PAR2, a 0.33 Mb region that is human-specific. To do so, we resequenced over 200 amplicons spanning the PAR regions in 32 unrelated chimpanzees and estimated recombination rates from patterns of linkage disequilibrium. We then compared the inferred chimpanzee recombination rates to those estimated from human sperm typing, and from human phase II HapMap data. Here, we discuss the results of this comparison, and the implications for the evolution of recombination rates.

1292/W

Patterns of Nucleotide Diversity and Potential Signatures of Natural Selection at *ICAM-1*. *F. Gomez[1,2], G. Tomas[3], F. Reed[1], S.A. Tishkoff[1,4], J. Rocha[3,4].* 1) Dept. of Biology, The University of Maryland, College Park, MD; 2) Hominid Paleobiology Doctoral Program, The George Washington University, Washington, DC; 3) Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal; 4) Equal contributors.

Variants at the gene that encodes ICAM-1 (intercellular adhesion molecule-1) are thought to play a role in malaria susceptibility. ICAM-1 is one of the primary vascular endothelial receptors to which *P. falciparum* parasitized erythrocytes adhere in the postcapillary venules of several organs. This observation has led many scholars to suggest that ICAM-1 is an important host receptor responsible for severe malaria pathogenesis. The clinical consequences of *ICAM-1* nucleotide diversity are under contention. A non-synonymous SNP within *ICAM-1*, termed *ICAM-1*^{Kilifi}, has been shown to be associated with either an increased risk or protection against severe malaria in Kenyan and Gabonese populations, respectively. Here we present a preliminary examination of the nucleotide variation within *ICAM-1*, a characterization of haplotype structure in a globally diverse sample of human populations, and a summary of tests of neutrality. A panel of ~100 individuals originating from Portugal, Mozambique, Sao Tome, Tanzania, and Thailand were analyzed for nucleotide variation across a ~6.8 kb region. Twenty-two SNPs were observed and three major haplotypes accounted for the majority of the haplotype variation within *ICAM-1*. From these data we have shown that the measures of nucleotide diversity are slightly lower than the genome average. However, the tests of neutrality do not yield statistically significant results. Haplotype network analyses indicate that the *ICAM-1*^{Kilifi}polymorphism is found in Africa and Thailand on divergent haplotype backgrounds. This result suggests that the Kilifi SNP either arose independently in Asia or was introduced during a migration of modern humans out of Africa, and has since undergone historic recombination events. We are currently examining nucleotide variation within a larger panel of diverse African populations to elucidate the evolutionary history of this locus.

1293/W

Demographic history and natural selection in Oceanic populations inferred from a genome-wide SNP analysis. *R. Kimura[1, 2], J. Ohashi[2], Y. Matsumura[3], M. Nakazawa[4], T. Inaoka[5], R. Ohtsuka[6], K. Tokunaga[2].* 1) Forensic Medicine, Tokai University School of Medicine, Kanagawa, Japan; 2) Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 3) Health Informatics and Education, National Institute of Health and Nutrition, Tokyo, Japan; 4) Socio-Environmental Health Sciences, Graduate School of Medicine, Gunma University, Gumna, Japan; 5) Environmental Sociology, Faculty of Agriculture, Saga University, Saga, Japan; 6) National Institute for Environmental Studies, Ibaraki, Japan.

It is thought that major prehistoric human migrations to Oceania occurred twice: 50K and 4K years ago. Indigenous people in Australia and New Guinea, who are anthropologically classified to Australoid, are considered as descendants of the former. Descendants of the latter are thought to be Austronesian-speaking people, who have characteristics of Mongoloid. In this study, we carried out a genome-wide SNP typing on an indigenous New Guinean population, Gidra, and a Polynesian population, Tongans, by using Affymetrix 500K Assay. The SNP data were analyzed together with the data of HapMap samples (YRI, CEU, CHB, JPT) provided by Affymetrix. In agreement with previous studies, our phylogenetic analysis suggested that indigenous New Guineans are closer to Asian populations than to African and European populations. A population structure analysis revealed that genetic contribution to the Tongan population is 70% Mongoloid and 30% Australoid. A high degree of linkage disequilibrium observed in the Gidra and Tongans implies that these populations have experienced population bottlenecks. We performed a scan for natural selection by examining haplotypic variation and identified candidates of locally selected loci, which may include "thrifty genes" in Oceania. Such an approach based on evolutionary medicine, providing a clue to understand how human beings have been adapted to our environments and lifestyles, must also be contribute to revealing gene functions if it is employed together with association analyses and following functional analyses.

1294/W

Molecular population genetics of *PCSK9*: a signature of positive selection. *I.J. Kullo, K. Ding.* Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN.

Proprotein convertase subtilisin-like kexin type 9 (*PCSK9*) is a newly discovered serine protease that plays a key role in regulating plasma low-density lipoprotein (LDL) cholesterol levels. Both rare mutations and common variants in the *PCSK9* coding regions influence LDL cholesterol levels and coronary heart disease risk, as well as response to lipid-lowering therapy. We characterized the pattern of variation at the *PCSK9* locus in African-Americans and European-Americans using resequenced data from the SeattleSNPs database (pga.gs.washington.edu). We performed tests for evolutionary neutrality, including tests based on nucleotide diversity, tests of population differentiation and the long-range haplotype (LRH) test, to detect signatures of recent position selection on *PCSK9*. No significant deviation from neutrality was found using the Tajima's D and Fay and Wu's test. However, using the LRH test, we found non-neutral evolution in two gain-of-function single nucleotide polymorphisms (SNPs) in *PCSK9*, with differential modes of selection in African-Americans and European-Americans. We observed signals of recent positive selection on the derived alleles of SNP rs562556 (I474V, $P = 0.0227$ in simulated distribution, and $P = 0.0001$ in empirical distribution) and rs505151 (E670G, $P = 0.0227$ and 0.0001) in African-Americans, but the ancestral allele of SNP rs562556 ($P = 0.1320$ and 0.0370) appeared to be under positive selection in European-Americans. A significantly high F_{ST} (a measure of population differentiation) between African-Americans and European-Americans was also noted for SNP rs505151 ($F_{ST} = 0.309$). Our findings suggest that evolutionary dynamics may underlie the gain-of-function mutations in *PCSK9* that influence inter-individuals variation in LDL cholesterol levels, susceptibility to coronary heart disease and response to lipid-lowering drugs therapy.

1295/W

The Role of Natural Selection in Shaping Genetic Variation at the N-acetyltransferase (NAT) Genes in African and Global Populations. *H.M. Mortensen[1], P. Awadalla[2], S.A. Tishkoff[1].* 1) Department of Biology, University of Maryland, College Park, College Park, MD; 2) North Carolina State University, Raleigh, NC.

Currently, studies of the possible role of natural selection in shaping the observed variation at drug metabolizing enzyme (DME) loci remain limited. Functional variability at the arylamine N-acetyltransferase (*NAT*) genes is associated with adverse drug reactions and cancer susceptibility in humans. The purpose of this study is: 1) to characterize nucleotide variation at the *NAT* drug-metabolizing genes (*NAT1*, *NAT2*) and the pseudo-gene (*NATP1*) in global human populations, including many previously underrepresented African populations and 2) to understand the role that natural selection has played in shaping variation at *NAT1* and *NAT2* in human populations living in different environmental settings. We resequenced ~3000 bp for each of the *NAT1*, *NATP1* and *NAT2* gene regions, in ~200 ethnically diverse African individuals and ~150 individuals from a representative global panel (HGDP-CEPH). We have identified Single Nucleotide Polymorphisms (SNPs) at each locus (*NAT1* (38), *NATP1* (42) and *NAT2* (40)), and have characterized long-range linkage disequilibrium for the entire ~175 kb region. We are currently testing several models of neutrality under a range of demographic scenarios. This work will contribute to our understanding of how variation at the *NAT* loci may have been adaptive in dealing with changes in diet and exposure to toxins during human evolution. H.M.M. is supported by an NSF grant IGERT-9987590 to S.A.T. Additional funding was provided by US-NSF grants BSC-0196183 and BSC-0552486, US-NIH grant R01GM076637, and David and Lucile Packard Career Award to S.A.T. .

1296/W

Rate of mutation accumulation in coding and noncoding elements during mammalian evolution. *L. Parand[1], S. Nikolaev[1], J. Montoya-Burgos[2], K. Popadin[3], E.H. Margulies[4], NISC Comparative Sequencing Program[4,5], S.E. Antonarakis[1].* 1) Department of Genetics & Dev., University of Geneva Medical School; Geneva, Switzerland; 2) Department of Animal Biology, University of Geneva; Geneva, Switzerland; 3) Department of Genetics, Moscow State University; Russia; 4) Genome Technology Branch, NHGRI, NIH; Bethesda, Maryland 20892, USA; 5) NIH Intramural Sequencing Center, NHGRI, NIH; Bethesda, Maryland 20892, USA.

A comprehensive phylogenetic framework is indispensable for investigating the evolution of constrained genomic features in mammals as a whole and particularly in humans. Using the ENCODE sequence data from 1% of each of 18 mammalian genomes, we reconstructed evolutionary rates for three genomic matrices: silent (dS) substitutions, non-synonymous (dN) substitutions and Conserved Non Coding (CNC) elements. We show that synonymous substitutions (approximating neutral evolutionary rates) evolve according to the Generation Time (GT) hypothesis. Consistent with the longer generation time within mammals, primates and especially humans display a slowdown of neutral evolutionary rates. Constrained elements, however, evolve under different mechanisms. We show that dN substitutions, regarded to be slightly deleterious, are fixed as effectively neutral substitutions in species with small populations (human, chimp) and counter selected in those with large populations (mouse). We found that CNCs are more conserved than dNs in the majority of stem branches, but despite it the average rate of evolution of CNCs is 1.7 times higher than the average dN substitution evolutionary rate. This observation suggests that the selective pressure acting on a fraction of CNCs has been relaxed in a lineage specific manner not predicted by the population size or generation time hypothesis. Using the ENCODE data we detected three cases (Chimpanzee, Shrew and Eutheria) with significant relaxation among the 20 longest CNCs. Thus only a fraction of the CNCs detectable over the entire mammalian tree undergo purifying selection, while another fraction is suggested to be gradually replaced by lineage specific CNCs or those sequences become temporally unconstrained.

1297/W

Molecular evolutionary study of the ionotropic glutamate-receptor gene family as schizophrenia susceptibility genes: human-specific non-neutral pattern observed in *GRIN2B* upstream region. *H. Shibata, K. Tanaka, K. Watanabe, H. Goto, Y. Fukumaki.* Med Inst Bioreg, Kyushu Univ, Fukuoka, Japan.

Schizophrenia is a common psychiatric disease with relatively strong genetic background ($\lambda_s = 10$). Typical preadolescent onset characterized by loss of sociality suggests severely reduced fitness. However, the disease prevalence is highly stable to be ~1% in any human populations. We hypothesized that the schizophrenia susceptibility alleles are maintained by non-neutral process such as balancing selection. To test this hypothesis, we started molecular evolutionary analyses on genes reported to be associated with schizophrenia. In this paper, we report the result of upstream regions of ionotropic glutamate receptor gene family. We collected the complete variation data from the target region by resequencing 50 unrelated humans and 50 unrelated chimpanzees as non-human controls. From the analyses of six ionotropic glutamate receptor genes: *GRIN1* (3.2 kb), *GRIN2A* (0.8 kb), *GRIN2B* (3.8 kb), *GRIN2D* (2.4 kb), *GRIA1* (5.1 kb) and *GRIK1* (4.5 kb), we identified, 123, 40, 41, 56, 131, 107 and 23 segregation sites, respectively. By window plot analysis, we identified significant positive values of Tajima's *D* (+2.16) at the 3.0 kb upstream region of *GRIN2B*. Since population contraction is unlikely for humans, this positive Tajima's *D* is a signature of balancing selection. In contrast, we observed no significant departure from neutral evolution in the same region in chimpanzees, suggesting that the pattern is specific to human lineage. The region harbors only two common SNPs closely located (rs12368476 and novel, 49 bp apart) and no other rare polymorphisms. By genotyping additional 250 samples, we observed modest LD ($r^2 = 0.4$) between the SNPs. We also found that the haplotype with derived alleles on both loci is missing, whereas the other three haplotypes observed in similar frequencies (22-48 %). Neutral process such as population expansion is unlikely to explain this unusual pattern. Since significant association of *GRIN2B* with schizophrenia has been frequently reported, this non-neutral pattern observed in the *GRIN2B* upstream region is potentially associated with schizophrenia.

1298/W

High altitude selection pressure on Angiotensin-I converting enzyme (*ACE*), Insertion(I)/Deletion(D) polymorphism. *T. Stobdan[1,2], A. Nejatizadeh[1,3], T. Norboo[4], G. Mohammad[5], G. Hemashree[6], T. Thinles[5], M.A.Q. Pasha[1].* 1) Functional Genomics Unit, Institute of Genomics and Integrative Biology, Delhi, India; 2) Dept of Biotechnology, Pune University, Pune, India; 3) Dept of Biochemistry, Jamia Hamdard University, Delhi, India; 4) Ladakh Inst of Prevention, Leh, J&K, India; 5) SNM Hospital, Leh, J&K, India; 6) High Alt Med Research Centre, 153 General Hospital, Leh, J&K, India.

The northern Himalaya, divides population of Indo-European(IE) linguistic groups to west and Tibeto-Burman(TB) to east. While most of the IE resides at an altitude of <800m, the TB occupies the Tibetan plateau (altitude >3500), depicting the fundamental model of human adaptation to high altitude (HA). One unique population i.e. Brokpa, an early branch of IE pastoral tribe, believed Aryan descent from IE linguistic family reside at this confluence (alt ~3000m) from time unknown. To investigate the genetic relatedness of Brokpa with its surrounding populations i.e. Ladakhi & Changpa(TB), Punjabi (IE) and one population from other ethnicity, Santhali (Austro-Asiatic), six *Alu* I/D polymorphisms were analysed. The genetic information was obtained by means of genetic distances (DA), PCA and AMOVA. We campared our findings with 32 different populations distributed worldwide. Since the role of *ACE* I/D polymorphism is implicated in various disease susceptibility, including HA adaptation and disorders, its importance in HA selection pressure was also investigated. Our findings suggest that when *ACE Alu* was included, the genetic distance between closely related populations i.e. Brokpas and Punjabi, with Brokpa subjected to HA selection pressure, was more than average. The PCA and phylogenetic analysis were consistent with linguistic pattern when *ACE* was excluded. We showed that Santhali, is a distinct ethnic group. In conclusion we demonstrated that the Ladakhi and Changpa share a common TB gene pool, clustering with the rest of East-Asian populations. Given the importance of the I allele in HA endurance, its over-representation in the geographically isolated Brokpa population in our study, advocates its association with adaptation to HA hypoxic environment.

1299/W

Genetic Polymorphism of Aldehyde Dehydrogenase (*ALDH2*) in Chinese Populations. *G.S. Wu[1, 2], H.R. Luo[1, 2], Y.P. Zhang[1, 2].* 1) Lab of Cellular & Molecular Evol, Kunming Inst Zoology, CAS, Kunming, China; 2) Lab for Conservation & Utilization of Bio-resource, Yunnan University, Kunming, China.

Atypical aldehyde dehydrogenase (*ALDH2*487Lys*), the defect form of ALDH2, caused by the G to A substitution (*Glu487Lys, ALDH2*487Lys*) on the exon 12 of *ALDH2*, is known to influence the drinking behavior because of higher accumulation of acetaldehyde in liver after alcohol intake. *ALDH2*487Lys* is highly prevalent in Asian. In this study, we examined the *ALDH2*487Lys*, four non-coding SNPs within *ALDH2*, and one downstream microsatellite locus, in total of 1,072 unrelated healthy individuals from 14 Chinese populations. Our results showed that the frequency for the atypical *ALDH2*487Lys* in the total samples was 15.11%. Coupled with the data reported previously, our analysis indicated that the frequency of the atypical *ALDH2*487Lys* allele of Guangdong Han population was significantly higher than other geographic populations ($P < 0.05$). The frequency of *ALDH2*487Lys*was decreased gradually from the highest in South China as center to other areas. Based on five SNPs across 40kb, five common haplotypes (with frequency higher than 5%) were found in all East Asian populations. The frequency of the ancestral haplotype (2211G) and the East Asian special haplotype (2211A) was 44.8% and 14.9%, respectively. Linkage disequilibrium extends at least 120kb in almost all populations, indicating a very low level of recombination. The F_{ST} values of the promoter region SNP and the functional SNP were at least two times higher than the other three SNPs. These high F_{ST} values may indicate selection has operated at these tightly linked sites, besides the effect from ancestral population, random genetic drift and genetic differentiation in subpopulation.

1300/W

A latitude dependent positive selection of the polymorphisms of p53 codon 72 and mdm2 SNP309 in Eastern Asian populations. *B. Su[1], H. Shi[1], S. Tan[2], H. Zhong[4], C.J. Xiao[2], Y. Peng[1], X.B. Qi[1], W. Shou[2], R.L. Ma[4], Y. Li[5], X. Lu[1,3].* 1) Kunming Institute of Zoology and Kunming Primate Research Center, Chinese Academy of Sciences, Kunming, Yunnan, China; 2) Human Genetics Center, Yunnan University, Kunming, Yunnan,China; 3) Ludwig Institute for Cancer Research, Oxford Branch, Oxford University, UK; 4) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 5) Qujing Normal College, Qujing, Yunnan, China.

The tumour suppressor p53 is one of the most important tumour suppressors, and mdm2 is the major inhibitor of p53 by acting through an autoregulation loop. Two of the human specific polymorphisms, p53 codon 72 and mdm2 SNP309 respectively, could influence the activities of p53 and mdm2. We screened 4,029 samples from 67 populations across eastern Asia, and we observed a tight link between p53Arg72 frequency and latitude ($r = 0.64$, $p<0.01$, two-tailed t test). Further analysis on ultraviolet radiation also indicated a strong correlation between UV strength and mdm2SNP309 polymorphism. This pattern suggests that the two genetic variations have recently been undergone positive selection in human populations due to the sensitivity of p53 and mdm2 to environmental stress. The data reported here is informative to a better understanding of cancer epidemiology in human populations.

1301/W

A Long-Range Haplotype of the SREBF1 Gene is Common in Europeans and Shows Signs of Recent Positive Selection. *S.D. Bailey[1], G. Paré[2], A. Montpetit[2], T.J. Hudson[1,2,3], D. Gaudet[4], J.C. Engert[1,3].* 1) Department of Human Genetics, McGill University, Montreal, Quebéc, Canada; 2) McGill University and Genome Quebéc Innovation Centre, Montreal, Quebéc, Canada; 3) Department of Medicine, McGill University, Montreal, Quebéc, Canada; 4) Dyslipidemia, Diabetes and Atherosclerosis Group and Community Genomics Research Centre, Université de Montreal and Chicoutimi Hospital, Quebéc, Canada.

The sterol regulatory element-binding transcription factor 1 (SREBF1) plays a pivotal role in cholesterol and lipid homeostasis. Genetic variation at the SREBF1 gene locus has been associated with obesity and type 2 diabetes. In order to capture common variation at the SREBF1 locus, we have genotyped 11 tagging single nucleotide polymorphisms (tSNPs) in 51 populations from the human diversity panel and a large French Canadian sample from the Saguenay-Lac St-Jean region region of Quebéc. Eight of the tSNPs passed quality control in all populations and were used in subsequent analysis. In the European derived populations and the HapMap CEU sample, we identified a common haplotype that spans the entire length, of the SREBF1 gene (~42.5kb). This eight SNP haplotype is the most prevalent haplotype in the European populations analyzed (except for Sardinia) (frequency range = 0.375-0.717). The second most frequent haplotype in all of these samples had the alternate allele at every SNP site (Yin Yang haplotypes)(1). This was the most common haplotype in the HapMap Asian samples (allele frequency = 0.829). In all populations the two Yin Yang haplotypes accounted for > 86% of the alleles. Of the eight SNPs comprising the haplotype, five alleles are ancestral and three alleles are derived. Two of the eight tSNPs were found to have an integrated haplotype score (iHS) of greater than 2 in the HapMap CEU sample, which is indicative of recent positive selection (2). This haplotype represents just a portion of a frequent haplotype (allele frequency = 0.517) that spans a ~250kb block of linkage disequilibrium and contains 98 SNPs with MAFs >0.05 in the HapMap CEU sample.1. Zhang J, et al. Am J Hum Genet 73:1073-1081, 2003; 2. Voight BF, et al. PLoS Biol 4:e72, 2006.

1302/W

First description of A Unique Genetic Isolate of Slavic Origin and possibilities for GWA studies of complex disorders with 500K technology. *P. Kovacs[1], A. Tonjes[1], I. Prokopenko[2], D. Brocklebank[2], Y. Bottcher[1], E. Zeggini[2], R. Rayner[2], J. Halbritter[1], F. Pettersson[2], M. Scholz[1], M.I. McCarthy[2], M. Loeffler[1], M. Stumvoll[1].* 1) University of Leipzig, Germany; 2) WTCHG, University of Oxford, UK.

The study of population isolates with limited genetic variability is predicted to be especially powerful for genome wide association studies offering increased LD and more homogeneous genetic and environmental background. Current technology can offer the opportunity of characterisation of such populations with 500k chip. Here we present a new isolated population of Slavonic origin - the Sorbs of Eastern Saxony, who have been culturally and politically isolated in the small area, lived in an ancient social order consisting of large, extended families for centuries and have a population of ~15,000 individuals living in 10 integrated villages today. External genetic influence is therefore not expected in the population, rendering it a very attractive population for genetic study. We have recruited and extensively phenotyped 1000 individuals. Reconstruction of genealogies is on-going based on church records. Phenotypic data containing laboratory blood tests, anthropometric measures, past medical history of the individual and up to third-degree relatives with focus on diabetes, obesity, hypertension, dyslipidemia, gallstones and goitre is available. All individuals are currently being genotyped using the 500K-Affymetrix-Chips. Based on this data we evaluate the extent of LD and haplotype diversity, and perform a population comparative analysis using HapMap and WTCCC samples. We describe the population using inbreeding coefficient, homozygosity measures and estimation of founding gene pool. Our project offers a unique opportunity to explore the advantages and statistical challenges presented by the study of population isolates, and to use recently developed methodology to incorporated information on relatedness into association analysis.

1303/W

Linkage Disequilibrium extension analysis on the Xq13 region in the island of Corsica. *M.S. Ristaldi[1], G. Sole[1], L. Varesi[2], G. Vona[3], A. Cao[1], V. Latini[1].* 1) INN-CNR, Consiglio Nazionale delle Ricerche, Monserrato , Cagliari, Italy; 2) Universitè de Corte, Corsica, France; 3) Dipt. Biologia Sperimentale, Università di Cagliari.

The identification of genes involved in the pathogenesis of multifactorial diseases would help to shed some light on their physiopathology with significant aid in on the prevention and development of new therapeutic approaches. Genetic isolates with a history of a small founder population, long-lasting isolation and population bottlenecks represent exceptional resources in the identification of disease genes. In these populations the disease allele reveals Linkage Disequilibrium (LD) with markers over significant genetic intervals, therefore facilitating disease locus identification. In a previous work we have examined the LD extension on the Xq13 region in three sub-populations of Corsica belonging to the internal mountainous region of the island. Here we have extended the analysis to the Corsican population of the coast. We found a decreasing of LD in this area. This result indicate a cline of LD inside the island which could be useful for the fine mapping of a gene contributing to a complex disease first mapped using the isolated, high LD, population of the same region. Moreover we reported the frequencies of a particular haplotype (DXS1225-DXS8082) in Corsican population which is typical of the island is not common in other European populations.

1304/W

Distribution of three VNTRs from intron 40 of the VWF gene in ten Mexican Mestizo families with von Willebrand disease. *J.J Palacios[1], R. Peñaloza[1], H. Benitez[2], M. Flores[1], F. Salamanca[1].* 1) Unidad de investigación en Genetica Humana, Centro Medico Nacional Siglo XXI, IMSS, Mexico City; 2) Servicio de Hematologia Pediatrica, Centro Medico Nacional Siglo XXI, IMSS, Mexico City.

Distribution of three VNTRs from intron 40 of the VWF gene in ten Mexican Mestizo families with von Willebrand disease. Palacios JJ1, Benitez H2, Flores M1, Salamanca F1, Peñaloza R1 1.Unidad de investigacion en Genética Humana, CMN SXX, IMSS, México City, MEXICO 2.Servicio de Hematología Pediatrica, CMN SXXI, IMSS, México City Background: von Willebrand disease (VWD) is the most common hemorrhagic alteration in humans, characterized by mucocutaneus bleeding in different intensities due to alterations in the VWF gene, located in chromosome 12p13.3. VWF protein polymerizes and participates in the coagulation system, forming links between the platelets and the site of vascular injury, besides protecting the VIII factor from an early degradation in plasma. Objective: To analyze VNTRs 1,2, and 3 located in intron 40 of VWF gene, which have shown to be useful in segregation studies in other populations, because they are very polymorphic, and usually, they show high heterozygosity levels, basic characteristics of good genetic markers. Materials and methods : Ten VWD patients and their families were studied, previous informed consent. DNA was isolated from blood leucocytes by standard methods, and the required sequences were amplified by PCR. The products were first analyzed in agarose gels to verify if they amplified, and then in polyacrilamide gels. Results and Conclusions: We found high heterozygosity levels in the three studied VNTRs; we also found a novel 4 ATCT repeat in the VNTR1 region, never reported before in Mexican Mestizo population. This results makes them useful in segregation studies and genetic assessment.

1305/W

Copy number variants detected by array-CGH in a Japanese population and their characteristics. *N. Takahashi[1], Y. Satoh[1], K. Sasaki[1], M. Kodaira[1], Y. Kodama[1], H. Omine[1], Y. Shimoichi[1], K. Sugita[2], H. Katayama[2], N. Tsuyama[3].* 1) Dept Genetics, Radiation Effects Research Foundation, Hiroshima, Japan; 2) Dept Information Technology, Radiation Effects Research Foundation, Hiroshima, Japan; 3) Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

[Purpose] We have studied the effects of atomic-bomb radiation on human germline cells at the DNA level. To conduct this study at the genomewide level, we have introduced DNA micro-array based comparative genomic hybridization (array-CGH). Preliminary experiments revealed that, using the optimum conditions established by us, copy number variants (CNVs) with the size of about 40 kb or more could be detected. Before launching a large-scale study, the feasibility of array-CGH was validated in a pilot study. We will report on various variants identified in the pilot study. [Experiment] We used an array with 2,238 Bac-clones. These target clones were distributed across human autosomes at an interval of about 1.2 Mb. We examined 40 offspring of A-bomb survivors and 40 controls. [Results and Discussion] We found a total of 251 CNVs at 30 different regions in the genome; of these, 14 (termed “rare” CNVs) were found individually located within distinct genomic regions of 14 individuals, while the remaining 16 CNV regions (termed “common” CNVs) were observed in two or more individuals. The rare CNVs were confirmed by quantitative PCR, and characterized more precisely than in previous reports using array CGH methods. Distinctive features of these CNVs were observed: Most prominent was that the majority of the rare CNVs presented on Bac-clones that did not overlap with regions of segmental duplication. About 90% of the common CNVs in this population had been previously identified, with the majority of those common CNVs located in regions of segmental duplication. It is likely, therefore, that rare and common CNVs arise through different genetic mechanisms. Since more than half of the rare CNVs are novel, it is also likely that different human populations bear different CNVs, as is the case for single-nucleotide-polymorphisms (SNPs) and insertion-deletion (indel) polymorphisms.

1306/W

Genomic Estimates of Inbreeding in the Old Order Amish. *C.V. Van Hout, J.A. Douglas.* Department of Human Genetics, University of Michigan, Ann Arbor, MI.

In population isolates, like the Old Order Amish (OOA), complex genealogies with multiple loops often exist. If the genealogies are not fully known, then the inbreeding coefficient may be underestimated. The inbreeding coefficient F is the probability that the two alleles at any autosomal locus in an individual are identical by descent. Underestimation of F may artificially increase the rate of false positive results, e.g., in the context of linkage analysis. Although matings between close relatives, e.g., first cousins, in the OOA are rare, the random component of inbreeding, i.e., the portion due to random mating in a finite population, is the same process as genetic drift and may contribute more to inbreeding through time than close consanguinity. We estimated inbreeding in the OOA of Lancaster County Pennsylvania by connecting 790 individuals into a single 14-generation pedigree with PedHunter (Agarwala et al. 1998) and the Amish Genealogy Database (AGDB 4.0) (Agarwala et al. 2001). These individuals were participants in a family-based genetic study of cardiovascular traits and were previously genotyped for a high-density map of single nucleotide polymorphisms (SNPs). We then estimated F from each individual’s genomic information using the maximum likelihood method recently proposed by Leutenegger et al. (2003; 2006) and implemented in their program FEstim. Simulation results suggest that FEstim accurately estimates F given dense marker maps and highly heterozygous markers (Leutenegger et al. 2003). Genomic data, including 2,408 SNPs distributed across the 22 autosomes, were used to estimate F for each individual. The median genealogy-derived F was 0.034 (range of 0.0003 to 0.076), and the median genomic-derived F was 0.032 (range of 0.001 to 0.095). Approximately 50% of FEstim estimates of F were significantly different from zero. However, less than 7% were significantly different from the genealogy-derived F. Notably, the median difference between the genealogy- and genomic-derived inbreeding coefficient was 0.001, and no absolute difference was greater than 0.06. Our results suggest that accurate knowledge of each individual’s inbreeding coefficient is given by the genealogy.

1307/W

The CanMap Project: Population Genetics and Whole Genome Association Mapping of Morphological and Behavioral Differences among Domestic Dog (Canis familiaris) Breeds. *C.D. Bustamante[1], T. Spady[2], H.G. Parker[2], B. vonHoldt[2,3], K. Bryc[1], M.H. Wright[1], N.B. Sutter[2], A. Reynolds[1], A.R. Boyko[1], M. Castelhano[1], E. Wang[4], K. Zhao[1,5], G. Johnson[6], M. Nordborg[5], R.K. Wayne[3], M. Cargill[4], E.A. Ostrander[2].* 1) Cornell University, Ithaca, NY; 2) NHGRI/NIH, Bethesda, MD; 3) UCLA, Los Angeles, CA; 4) Affymetrix, Santa Clara, CA; 5) U. Southern California, Los Angeles, CA; 6) U. of Missouri, Columbia, MO.

Domestic dog breeds exhibit great variation in behavior and morphology among breeds and low phenotypic and genetic diversity within breeds, making the dog an excellent genetic system for mapping traits of interest. Here, we present population genetic analyses and preliminary results for simultaneous whole-genome association mapping of morphological and behavioral trait differences among breeds using a panel of 1,000 dogs from 80 breeds genotyped on the Affymetrix Canine Array v.2.0 (~100,000 SNP). Population genetic analyses reveal clear genetic clustering of dogs into breeds with well defined boundaries, and shallow clustering of breeds into higher order groups. We use fine-scale recombination rate estimates across the genome to identify regions of unusually high linkage-disequilibrium within a breed, which may identify recent targets of selection during breed formation. We also estimate the domestication bottleneck size for dog as well as breed-specific bottleneck and inbreeding rates which account for dramatic differences in effective population size among popular breeds. Using a mapping strategy that accounts for expected high genetic relatedness within a breed, we aim to identify regions of the dog genome associated with skeletal conformation, hair pigmentation and texture, and behavioral trait differences including: body size, foreshortened limbs, foreshortened face, compact face and cranimum, proportional dwarfism, wire hair, curly hair, corded coat, face mask color, and prey drive. For several traits, overlying “peaks” of association with signatures of selection allows us to refine our signals to a just a few candidate genes. The approach we employ replicates previously identifies gene-trait association, including the link between IGF1 and body size.

1308/W

Improving tag SNP portability in India using optimal mixtures of database samples. *T.J. Pemberton[1], M. Jakobsson[2], D.F. Conrad[3], G. Coop[3], J.D. Wall[4], J.K. Pritchard[3], P.I. Patel[1], N.A. Rosenberg[2].* 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) Department of Epidemiology and Biostatistics, University of California, San Francisco, CA.

When performing tag SNP association studies in populations that have not been the focus of large-scale studies of haplotype variation, it is necessary to rely on genomic databases in other populations for the selection of suitable tag SNPs. Recent studies have found that among the populations for which such databases are least effective in tag SNP selection are populations of low or intermediate linkage disequilibrium that are genetically distant from populations in the databases. One important geographic region that has not been the focus of major SNP genotyping efforts is India. To improve the performance of tag SNPs in India - and in non-HapMap populations more generally - we study tag SNP portability using genotypes at 2,810 SNPs spanning 12 Mb of DNA sequence in a worldwide sample of 957 individuals, including 30 individuals from India. We show that a strategy that uses tag SNPs chosen based on mixtures of HapMap populations has the potential to produce improved tagging compared to a strategy that relies only on the most similar HapMap population. The difference in composition between optimal mixtures in different populations from across Asia is compatible with the differing geographic positions of the groups. These results are important both for association studies in India and more generally for improving tag SNP portability in non-HapMap populations.

1309/W

Further SNP mapping of 10q26 supports strong association of rs11200638 in the HTRA1 promoter to age-related macular degeneration. *D. Gibbs[1], Z. Yang[1], D.J. Cameron[1], C. Stratton[1], A. DeWan[2], J. Hoh[2], K. Zhang[1].* 1) Department of Ophthalmology and Visual Sciences, University of Utah, Salt Lake City, UT; 2) Department of Epidemiology and Public Health, Yale University, New Have, CT.

Purpose: Age related macular degeneration (AMD) is the leading cause of blindness in the developed world. While this disease is known to have a genetic basis, few genes have been consistently implicated. One locus at chromosome 10q26 has been shown in multiple independent studies to confer risk. Recently a SNP in the 10q region in a transcription factor binding site of the promoter of HTRA1 gene was described as a causal variant for AMD. The purpose of this study is to investigate addition variants in chromosome 10q26 in order to refine understanding of the region. Methods: Using DNA extracted from peripheral blood leukocytes, additional single nucleotide polymorphisms (SNPs) were genotyped by PCR and the SNPSHOT method on an ABI 3130xl Sequencer. A Utah cohort, of 546 AMD patients, was genotyped for addition SNPs in chromosome 10q26 and allele frequencies were compared to 294 age and ethnicity matched normal controls by lab personnel blinded to case/control status. Results: We genotyped 18 single nucleotide polymorphisms (SNPs) in the 10q26 region encompassing the genes PLEKHA1, ARMS2 (formerly LOC387715), and HTRA1. Many variants throughout this region were associated with AMD but rs11200638 was the most significantly associated polymorphism with a chi-squared test for trend p-value of 5.3X10-15. Conclusions: From this comparative data the HTRA1 promoter SNP rs11200638 still remains the most significantly associated SNP with AMD. These findings do not exclude the possibility that there may be multiple causal variants. However rs11200638 remains the best candidate presented thus far.

1310/W

A multiple founder effect of HFE C282Y mutation explains the hereditary hemochromatosis in Azorean island of Sao Miguel (Portugal). *C.T. Gomes[1], P.R. Pacheco[1,2], M. Sao-Bento[1], R. Cabral[1,2], C.C. Branco[1,2], L. Mota-Vieira[1,2].* 1) Molecular Genetics Pathology Unit, Hospital of Divino Espirito Santo, Ponta Delgada, Azores, Portugal; 2) Institute Gulbenkian of Science, Oeiras, Portugal.

Hereditary hemocromatosis (HH) is an autosomal recessive disorder of the iron metabolism. It is typically associated with homozygosity for the C282Y mutation of the HFE gene, which is located on the HLA region (6p21.3). Generally, this mutation lies within the celtic ancestral HLA-A*03-B*07 haplotype. Here, C282Y mutation was selected as a model to study the diversity and origin of recessive mutations in a geographic isolated population. A total of 130 individuals from Sao Miguel Island (Azores) were genotyped for HLA-A and -B by PCR-SSP, and for HFE mutations (C282Y, H63D and S65C) by PCR-RFLP. Data analysis was performed using Arlequin v3.1 and Graphpad Prism v5.0 softwares, after dividing the sample in two groups: 48 homozygous or carriers for the C282Y mutation and 82 with no mutations. Statistical analysis revealed that four alleles - HLA-A*03 (20.8%), HLA-A*26 (2.1%), HLA-B*29 (10.4%) and HLA-B*45 (9.4%) - protrude in the C282Y mutation group, when compared to the control subjects ($p<0.05$). Moreover, three haplotypes, identified by computational inference, were found to be significantly associated with the C282Y: HLA-A*02-B*58 (5.2%; OR=19.78, 95% CI: 1.08-362.0), HLA-A*03-B*07 (5.2%; OR=8.96, 95% CI: 1.03-77.84) and HLA-A*29-B*45 (7.3%; OR=27.57, 95% CI: 1.56-488.70). Another haplotype HLA-A*24-B*15 (3.1%) was also identified by direct inference in an individual homozygous for HLA region and C282Y mutation. These haplotypes probably have several geographical origins. Overall, these findings suggest that HH in the São Miguel Island can be explained by a multiple founder effect. (lmotavieira@hdes.pt), Azorean Government founded.

1311/W

***LRRK2* R1441G: evidence of a common founding event in the 9th century in northern Spain.** *I.F. Mata[1, 2], C.M. Hutter[3], C. Huerta[4], M. Blazquez[5], R. Ribacoba[5], L.M. Guisasola[5], C. Salvador[5], J. Infante[6], M.C. Gonzalez-Fernandez[7], M.M. Pancorbo[7], E. Lezcano[8], J. Jankovic[9], H. Deng[9], K.L. Edwards[3], V. Alvarez[4], C.P. Zabetian[1, 2].* 1) GRECC, VA Puget Sound HCS; 2) Neurology Dept, Univ of Washington; 3) Epidemiology Dept, Univ of Washington, Seattle, WA; 4) Genetica Molecular-Instituto de Investigacion Nefrologica, Hospital; 5) Servicio de Neurología, Hospital Univ Central de Asturias, Oviedo; 6) Neurology Service, Univ Hospital "Marqués de Valdecilla", Univ of Cantabria, Santander; 7) Servicio General de Investigación Genómica: Banco de ADN, Univ of the Basque Country, Vitoria-Gasteiz; 8) Servicio de Neurología, Hospital de Cruces, Barakaldo, Spain; 9) Neurology Dept, Baylor College of Medicine, Houston, TX.

The Leucine-Rich Repeat Kinase 2 (*LRRK2*) gene represents the most common genetic determinant of Parkinson's disease (PD) identified to date. The vast majority of patients with *LRRK2*-related PD carry one of three pathogenic mutations: G2019S, R1441C, or R1441G. While G2019S and R1441C are geographically widespread and have each arisen from at least three different founders, R1441G appears to have arisen from a single founder and is largely restricted to northern Spain. We sought to better understand the genetic and demographic processes that have shaped the current distribution of R1441G. We genotyped 10 microsatellites and one SNP spanning a distance of 15.1 Mb across the *LRRK2* region in 29 unrelated PD patients heterozygous for R1441G and 37 wild-type controls. All controls and all but one patient were from northern Spain. We used PHASE v2.1.1 to infer haplotypes and a maximum-likelihood method to estimate the age of the most recent common founder. Significant allele sharing was observed over a region approximately 9.0 Mb in length bounded by markers D12S2080 and D12S1301. A single, rare background haplotype was seen in all patients indicative of a common ancestor. We estimate that the founding event occurred 1,170 (95% CI, 900-1,530) years ago in approximately the 9th century. This, coupled with limited migration might explain the restricted distribution and relatively high frequency of R1441G in northern Spain.

1312/W

A complex evolutionary pattern of haplotypes at *RET*. *N. Mukherjee, J.R. Kidd, W.C. Speed, A.J. Pakstis, K.K. Kidd.* Dept Genetics, Yale Univ Sch Medicine, New Haven, CT.

RET is involved in the etiology of complex disorder Hirschprung disease (HSCR) and the Mendelian disorder Multiple Endocrine Neoplasia2 (MEN2). We have studied the genomic region around the *RET* protooncogene on chromosome 10q11.2 in order to understand its pattern of haplotype diversity across global populations. We have genotyped 92 single nucleotide polymorphisms (SNPs) spanning 339 kb in 38 global populations. We identified a 134 kb region including the 3' half of the *RET* gene and extending to 5' of the *RASGEF1A* gene, in which 17 SNPs, interspersed among 20 other SNPs, have almost perfectly correlated (>0.98) allele frequencies in all the populations of non-African ancestry, i.e. have nearly identical allele frequencies in each population of non-African ancestry. Analyzing all the SNPs across this region shows that two distinct haplotype lineages have formed independent of each other by mutations of different sets of SNPs. The most common haplotypes do not show evidence of recombination. In the populations of African ancestry derivatives of only one haplotype lineage predominate while in the populations of non-African ancestry derivatives of both the haplotype lineages comprise 80% or more chromosomes. Though LD exists between SNPs within this region and those flanking, LD is much lower at either end of the 134 kb region indicating greater historical recombination. The primary MEN2 mutations lie in exon 11, only 180 nucleotides from the first of these SNPs. The SNP thought most strongly associated with HSCR lies in intron 1, 28.3 kb from this downstream region but show significant LD with the common 17 SNP haplotypes. The HSCR-associated allele is predominantly associated with the haplotype that is essentially non-African, consisting of mostly derived alleles, and the most common haplotype in East Asia. The increased frequency of the non-African haplotype seems to be an effect of random genetic drift. However, maintenance of the HSCR associated allele in high frequency in some popualtions is intriguing and reamins open for further investigation. Supported by NIH grant GM057672 to KKK.

1313/W

Origins of regulatory mutations at the LCT locus in African populations. *A. Ranciaro[1,2], F. Reed[2], J. Hirbo[2], K. Powell[2], O. Sabah[3], M. Osman[4], H. Muntaser[4], S.A. Tishkoff[2].* 1) Dept. of Biology, University of Ferrara-Italy; 2) Dept. of Biology University of Maryland, College Park, MD; 3) Kenya Medical Research Institute, Centre of Biotechnology Research and Development, Nairobi, Kenya; 4) Dept. of Molecular Biology, Institute of Endemic Diseases, University of Khartoum-Sudan.

In most human populations, the ability to digest lactose, the sugar present in milk, declines rapidly after weaning because of decreasing levels of the enzyme lactase (lactase-phlorizine hydrolase, LPH) in the small intestine. However, there are individuals who maintain the ability to digest milk and other dairy products into adulthood due to a genetic adaptation, primarily in populations that herd cattle and have a history of drinking fresh milk. The goal of the current project is to identify new variants that may be associated with Lactase Persistence (LP) in ethnically diverse populations, to characterize nucleotide diversity in a diverse set of African and Middle Eastern populations and to reconstruct the evolutionary history of this region. We collected phenotype data from from Tanzania, Kenya and the Sudan. We resequenced 1.7 kb of intron 9 and 3.3 kb of intron 13 of the MCM6 gene (previously found to be associated with lactase persistence in European populations) and 2.2kb of the promoter region of the LCT gene in 280 Africans with phenotypic data and in 300 African and Middle Eastern individuals without phenotypic data. Four SNPs located in intron 13 (at position -14010, -13915, -13910, -13907 from the start of the LCT gene) showed a significant association with the LP trait. Resequencing of these regions in a panel of great apes indicated that the alleles associated with LP are derived. This result suggests that multiple mutations arose independently in different African populations due to convergent evolution. These results have implications for understanding the origins of pastoralism as well as historic migration events within Africa.

1314/W

Balancing selection maintains allelic diversity at MBL2 and TLR6 loci. *P. Majumder[1], D. Wagener[2], U.S.A.-India Research Group on Vaccine Response (RTI, Duke, CpG, TCGA, NICED).* 1) Human Genetics Unit, Indian Statistical Inst, Kolkata, India; 2) RTI International, Research Triangle Park, North Carolina, USA.

Because innate immunity (InnImm) genes play a vital role in microbial recognition and activation of the adaptive immune system, natural selection may play a crucial role in shaping the genotype and allele frequencies among individuals inhabiting areas that have a high load of bacterial and other microbial pathogens. We sampled ~175 unrelated individuals from two communities (Muslim and Hindu) inhabiting slums of Kolkata, India, with annual outbreaks of typhoid, cholera and other diarrheal diseases. We resequenced (double-pass) 11 InnImm genes (DEFA4, DEFA5, DEFA6, DEFB1, MBL2, TLR1, TLR2, TLR4, TLR5, TLR6, TLR9). We found many unreported SNPs. Allele and haplotype frequency are similar for both communities. Haplotype diversities are high (0.6-0.9). The correlation coefficient of LD values of adjacent SNPs in all genes between Muslim and Hindu is 0.98, indicating a strong similarity of LD structures in both communities. Analyses of these data revealed (a) Tajima's D, and Fu & Li's D* and F* values are all significant ($p<0.05$) and positive for MBL2 and TLR6, indicating an excess of intermediate frequency variants that is observed under balancing selection, and (b) two or more high-frequency haplotypes are separated by long branches in a median-joining network - a signature of balancing selection - for both genes. For MBL2, 4 of 20 haplotypes and, for TLR6, 3 of 14 haplotypes have high frequencies (>15%). The MBL2 and TLR6 produces proteins at the cell surface that are crucial for recognition of a wide range of pathogens. Thus, our finding that the overall allelic diversity at these loci is maintained by balancing (diversifying) selection is consistent with their function, which is similar to earlier findings for the MHC locus that is also involved in pathogen recognition. Supported by: NIAID, NIH, USA, Contract No.: HHSN200400067C.

1315/W

Living in a box: Three cosegregating genes as determinants of heart failure. *F. Friedrichs[1, 2], C. Zugck[2], G.-J. Rauch[2], B. Ivandic[2], D. Weichenhan[2], M. Mueller-Bardorff[3], N.E. El Mokhtari[4], V. Regitz-Zagrosek[5], R. Hetzer[5], A. Schaefer[4], S. Schreiber[4], J. Chen[6], I. Neuhaus[6], R. Ji[6], N.O. Siemers[6], N. Frey[2], W. Rottbauer[2], H. Katus[2], M. Stoll[1].* 1) Leibniz-Institute for Arteriosclerosis Research, Münster, Germany; 2) University Hospital Heidelberg, Heidelberg, Germany; 3) University Clinics Schleswig-Holstein Lübeck, Lübeck, Germany; 4) University Clinics Schleswig-Holstein Kiel, Kiel, Germany; 5) Deutsches Herzzentrum Berlin, Berlin, Germany; 6) Bristol-Myers Squibb Research and Development, USA.

The finding of multiple cosegregating susceptibility genes is considered a limitation for identification of the underlying disease gene. We performed a comprehensive linkage disequilibrium (LD) mapping study for human cardiomyopathy in three independent Caucasian study samples and show replicated association of a 600 kilobases LD block on 5q31.2-3 with heart failure. We analyzed evolutionary relationships of the haplotypes using a median joining network to identify SNPs representing groups of related haplotypes (e.g. a group of risk related haplotypes tagged by rs2569193; first sample: odds ratio (OR)=0.73, 95% CI=0.55-0.96, p= 0.024; second sample: OR=0.81, 95% CI=0.66-0.99, p=0.039; third sample: OR=0.64, 95% CI=0.45-0.91, p=0.012). The associated cluster harbors several co-expressed genes and is conserved in syntenic blocks in other mammalian genomes. Estimates of Ka/Ks evolutionary characteristics comparing human, rodent, chicken, and frog are consistent with high functional conservation of the loci within the region. Synteny is largely intact in birds, detectable in amphibians, but not present in fish. To elucidate the individual contribution of the clustered genes the human genetic studies were complemented by functional studies using antisense oligonucleotide mediated knock-down in zebrafish. We show that three of the clustered genes, HBEGF, IK and SRA1, independently result in a myocardial phenotype of contractile dysfunction. We hypothesize that the emergence and conservation of LD in the genome reflects clusters of functionally cooperating genes that synergize to determine a complex trait.

1316/W

Mitochondrial DNA Mutations Found in Native Central and South American Samples Provide Evidence for Mitochondrial Adaptation to New Environments. *O. Derbeneva, K. Von Hasseln, M. Brandon, M. Lvova, D.C. Wallace.* MAMMAG, University of California, Irvine, Irvine, CA.

Human mitochondrial DNA (mtDNA) lineages (haplogroups, hplgrs) show striking geographic associations. This led us to hypothesize that ancient mtDNA mutations which altered the mitochondrial OXPHOS energy allocation between ATP production and heat generation (coupling efficiency) permitted humans to adapt to new climatic zones. Analysis of mtDNA sequences revealed that the mtDNA COI gene is variable in the tropics, the cytochrome b gene is variable in the temperate zone, and the ATP6 gene is variable in the arctic (Mishmar et al., 2003, PNAS 100:171-176). The mtDNA hplgrs A2, C1, and D1 arose in Siberia and subsequently cross the Bering land bridge to colonize the Americas. Therefore, these cold adapted Siberian mtDNAs had to readapt to the tropical conditions of Central and South America. To determine if these mtDNA lineages acquired new mtDNA mutations permitting topical survival, we sequenced 91 A2, C1 and D1 mtDNAs from 9 South and Central American Amerindian tribes. Both novel and recurrent polypeptide, tRNA & rRNA, and control region mutations were found to have been acquired as the Siberian mtDNAs became established in the tropics. The ratio of non-synonymous to synonymous variants was found to be 2.5 times higher at the base of the branches of the tropic D1 tree than at the ends consistent with early adaptive selection. Furthermore, missense mutations were elevated 1.5-3 fold in the ATP6 and COII genes relative to arctic and non-arctic mtDNAs, and many of the acquired missense mutations had previously been observed in other populations on different mtDNA hplgrs, e.g. nucleotide (nt) 4216 previously found in European hplgrs T & J; nt 13708 in J; nt 5460 in W, Q1, Q2; nt 1719 in I & X; nt 1888 in T; nt 15924 in L; etc. Such convergent evolution can only be explained by adaptive selection. Thus, Siberian mtDNA lineages A2, C1, and D1 acquired adaptive mutations as they migrated into tropical America, thus proving that mtDNA variation has permitted human adaptation to new environments.

1317/W

Mitochondrial DNA polymorphism and its association with longevity in the Latvian population. *A. Krumina[1], L. Pliss[2], A. Brakmanis[1], V. Baumanis[2].* 1) Medical Biology & Genetics, Riga Stradins University, Riga, Latvia; 2) Latvian Biomedical Research and Study Centre, Riga, Latvia.

There is increasing evidence that some mitochondrial DNA (mtDNA) polymorphisms of coding region and control regions HVSI and HVSII could affect rate of ageing (Wallace, 2005). mtDNA haplogroup J has been reported to increase the chance to attain longevity in northern Italians and Finns, haplogroup K - in the French and Irish, haplogroup D - in the Japanese. These findings allow to suggest that the association of mtDNA variability with longevity may be population specific, depending on both genetic and environmental background. Studies of the control region HVSII polymorphism in association with longevity so far are very limited.

The aim of our study was to verify if there is any association between mtDNA coding and control region polymorphisms and longevity in the Latvian population.

Objects were 351 healthy unrelated Latvians 18 - 40 years old, 98 individuals aged 74 - 89 years and 44 centenarians. Material of the research was DNA isolated from leukocytes. mtDNA haplogroups depending on mutations in the coding region of mtDNA were determined by PCR - RFLP analysis. Polymorphisms in the control regions HVSI and HVSII were analysed by direct DNA sequencing.

The frequencies of haplogroup I and subhaplogroup U4 were significantly lower and that of subhaplogroup U5a significantly higher in the older age group (74 - 89 years) than in younger individuals. Frequency of HVSII polymorphism at the site 00068 was significantly higher in centenarians with no cases of this type of polymorphism observed in the youngest age group.

Our results support hypothesis that certain population specific inherited mtDNA polymorphisms may promote human longevity.

1318/W

Construction of a SNP-Associated, Mitochondrial Haplogroup Database. *E.L. Stevens, R.I. Sanchez, M.V. Osier, D.L. Newman.* Biological Sciences, Rochester Institute of Technology, Rochester, NY.

The 16.5 kb mitochondrial (mt) genome contains thousands of SNPs due to its high mutation rate. A subset of these SNPs have been used to define haplogroups that correlate with populations and are used to study population genetics, ancient human migrations, and disease associations. Much literature has been published that associates certain alleles with one or more haplogroups, but the public databases (e.g. Mitomap) do not associate particular SNPs with specific haplogroups. We are constructing a searchable, Web-based database that links SNP alleles with observed haplogroups and the location/functional consequences of each variant. We have already begun to populate the database using data gathered from literature and online databases to create a user-friendly, publicly accessible database. We are also adding information from our own research and will accept contributions from other investigators. The database includes links to references that document the sources of information. We have begun with the European haplogroups (H, I, J, K, T, U, V, W, X) but eventually plan to include all haplogroups. A search tool will enable the user to determine all alleles that have been associated with any particular haplogroup or sub-haplogroup, or to determine which haplogroups or sub-haplogroups are known to be associated with any particular SNP allele (note that not every SNP allele belongs exclusively to a single haplogroup). This information will be useful to researchers investigating inherited diseases associated with the mt genome. For example, if members of a certain haplogroup had increased susceptibility to a disease, this tool would be useful for quickly identifying candidate loci to investigate for potential functional consequences in the development of the disease.

1319/W

Can environmental contamination affect mtDNA pedigree mutation rate? *K. López-Agívarez, N. Pérez-Nazario, C. Torres-Vargas, E. Guzmán-Morales, L. Rivera del Toro, V. Figueroa-Tañón, J. Concepción-Acevedo, T. Toro-Ramos, G. González-Guardiola, D. Morales-Hernández, J.C. Martínez-Cruzado.* Dept. of Biology, University of Puerto Rico at Mayagüez, Mayagüez, PR.

Cancer rate in Vieques, an island municipality of Puerto Rico, is far higher than in the big island of Puerto Rico. Environmental contamination with mutagenic substances that may increase the DNA mutation rate is a reasonable but unproven hypothesis explaining this observation. We propose that if the underlying mutation rate in Vieques is higher than in Puerto Rico, this effect may be observable in the mtDNA control region in the form of a higher frequency of heteroplasmies as manifested by peak heights in chromatograms. We collected mouthwash samples from 42 maternal families each in Vieques and Puerto Rico, totaling 569 samples and the same number of generational transmissions. As phylogenetic evidence suggests that mutability at some mtDNA sites can vary with haplogroup identity, the same haplogroups were collected at each island except that one haplogroup T mtDNA in Vieques was substituted for a haplogroup J in Puerto Rico. We have sequenced the control region of 140 and 101 samples from Puerto Rico and Vieques, covering 135 and 95 transmissions, respectively. We have found one hereditary heteroplasmy involving a nucleotide substitution for each population. One involved a pyrimidine transition and the other a purine transition at the haplogroup L2-defining position 16390 in a haplogroup U5 background. Preliminarily, the findings do not support a significant mutation rate difference between the islands nor relative to populations studied previously by other investigators. Assuming 20 years per generation, the pedigree divergence rate for the entire control region is estimated at 0.78 mutations/site/Myr (95% CI: 0-1.85).

1320/W

Inferring selection intensity and allele age from haplotype structure. *H. Chen, M. Slatkin.* Department of Integrative Biology,University of California, Berkeley, CA.

It is a challenging task to infer selection coefficient and allele age from population genetic data. Here we present a method that can efficiently estimate selection coefficient from the haplotype structure around the vicinity of the segregating selected allele. A subdivided population model with varying population sizes is used to model the historical frequency trajectories of the selected allele. Given the trajectories and the selected mutant position, the genealogies of the haplotypes are modeled with random mutation, coalescent and recombination events. The importance sampling algorithms are adopted to explore both frequency trajectories and gene genealogies consistent with the sample. By the simulation data, we demonstrate that the method can estimate the selection intensity for moderate selection. We also applied the method to a real data set G6PD. The proposed method is highlighted in handling haplotype data from recombination regions and from populations with exponential growth or other arbitrary histories.

1321/W

Tooth enamel thickness and adaptive evolution of *enamelin* in Humans and among Primates. *J.L. Kelley, W.J. Swanson.* Genome Sciences, University of Washington, Seattle, WA.

Scans of the human genome have identified many loci as potential targets of recent selection, but exploration of these candidates is required to verify the accuracy of genome-wide scans and clarify the importance of adaptive evolution in recent human history. We present analyses of one such candidate, *enamelin*, whose protein product operates in tooth enamel formation. *enamelin* sequences of 100 individuals from 10 populations show lower than expected levels of nucleotide polymorphism. Evidence of a recent selective sweep at this locus confirms the signal of selection found by genome-wide scans. Patterns of polymorphism in *enamelin* correspond with population-level differences in tooth enamel thickness, and selection on enamel thickness may drive adaptive *enamelin* evolution in human populations. Sequences of exons from 12 primate species show evidence of historical selection on *enamelin*. In primates enamel thickness correlates with diet, and bursts of adaptive *enamelin* evolution occur on primate lineages with dietary changes and evolved differences in enamel thickness. Our hypothesis is that among human populations, and among primate species, evolution of tooth enamel thickness is associated with the adaptive evolution of *enamelin*.

1322/W

Tracing the Selection on Human ADH1B Gene. *H. Li, S. Gu, K.K. Kidd.* Department of Genetics, Yale School of Medicine, New Haven, CT.

Alcohol dehydrogenase (ADH) is a widely studied enzyme as is the gene family encoding the focus of this enzyme. Previous studies have shown that the *ADH1B*47His* allele is associated with a decrease in the risk of alcoholism and the core region with this allele has undergone positive selection in some populations. A literature review identified studies reporting allele frequencies of this polymorphism for 131 population samples (for a total of 168 when combined with our new data on 37 populations. The derived *ADH1B*47His* allele reaches high frequencies only in West and East Asia, but has a low frequency in the region between East and West Asia, suggesting that the derived allele increased in frequency independently in the two regions. We tested seven single nucleotide polymorphisms (SNPs) and two short tandem repeat polymorphisms (STRPs) in the *ADH1B* region in the world sample to form the haplotypes. Seven haplogroups were defined with different SNP allele patterns. H5, H6, and H7 are haplogroups with the derived *ADH1B*47His* allele. H5 is restricted in West Asia and H6 is in East Asia and Pacific region. H7 has in addition to H6 the derived allele of rs3811801 in the regulatory region, and is restricted to East Asia. We analyzed 24 population samples from East Asia covering six ethnic families and find H7 is enriched in the Hmong, Han Chinese, and Altaic families. We typed 23 more SNPs in about 170kb flanking region of *ADH1B*. The extended haplotype homozygosity (EHH) and relative EHH tests for the *ADH1B* core were consistent with selection for the haplogroups with derived SNP alleles in the Hmong and Altaic. Other populations showed only a weak signal at best. The selection distribution is significantly correlated with the frequency of the derived regulatory polymorphism rs3811801, not the derived amino-acid altering allele *ADH1B*47His*. Thus, the real focus of selection may be the regulatory region of H7. The *ADH1B* downstream STRP provides relative age estimates for the SNP-based haplogroups that are in general agreement with the Out of Africa pattern and a recent expansion of H7 in East Asia. The refined pattern of variation for *ADH1B* haplogroups will help design studies to understand the selective force(s) that may have operated.

1323/W

Identification of potential functioning variants in COMT through high frequency derived allele haplotypes. *J.B. Listman[1], H.R. Kranzler[2], R. Anton[3], J. Gelernter[4,5].* 1) Dept. Anthropology, New York Univ., New York, NY; 2) Dept. Psychiatry, Univ. of CT Sch. Medicine, Farmington, CT; 3) Dept. Psychiatry, Med. Univ SC, Charleston, SC; 4) Depts. Psychiatry, Genetics, and Neurobiology, Yale Univ. Sch. Medicine, New Haven, CT; 5) VA CT West Haven, CT.

Variation in the gene encoding the enzyme catechol-O-methyltransferase (COMT) has been investigated in relation to phenotypes including schizophrenia, suicidal behavior, pain response, substance dependence, anxiety, and intelligence, with a focus on the functional val108/158met polymorphism (the val allele is the ancestral allele as determined by comparison with the chimp genome); however, this variant alone cannot account for the effects of the locus on the above phenotypes. We genotyped 149 European Americans (EA) and 165 African Americans (AA) for val108/158met and an additional 14 SNPs spanning 25.583 kb of COMT. The most common reconstructed haplotype extending across all 15 SNPs in EAs (14%) and the second highest in AAs (5%) includes the derived met allele but also the derived alleles at three other SNPs (rs933271, rs5993883, and rs740603). The most common 15-SNP haplotype in AAs (7%) contains the four ancestral alleles at the same loci. In EAs, this haplotype has a frequency of 3%. While we did not find high linkage disequilibrium in this region overall, for a core region spanning these three additional loci we found in EAs higher Relative Extended Haplotype Homozygosity (high LD extending from a high frequency haplotype - a departure from neutrality) for the three-derived-allele haplotype than for the three-ancestral-allele haplotype. In AAs we found the opposite. The three additional derived alleles in the most common EA15-SNP haplotype are potentially functional variants for phenotypes in which the val108/158met allele has been implicated but does not fully explain phenotypic variation, as they may show a signature of positive selection. These findings bear further investigation, particularly in the EA population.

1324/W

Rapid Selection for HLA alleles that Protect against HIV-1 Infection Correlates Significantly to the Declining Incidence of HIV-1 in an East African Sex Worker Population. *M. Luo[1,2], J. Kimani[1,2], N.J.D. Nagelkerke[3], T. Ball[1], K. MacDonald[4], J. Ndinya-Achola[2], S. Njenga[2], J. Bwayo[1], S. Ramdahin[1], T. Bielawny[5], L. Mendoza[5], J. Tuff[5], S. Thavaneswaran[1], M. Narayansingh[1], J. Rutherford[1], L. Slaney[1], K. Fowke[1], E. Ngugi[2], J. Embree[1], F. Plummer[1,5].* 1) University of Manitoba, Canada; 2) University of Nairobi, Nairobi, Kenya; 3) United Arab Emirates University, UAE; 4) Mount Sinai Hospital, Toronto, Canada; 5) National Microbiology Laboratory, Winnipeg, Canada.

Human Leukocyte Antigens (HLA) present antigens to T cells and are centrally involved in acquired immunity against infectious pathogens. The extreme diversity of HLA system is thought to be the result of selection by infectious pathogens and is a population's ecologic defence mechanisms against epidemics. Like other pandemics in history the selective pressure of current HIV-1 epidemic in sub-Saharan Africa may influence HLA genotype frequencies in the population. We examined the effect of HIV epidemic on HLA genotype frequency of two African research cohorts (n=1345) with very different exposures to HIV-1 over two decades. The frequencies of HLA genotypes associated with resistance to HIV-1 infection increased significantly (p=0.003, odds ratio: 1.42, 95%CI: 1.13-1.79) over time in the sexworker cohort (1985-2001). This change in sexworker cohort is independent of ethnic makeup and country of origin and significantly correlated to the decrease of seroconversion over time (p=0.00003). Multivariate analysis with time-dependent covariates including condom usage, number of partners per day, age at enrolment, duration of prostitution before enrolment and resistant HLA genotype showed that the increase of resistant genotypes in the population is one of the factors significantly correlated with the reduced HIV-1 infection risk in the highly exposed population (p=0.004). This study provides the first insight into host population genetic changes as a result of selection by HIV-1 in Sub-Saharan Africa and shows that natural selection may ultimately play an important role in controlling the HIV-1 epidemic.

1325/W

Contrasting patterns of variation in two pigmentation candidate genes: *TYR* and *LYST*. *H. Norton, M. Hammer.* ARL-Biotechnology, Univ Arizona, Tucson, AZ.

Skin pigmentation is a complex trait that has been shaped by natural selection as humans expanded out of Africa and into different UVR environments across the globe. This process of local adaptation is expected to leave identifiable signatures in the DNA sequence of pigmentation genes, such as a reduction in heterozygosity, an excess of high-frequency derived alleles, and strong population differentiation between populations subject to different UVR environments. However, as processes related to changes in population structure, size and distribution can also have similar effects it is important to be able to distinguish between patterns due to selection and those arising from non-neutral demography. Here we compare sequence variation in two pigmentation candidate loci, *TYR* and *LYST*, to variation in 21 neutral autosomal loci sequenced in the same panel of 90 individuals representing 3 African (Biaka, Mandenka, and San) and 3 non-African populations (Han Chinese, French Basque, and Melanesians). *TYR* shows a significantly elevated Tajima's D value in our Melanesian sample ($D = 2.57$, $p < 0.01$), but does not appear to be an outlier for any other statistic in this population or any of the five others studied. On the other hand, *LYST* shows reduced heterozygosity in both the Han Chinese and Melanesian samples relative to the comparison autosomal loci, reduced haplotype diversity in both populations, and a Tajima's D value (-1.73) that falls at the extreme of the empirical distribution in the Han population. *LYST* F_{ST} values between the Han and Melanesians and all other populations are also relatively high (0.19 - 0.45), indicating relatively strong inter-population divergence at this locus. The patterns suggest that polymorphisms in *LYST*, but not *TYR*, may have been favored by natural selection in the Han population.

1326/W

Confounding between recombination and selection, and a novel genome-wide method for detecting selection. *P.F. O'Reilly[1], E. Birney[2], D.J. Balding[1].* 1) Epidemiology and Public Health, Imperial College London, London, United Kingdom; 2) European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom.

In recent years there have been major developments of population genetics methods to estimate both rates of recombination and levels of natural selection. However, genomic variants subject to positive selection are likely to have arisen recently, and consequently had less opportunity to be affected by recombination. Thus, the two processes have an intimately-related impact on genetic variation, and inference of either may be vulnerable to confounding by the other. We illustrate here that even modest levels of positive selection can substantially reduce population-based recombination rate estimates in humans. We also show that genome-wide scans to detect loci under recent selection in humans have tended to highlight loci in regions of low recombination, suggesting that confounding with recombination rate may have reduced the power of these studies. Motivated by these findings we introduce a new genome-wide approach for detecting selection, based on the ratio of pedigree-based to population-based estimates of recombination rate. Simulations suggest that this "Ped/Pop" approach has good power to discriminate between neutral and adaptive evolution. The LRH method (Sabeti et al. 2002), which does allow for the confounding effects of recombination, has good power only for partial selective sweeps. Since selective sweeps are often rapid, the relevant time interval may be short. In contrast, the power of the Ped/Pop method is maintained for many generations after the fixation of an advantageous variant. Unusually for a multi-marker method our approach also shows good power in regions of high recombination. We apply the method to human HapMap and Perlegen data sets, finding confirmation of reported candidates as well as identifying new loci that may have undergone recent intense selection.

1327/W

MICA and MICB polymorphism and linkage disequilibrium with HLA-B and -DRB1 in Koreans. *M.H. Kim, H.J. Chung, S.E. Choi, C.H. Cha, O.J. Kwon, H.B. Oh.* Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Allele and haplotype frequencies of MICA and MICB genes whose loci are located within the major histocompatibility complex are different according to ethnic groups. In this study, MICA and MICB polymorphism was assessed in 139 unrelated healthy Koreans by means of sequence-based typing (SBT) of exons 2, 3, 4, and 5. Seventeen different MICA alleles of extracellular domains (α1 -3) were identified; MICA*010 was noted as the highest frequency (20.9%), followed by MICA*00201 and *00801. Eight different MICB alleles were identified; MICB*00502 was noted as the highest frequency (57.2%), followed by MICB*002 and *004. Five different MICA alleles of transmembrane domains (exon 5) were identified and allele A5 was the most common (29.5%), followed by allele A6. MICA*010 showed strong linkage disequilibrium with MICB*00502 (19.8% of HF). The frequencies of three loci haplotype extending from HLA to MIC gene were also analyzed; the most common haplotypes were B*1501-MICA*010-DRB1*0406 (5.8%), B*1501-MICA*010-MICB*00502 (10.4%), MICA*010-MICB*00502-DRB1*0406 (5.8%). Among B-MICA-MICB-DRB1 haplotypes, the most common haplotype was B*1501-MICA*010-MICB*00502-DRB1*0406 (5.8%), followed by B*4403-MICA*004-MICB*00502-DRB1*1302 (4.6%). This is the first report on the frequencies of MICB alleles and of haplotypes extending from HLA-B and -DRB1 in high-resolution to MICA and MICB genes in Koreans. These results will be of great use in elucidating association between HLA or MIC genes and autoimmune or infectious diseases in Koreans.

1328/W

Frequency of the Thr399Ile single nucleotide polymorphism of the Toll-like Receptor 4 gene in obese mestizo women of Durango, Mexico. *B. Lazalde[1], M.R. Reyes[2], H. Rodriguez Hernandez[1], M. Rodriguez Moran[1], F. Guerrero Romero[1], G. Zambrano[1].* 1) Biomedical Research Unit, Mexican Institute of Social Security, Durango, Durango, Mexico; 2) Faculty of Medicine U.J.E.D., Durango, Dgo., Mexico.

Background. Obesity is associated to insulin resistance and chronic inflammation. The Toll-like receptor 4 (TLR4) mediates inflammatory events and insulin resistance in peripheral organs. A single nucleotide polymorphism in the TLR4 gene, Thr399Ile, has been associated with hyporesponsiveness for signal transduction, hence the carriers of this polymorphism could have a lower risk for developing obesity and chronic inflammation. The prevalence of this polymorphism in distinct populations is between 6% and 10% according to various reports. Aim. The aim of this work was to determine the allelic and genotypic frequencies of the Thr399Ile TLR4 polymorphism in a sample of obese mestizo women of Durango, Mexico. Methods. Previous informed consent from participant women, a sample of venous blood was obtained for DNA isolation and the Thr399Ile polymorphism was determined by HinfI RFLP after PCR amplification of the polymorphic site using primers and amplification conditions published elsewhere (Biotechniques 31: 22-24, 2001) Amplification products were resolved by electrophoresis in agarose gels stained with Et-Br. Results. The allelic and genotypic frequencies found were as follows: Thr allele, 97.71%; Ile allele, 2.29%; Thr/Thr genotype, 95.42%, Thr/Ile genotype, 4.58%; the Ile/Ile genotype was not found. The sample was in Hardy-Weinberg equilibrium. Conclusions. Our results showed that the wild Thr allele and the Thr/Thr genotype were overrepresented in the women studied; on the contrary, the mutated Ile allele was underrepresented when compared to the frequencies reported for other populations. Whether these findings reflect the frequencies of the general population of Durango remains to be elucidated. Further studies are warranted since the determination of this polymorphism could have implications with preventive aim in individuals at risk for developing obesity and chronic inflammation. (Partially supported by grant DGO-2007-C01-66735 from COCYTED-FOMIX to B. L.).

1329/W

The age, distribution, and molecular evolution of the MAPT inversion. *M.P. Donnelly, W.C. Speed, J.R. Kidd, A.J. Pakstis, K.K. Kidd.* Department of Genetics, Yale University School of Medicine, New Haven CT, USA.

The 17q21 inversion, sometimes called the MAPT inversion, is a ~900 kb inversion found primarily in Europeans and Southwest Asians. There is no recombination between the H2 (inverted sequence) and H1 (non-inverted sequence). The H2 haplotype is found at frequencies of up to 35%. We have identified 20 SNPs that act as inversion markers. Using subsets of these markers, we are able to show that the inversion is found at the highest frequencies in Southwest Asia (frequencies of ~30%) with smaller frequencies in Eastern Europe, reaching as low as <5% in Finns, and rising again in Western Europe (frequencies of ~20%). The H2 inversion haplotype also occurs at low frequencies in Africa, Central Asia, India, East Asia, and the Americas, though the East Asian and American alleles are likely due to European admixture. We then used SNPs that were variable on either only one of the orientations or that were variable on both, in conjunction with the previous inversion marking SNPs, to trace the molecular evolution of the inversion. These SNPs can form haplotype networks that suggest the H2 haplotype may have originally arisen in Africa or Southwest Asia. Though reciprocal recombination between the H1 and H2 haplotypes is not seen (or expected) there is some evidence of gene conversion between the two alleles. With four STRPs and the method described by Stephens *et al.* (1998) we estimated the age of the inversion to 13,600-27,200 years. Though the H2 inversion has many fixed differences across the ~900 kb, the STRP data indicate the most recent common ancestor (MRCA) is very recent, much different from the 3 million year age estimated by Stefansson *et al.* (2005). Supported in part by NIH GM57672.

1330/W

Molecular Study of Five Ethnic Groups of Rajasthan. *M. Ghaznavi Idris[1], M. Rupak[3], K. Harpreet[3], K.N. Saraswati[3], S.S. Kamboj[1], S. Bhardwaj[4], K. Kucheria[2], R. Dada[1].* 1) Department of Anatomy,All India institue of Medical Sciences, New Delhi, India; 2) Department of Endocrinology,All India institue of Medical Sciences, New Delhi, India; 3) Department of Anthropology,Delhi University,Delhi,India; 4) Department of Zoology,Government College,-Banswara, Rajasthan,India.

INTRODUCTION: Rajasthan is a state in north-western India with several ethnic and tribal groups such as the Rajputs, Minas, Bhils, Sahariyas, Damaria and Garasiya. These groups still maintain their ethnic identity which makes a very useful cohort in studying their genetic imprinting. In the present study seven human-specific Alu insertion / deletion and five Restriction Fragment Length Polymorphisms (RFLPs) have been analyzed. MATERIAL AND METHODS: Intra venous blood samples were taken after written consent from 26 Damaria, 34 Rajput, 30 Saharia, 34 Mina, 53 Garasiya, and 47 Bhil individuals and DNA was isolated according to the standard protocol of phenol chloroform given by Maniatis. PCR amplification was done for Alu insertion / deletion polymorphism and RFLP. Five different restriction enzymes were used to digest the PCR products for RFLP markers. The results were visualized in UV light after running them in agarose gel. RESULTS AND DISCUSSION: Gene counting method was used for estimating allele frequencies. Ht, Hs and Gst values were computed using the programme DISPAN. A phylogenetic tree was constructed on the basis of genetic distance using the programme DISPAN. The Gst value which is the genetic differentiation between the 6 populations groups is found to be 4.9%. The Ht and Hs values are 0.457 and 0.435 respectively. The marker wise Ht values are found to be very high, all of them approaching the theoretical maximum i.e. 0.5, except for CD4 (0.36). These high heterozygosity values indicate the fixation of these alleles in the population groups under study.

1331/W

Evidence for natural selection at HLA class I-recognizing leukocyte immunoglobulin-like receptor (*LILR*) in Northeast Asians. *K. Hirayasu[1], J. Ohashi[1], H. Tanaka[2], K. Kashiwase[2], M. Takanashi[2], M. Satake[2], K. Tokunaga[1], T. Yabe[2].* 1) Human Genetics, University of Tokyo, Tokyo, Japan; 2) Tokyo Metropolitan Red Cross Blood Center, Tokyo, Japan.

[Purpose] On the basis of the structural feature, the LILR family is divided into activating, inhibitory, and soluble forms. Both LILRB1 and LILRB2 bind to a broad range of classical and non-classical HLA class I molecules. Last year at this meeting, we reported that high allele frequencies of the *LILRA3* deletion were observed in Northeast Asians, and novel alleles with a premature termination codon in exon 3 were detected only in Northeast Asians. In this study, we examined the linkage disequilibrium (LD) around the *LILRA3* gene.

[Methods] The PCR-SSP and sequencing-based typing method were performed in HapMap population samples (JPT, CHB, CEU, and YRI). LD analysis was performed using the Haploview program. F_{ST} statistic was calculated to measure the genetic differentiation between populations.

[Results] Strong LD was observed between *LILRA3* and *LILRB2*. Furthermore, East Asian and non-East Asian were significantly differentiated for the SNPs both in *LILRA3* and *LILRB2*.

[Conclusion] Our results suggest that natural selection has acted on *LILRA3*, or *LILRB2*, or both of them in East Asians, and lead us to speculate that the *LILR* genes may be involved in pathogen-host interaction.

1332/W

Comparisons of genome diversity between the Okinawan and four HapMap populations. *W.-C. Hsueh[1], Q. He[2], D.C. Willcox[2,3,4], M. Suzuki[4], J.W. Chen[1], R. Chen[2], K. Yano[2], T. Donlon[2], J.D. Curb[2], J. Grove[2], W.S. Browner[1,5], S.R. Cummings[1,5], M. Boehnke[6], P.-Y. Kwok[1], B.J. Willcox[2].* 1) UCSF, San Francisco, CA; 2) Pacific Health Research Institute, Kuakini Medical Center & Univ. of Hawaii, Honolulu, HI; 3) Okinawa International Univ., Okinawa, Japan; 4) Okinawa Research Center for Longevity Science, Okinawa, Japan; 5) California Pacific Research Institute, San Francisco, CA; 6) Univ. of Michigan, Ann Arbor, MI.

The Okinawans reside on an island prefecture of Japan and have among the world's greatest longevity. We conducted a pilot genome-wide study to investigate (a) whether Okinawans are genetically similar to Japanese and Chinese, as has been historically documented, and (b) whether they may be more homogeneous than these two populations.

We genotyped 26 DNA samples from randomly selected Okinawans using the Affymetrix 500k GeneChips and compared the genetic diversity of their genomes to 26 randomly selected subjects from each of 4 HapMap samples (West Africans, Caucasians, Japanese, and Chinese; or YRI, CEU, JPT and CHB). Previous studies have shown strong similarity between the JPT and CHB genomes compared to the CEU, and the YRI genome is more diverse. Based on data from ~345k SNPs available in all 5 samples, we observed that Okinawan allele & haplotype frequencies are similar to those of the JPT and CHB than the CEU or YRI, but that the Okinawan samples showed increased linkage disequilibrium (LD) and somewhat decreased haplotype diversity compared to the JPT and CHB samples. For instance, in 300 kb chromosomal segments, the 10 most common haplotypes on average account for 83% of haplotypes present in Okinawans, compared to 61% in JPT and CHB, 55% in CEU and only 38% in YRI.

Our findings suggest that information from the HapMap Project and commercial high-throughput genotyping platforms will be useful for genetic studies in the Okinawans, and that while Okinawans are genetically similar to Chinese and Japanese, they show reduced genetic diversity. This reduced genetic diversity may increase the coverage and hence the power of LD-based genetic association studies for complex traits such as longevity.

1333/W

Inferring the evolutionary history of the Duffy-O mutation. *C.A. Lambert[1], J.M. Akey[1], R. Qiu[2], J. Madeoy[1], D.G. Buckley[2], M.V. Olson[1,2].* 1) University of Washington Department of Genome Sciences, Seattle, WA; 2) University of Washington Genome Center, Seattle, WA.

The Duffy-O mutation, which confers complete resistance to malaria caused by the parasite *Plasmodium vivax*, is a well-accepted example of positive natural selection in African populations. However, many details about the evolutionary history of the Duffy-O mutation remain unknown. To this end, we resequenced 10.5 kb surrounding the site of Duffy-O in 65 geographically-diverse individuals. Our goal was to infer possible evolutionary histories for the Duffy-O mutation by analyzing patterns of DNA variation at the locus. The signature of positive selection we observed is a 6.4-kb region of dramatically reduced diversity among Duffy-O chromosomes; all variation outside this region can be explained by mutation and historical recombination events. Our results are consistent with the Duffy-O mutation arising on a single ancestral chromosome in our sample, which then became fixed in sub-Saharan Africa sometime after the diaspora of modern humans.

To determine how unusual the patterns of variation are surrounding Duffy-O, we also performed a genome-wide scan for positive selection using publicly available data from the HapMap Project. In our scan, we identified SNPs with allele-frequency distributions similar to or more extreme than the Duffy-O mutation. The scan produced an additional 15 loci that have patterns of genetic variation consistent with strong, recent positive selection in the Yoruban population. While only one of the SNPs we identified occurs at a protein-coding site, 12 others occur in introns or just upstream of known genes. Interestingly, 3 of the non-coding SNPs occur in regions of extremely high interspecies conservation, suggesting that regulatory sites may be an important substrate of recent adaptive evolution. All fifteen loci are currently being resequenced in a panel of geographically diverse individuals to facilitate detailed evolutionary analyses. More generally, our data and approaches are providing new insights into the history of the Duffy-O mutation, as well as additional targets of African-specific positive selection.

1334/W

Haplotypic background of a high-frequency Native American private allele. *K.B. Schroeder[1], M. Jakobsson[2], T.G. Schurr[3], M.H. Crawford[4], D.F. Conrad[5], L.P. Osipova[6], L.A. Tarskaia[7], S.I. Zhadanov[3,6], J.D. Wall[8], J.K. Pritchard[5], D.G. Smith[1], N.A. Rosenberg[2].* 1) Dept. of Anthropology, University of California, Davis, Davis, CA; 2) Dept. of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Dept. of Anthropology, University of Pennsylvania, Philadelphia, PA; 4) Dept. of Anthropology, University of Kansas, Lawrence, KS; 5) Dept. of Human Genetics, University of Chicago, Chicago, IL; 6) Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia; 7) Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia; 8) Dept. of Biological Sciences, University of Southern California, Los Angeles, CA.

Previous research has shown that a nine-repeat allele (9RA) at microsatellite D9S1120 is present in all 20 sampled Native American and West Beringian populations and is absent from all 53 other populations sampled. The distribution of this allele has been used to support the hypothesis that most Native Americans descend from a single founding population. This inference assumes that copies of the 9RA are identical by descent and that the allele is not under selection. We have genotyped 34 SNPs spanning a 499 kb region around D9S1120 in 1252 individuals from 72 populations worldwide, including 19 Native American, 2 West Beringian, and 22 East/Central Asian. 89.7% of haplotypes with the 9RA share a 76 kb haplotype, suggesting most or all copies of the 9RA are identical by descent. Most of the individuals we genotyped have been genotyped for 2834 SNPs in 36 genomic regions, allowing a genomic comparison. Although a 76 kb haplotype at a frequency of 54% would be highly unusual in European or African populations, we found that, due to the high levels of LD, it is not highly unusual in Native American populations. Thus, the length and frequency of the 9RA haplotype do not support the hypothesis that its distribution results from positive selection. Recombination within the 9RA haplotype allows us to estimate the length of the intraallelic genealogy, and, thereby, the age of the MRCA of sampled copies of the 9RA. The results have implications for the peopling of the Americas and for detecting positive selection in Native Americans.

1335/W

Significant difference in the distribution of allele frequency among independent Japanese populations including HapMap-JPT. *T. Taniguchi[1], M. Nakano[1], Y. Ikeda[2], N. Omi[1], M. Tanaka[1], K. Mori[2], S. Kinoshita[2], K. Tashiro[1].* 1) Department of Genomic Medical Sciences; 2) Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Purpose: The International HapMap Project provides a haplotype map from four human populations to share common “tags” on the human genome as a resource for all populations in the world. Although most of the haplotypes occur in all populations, their frequencies differ among each, and thus it is necessary to refer each data of minor allele frequency (MAF) to help clarifying disease-related genes by whole-genome association studies using a chip-based SNPs genotyping system. Each HapMap data is, however, relying on the data from a single group of not more than a hundred individuals. In this study, to confirm the reliability of the HapMap data, we prepared two independent groups of Japanese and precisely compared with the HapMap counterpart, HapMap-JPT. **Methods:** We recruited 718 volunteers of unrelated Japanese from Kyoto, Japan, with written informed consent. SNPs were genotyped by Affymetrix Mapping 500K Array Set. We selected 5,805 and 5,036 SNPs on chromosome 21 and 22, respectively, with over 95% call rate/SNP to exclude SNPs that showed poor clustering. The MAF for each SNP between our two groups (n=300 or 418) and HapMap-JPT (n=45), obtained from the NetAffx Analysis Center, was compared quantitatively. **Results:** We observed a tight correlation of MAF between our two groups and HapMap-JPT (r^2>0.93), irrespective of the difference in groups or chromosomes. No significant difference of the mean MAF was observed among three groups. However, the distribution of MAF significantly differed between our groups and HapMap-JPT, especially in the range of 0 to 5% (χ^2 test, p<0.05). **Conclusion:** The overall correlation among three Japanese populations supported the usefulness of the data from HapMap-JPT. However, although the Japanese is less heterogeneous than the other populations, we found a slight, but a significant difference in the distribution of the low MAF. We should be aware of it that such difference would also appear in the other populations, especially when we focus on the SNPs of low MAF.

1336/W

Population sub-structure revealed from genealogical and genetic data in two isolated populations in South Italy. *V. Colonna[1], T. Nutile[1], M. Aversano[1], G. Fardella[1], S. Bracco[1,3], L. Dionisi[1], M. Borra[4], M.F. Carfora[2], C. Angelini[2], M. Ciullo[1], M.G. Persico[1].* 1) IGB-ABT, CNR, Naples, Italy; 2) IAC, CNR, Naples, Italy; 3) EURAC, Bolzano, Italy; 4) Stazione Zoologica Anton Dohrn , Naples, Italy.

We investigated the genealogical structure in the populations of two isolated villages, Gioi and Cardile in order to use them effectively in gene mapping studies. Study samples consist of individuals living in Gioi (N=882) and Cardile (N=474) of which 94% are in a single 5165-member pedigree. Despite the single pedigree and the fact that villages lie only 6 km apart, we suspected a potential sub-structure and thus we investigated this hypothesis, quantifying admixture between the villages according to genealogical data. For this purpose a sub-pedigree was reconstructed for each sample on the basis of genealogical records, going back 10 meiotic steps. Shared individuals between the sub-pedigrees per meiotic step were determined in a matrix described by an index. To asses the significance of this index, samples were mixed in a single data set and 10K more matrices with relative indexes were generated by randomly permuting the membership of this data set. We found that the index of the true matrix is significantly different from those obtained by resampling (p-value < 10e-5). This indicates that, despite the fact that Gioi and Cardile have shared a number of ancestors, they could represent two sub-populations rather than a single population. In agreement with this finding, higher average kinship values (k) were found both in the sub-genealogies of Gioi (pedigree size= 4182; kG= 0.004) and Cardile (pedigree size = 2380; kC = 0.009) compared to that observed in the genealogy built from the whole study sample (pedigree size = 5255; kGC = 0.003). Haplotype analysis of the mtDNA Hypervariable Region II in Gioi and Cardile populations shows that very few haplotypes are shared between the populations while the majority are village-specific. This result gives further evidence of population substructure With this work we have shown for the first time, a powerful use of genealogical records to investigate population sub-structure.

1337/W

Comparison of different methods to estimate genetic ancestry and control for stratification in genome-wide association studies. *E. Salvi[1,2], G. Guffanti[1], A. Orro[2], L. Milanesi[2], J. Turner[3], D. Keator[3], J. Fallon[3], S. Potkin[3], F. Macciardi[1].* 1) Department of Science and Biomedical Tecnology, University of Milan, Italy; 2) Institute of Biomedical Technologies CNR, Milan, Italy; 3) Department of Psychiatry and Human Behavior University of California, Irvine.

Population stratification can occur in case-control association studies when allele frequencies differ between cases and controls because of systematic differences in ancestry. It may lead to spurious associations due to population structure rather than association of genes with disease. The prevailing methods for dealing with stratification were Fst test, Genomic Control (GC) and STRUCTURE that are based on the usage of unlinked genetic markers. Recently new methods have been proposed that enable explicit detection and correction of population stratification on a genome-wide scale. EIGENSTRAT and PLINK detect population structure using data reduction techniques to model population genetic variability. We evaluate these methods using 317K SNPs (Illumina HumapHap300) in a case-control sample of about 200 subjects. Fst, STRUCTURE and GC did not detect a significant stratification in our sample, as well as EIGENSTRAT and PLINK. However, these last two methods, using a much larger information from the whole set of SNPs, suggested the presence of a not completely homogeneous population, probably due to admixture rather than to stratification. We used STRUCTURE software to assess the degree of admixture of our sample and we detected an alpha>1 that seems to suggest admixed individuals. When we correct our data adjusting association statistics by 1) a uniform overall inflation factor (lambda) calculated on 400 unlinked genetic markers; 2) a uniform overall lambda calculated on 317K markers; 3) marker specific factors (ancestry based) obtained via computing residuals of linear regressions, we found a larger lambda value for those methods with the highest information content (EIGENSTRAT and PLINK).Even if these different strategies provide apparently similar informations, the larger amount of details of informations allows a more accurate estimate to control for heterogeneity factors although we can’t identify precisely them.

1338/W

Intraspecific *Cis*-Regulatory Variation Underlying Functional Differences in *PDYN* Expression. *C.C. Babbitt[1], J.S. Silverman[2], R. Haygood[2], M.V. Rockman[2], G.A. Wray[1,2].* 1) Institute of Genome Science and Policy, Duke University, Durham, NC; 2) Department of Biology, Duke University, Durham, NC.

Previous work has shown that the regulatory region of *prodynorphin* (*PDYN*) has been under selection in modern and ancient human populations, indicating that there may be segregating variation in populations that is responsible for differences in phenotype. We present a functional analysis of 11 polymorphisms in the *cis*-regulatory region of *PDYN,* an endogenous opioid precursor known to play a role in cognitive function and disease. To address the functional consequences of the polymorphisms we transiently transfected 19 naturally occurring *PDYN cis*-regulatory haplotypes into 2 neuroblastoma cell lines. This approach was chosen in order to explore all of the known *cis*-regulatory polymorphisms and also *trans* factors that influence expression in the many brain regions *PDYN* is found in. For each cell line, we performed a cross-validated regression tree analysis, which identified the polymorphisms most strongly associated with variation in expression level among the haplotypes. Regression tree analyses find the variants that statistically best explain expression differences and to partition out the separate affects of epistatic interactions that may be occurring between different parts of the *cis*-regulatory region. Expression results supports cell-type dependent effects and overall implicated 4 of the polymorphisms as explanatory genetic polymorphisms for differences in expression. These polymorphisms were a previously identified 68-bp tandem repeat, two microsatellites and a SNP. Despite the extensive work on *PDYN*, this is the first study to characterize the standing functional haplotype variation that exists in human populations. Our study helps to bridge the gap between knowledge of the phenotypic consequences of changes in *PDYN* expression and the *cis*-regulatory variation that causes these changes. The results of this study provide new candidate regions that will allow future clinical studies to further elucidate the genotype/phenotype relationship of *PDYN.*

1339/W

Retrotransposition rates of L1 elements in human germline DNA. *C.M. Macfarlane, P. Collier, A.J. Jeffreys, R.M. Badge.* Department of Genetics, University of Leicester, Leicester, LE1 7RH, United Kingdom.

The Long Interspersed Nuclear Element-1 (LINE-1 or L1) family are non-LTR retrotransposons which are acknowledged to be the most prolific class of mobile or transposable elements within mammalian genomes. Within the human genome, the efficiency of autonomous L1 retrotransposition has led to 17% of the genome sequence being composed of L1 with a further 10% being comprised of transposable elements which utilise the L1 machinery in order to replicate (Alu and SVA). Although the contribution of L1 to genome plasticity is recognised, very little is known about the evolutionary dynamics of their mobilisation within the germline, for example their rate of de novo insertion. Current estimates are that between 1 in 8 and 1 in 100 humans carry a novel retrotransposon insertion somewhere in their genome. However, these estimates are subject to acquisition bias as they are based upon retrotransposon insertions that manifest a disease phenotype. In order to directly monitor endogenous L1 retrotransposition we have been using a genome-wide molecular approach to try to capture de novo retrotransposition events in human germline DNA (derived from ejaculated sperm). The technique is based upon ATLAS, a genomic display technique that selectively displays full-length L1 terminus/genomic DNA junctions from the most active L1 subfamilies. We have modified ATLAS to operate at the single molecule level. This has been confirmed through limiting dilution and poisson analysis of specific L1 insertions that are known to be recent evolutionary acquisitions within the human genome. In addition, by performing limiting dilution of display inputs we are able to estimate the amount of DNA scanned per reaction, thus placing an upper limit on the rate of de novo insertion. Finally, the technique has been developed to the point of high throughput (>1000 sperm genome/experiment) screening of human sperm DNA and can also detect low levels of genomic DNA mosaicism.

1340/W

The impact of Alu insertions on local recombination rates. *J. Xing, D.J. Witherspoon, L.B. Jorde.* Eccles Institute of Human Genetics, Univ. of Utah, Salt Lake City, UT.

Alu elements are the most successful primate Short Interspersed Elements (SINEs) and currently more than one million Alu elements are present in the human genome. Many young Alu elements are still polymorphic for presence and absence among human populations. This heterozygosity may pose a problem during the pairing of homologous chromosome pairs and influence the probability of recombination. On the other hand, fixed Alu elements have high GC content and may promote local recombination. Using single nucleotide polymorphism (SNP) data from the HapMap project, we examined recombination rates around all Alu elements belonging to the AluY subfamily (the youngest major Alu subfamily). Our recombination rate (Rho) estimates based on ~140,000 AluY loci indicate that on average the recombination rate in intervals containing AluYs is only 80% of the rate of other intervals within 50kb of the AluY locus. However, we found that SNP pairs directly adjacent to AluY elements are separated by larger physical distances compared with the average inter-SNP distance in the 50kb flanking regions. This probably reflects difficulties in ascertaining and typing SNPs very close to these repeat elements. Estimates of rho are in turn affected by the size of inter-SNP intervals. To determine to what extent a SNP ascertainment bias can account for local recombination rate variation, we resampled the 50kb AluY flanking genomic regions to match the Alu-containing SNP-pair interval sizes. We show that after taking into account the SNP interval size difference, recombination rates in AluY insertion loci are not different from their proximate regions. We conclude that the initial reduced recombination rate estimates around AluY insertions appears to be due to the strong association of AluYs with longer intervals, which are in turn correlated with biased upward estimates of rho. Researchers using the HapMap data for whole genome analysis should be aware of this type of systematic ascertainment bias and take it into account during their analyses. Supported by NIH Grant GM-59290 and NSF Grant BCS-0218370.

1341/T

Population structure of human linkage disequilibrium patterns. *D.J. Witherspoon, J. Xing, W.S. Watkins, Y. Zhang, W. Tolpinrud, L.B. Jorde.* Human Genetics, University of Utah, Salt Lake City, UT.

Patterns of linkage disequilibrium (LD) vary between human populations due to their different demographic histories. As a result, SNPs chosen for their utility in one population may prove less useful in another population. The degree to which different populations share a common LD structure must be understood in order to perform genome-wide association studies in different populations. We examined patterns of linkage disequilibrium in 19 independent 100-kb regions of the genome in 20 populations (334 individuals) from Europe, East Asia, and sub-Saharan Africa, including several African Pygmy populations. As expected, LD is lowest in the sub-Saharan group, where the average r2 between markers is 2/3 of that observed in the group with the highest LD (East Asians.) By comparing measures of LD obtained using our samples with those derived from the HapMap samples, we are able to determine how well they generalize to independent samples from the same or different populations. Estimates of LD patterns are quite similar for samples drawn from the same populations: r2 estimates for the same marker pairs in different samples from the same continents (i.e., our African samples vs. HapMap Yoruban YRI, our Asian samples vs. HapMap Japanese and Chinese JPT/CHB, and our European samples vs. HapMap CEPH CEU) show product-moment correlations exceeding 95%. These correlations decrease significantly for comparisons between continental populations. The correlation between r2 estimates for the same marker pairs in Pgymy vs. Asian or European populations is ~75%. That correlation rises to >90% between the YRI HapMap population sample and our Pygmy sample. These results suggest that the HapMap information will generalize well to most human populations, including even genetically distinct Pygmy populations. Supported by NIH Grant GM-59290 and NSF Grant BCS-0218370.

1342/T

A more severe Out-of-Africa population bottleneck on chromosome X. *A. Keinan[1,2], J. Mullikin[3], N. Patterson[2], D. Reich[1,2].* 1) Dept. of Genetics, Harvard Medical School, Boston, MA; 2) The Broad Institute, Cambridge, MA; 3) National Human Genome Research Institute, NIH, Bethesda, MD.

We generated and analyzed two large population genetic data sets, each spanning both chromosome X and the autosomes, permitting us to learn how demographic history varies by gender. For the first data set, we identified subsets of SNPs from HapMap that are free of ascertainment bias. For the second data set, we aligned hundreds of millions of base pairs from individuals of known ancestry and estimated the average time since divergence.

Both data sets point to more X-chromosomal than autosomal genetic drift since migration from Africa, but before the European-Asian split: (1) Genetic diversity in non-African populations is reduced on chromosome X compared with the autosomes, more than would be expected based on the autosomal demographic history, combined with the 3/4 effective population size and the different mutation rate. (2) Allele frequency differentiation (F_{ST}) between Africans and non-Africans is much greater on chromosome X than would be expected based on the premise that X-chromosomal genetic drift should be 4/3 that of the autosomes. (3) The X-chromosomal allele frequency spectrum of non-Africans exhibits an unexpected deviation from that of the autosomes, which modeling reveals to be consistent with a more severe Out-of-Africa population bottleneck on chromosome X.

These results can be explained if the Out-of-Africa dispersal involved a larger effective population size of men than women. Due to the higher variability in reproductive success in men, it is difficult to explain the larger effective population size if non-African populations were colonized by a single migration. However, if there were multiple waves of male-biased migration out of Africaso that the non-African gene pool was mostly contributed by male ancestorsthen non-Africans would exhibit a lower effective population size of women. An alternative possibility, extensive X-chromosomal selection in non-Africans, is unlikely to be a full explanation since the results hold after excluding genes and loci identified as being under selection.

1343/T

Endogamic exogamy in Gujarati Patels. *F-Y. Li[1], T.J. Pemberton[1], N.U. Mehta[1,2], S. Wong[1], J.W. Belmont[3], C. Tyler-Smith[4], N.A. Rosenberg[5], P.I. Patel[1,2].* 1) Inst Genetic Medicine, Univ Southern California, Los Angeles, CA; 2) Dept of Biochem and Mol Biology, Univ of Southern California, Los Angeles, CA; 3) Dept of Mol and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 5) Dept of Human Genetics, Univ of Michigan, Ann Arbor, MI.

Social stratification in India is evident as social classes that are defined by a number of endogamous groups often termed as jātis or castes. The jātis themselves exist among one of four varnas or classes: Brahmin, Kshatriya, Vaishya and Shudra. Within a jāti, there exist exogamous groups known as gotras, or gols, which refer to the lineage or clan of a person. Societal rules governing marriage are similar in diverse regions of India. There is typically a strict definition of the clan, gol or gotra from within which an individual's mate may be selected and a sanction against marriage to any individual from within his or her own gotra. This practice typically translates into a surname or gotra endogamy. Thus, while consanguinity is strictly avoided and there is some randomness in mate selection, there is likely a degree of gene flow restriction. Patels who originate from the state of Gujarat practice this form of "endogamic exogamy." Members of a village do not marry anybody from their own village and may only marry an individual from one of the other villages within a specified group of villages. We have studied one such group, the Chh Gaam Patels. This group, which constitutes the largest gol among Patels, comprises Patels from six villages. In order to determine the genetic structure of this group, we have obtained genotypes at ~800 microsatellites and ~400 indel loci. Y-chromosome and mitochondrial haplogroup analyses have also been conducted. These data are being analyzed to determine if the restricted marital practice within the same geographic region has resulted in limited genetic differentiation and effective "genetic isolation". The number of founders and the extent of admixture with neighboring groups will also be investigated.

1344/T

The Autochthonous Origin and a Tribal Link of Indian Brahmins: Evaluation Through Molecular Genetic Markers. *S. Sharma[1,2], E. Rai[1,2], S. Singh[1,2], P.R. Sharma[1,3], A.K. Bhat[1], K. Darvishi[1], A.J.S. Bhanwer[2], P.K. Tiwari[3], R.N.K. Bamezai[1].* 1) NCAHG, SLS, JNU, New delhi; 2) Department of Human Genetics, GNDU, Amritsar; 3) Centre for Genomics, SOS zoology, JU,Gwalior.

The co-existence and associated genetic evidences for the major rival models: i) recent Central Asian introduction of Indian caste system, ii) rank related west Eurasian admixture, iii) South Asian origin for Indian caste communities, and iv) late Pleistocene heritage of tribal and caste populations, leave the question of the origin of caste system in India hazy and obscure. To resolve the issue, we screened 621 Y-chromosomes (of Brahmins, occupying upper most caste position and Dalits and Tribals with the lower most positions in the Indian caste hierarchical system) with fifty-five Y-chromosomal binary markers and Y-microsatellite markers and compiled a data set of 2809 Y-chromosomes (681 Brahmins, 2128 Tribals and Dalits) for conclusions. Overall, no consistent difference was observed in Y-haplogroups distribution between Brahmins, Dalits and Tribals, except for some differences confined to a given geographical region. A peculiar observation of highest frequency (upto 72.22%) of Y-haplogroups R1a1* in Brahmins, hinted at its presence as a founder lineage for this caste group. The widespread distribution and high frequency across Eurasia and Central Asia of R1a1* as well as scanty representation of its ancestral (R*, R1* and R1a*) and derived lineages across the region has kept the origin of this haplogroup unresolved. The analyses of a pooled dataset of 530 Indians, 224 Pakistanis and 276 Central Asians and Eurasians, bearing R1a1* haplogroup resolved the controversy of origin of R1a1*. The conclusion was drawn on the basis of: i) presence of this haplogroup in many of the tribal populations such as, Saharia (present study) and Chenchu tribe in high frequency, ii) the highest ever reported presence of R1a* (ancestral haplogroup of R1a1*) in Kashmiri Pandits (Brahmins) and Saharia tribe, and iii) associated averaged phylogenetic ages of R1a* (~18,478 years) and R1a1* (~13,768 years) in India. The study supported the autochthonous origin of R1a1 lineage and a tribal link to Indian Brahmins.

1345/T

Genetic diversity of global human populations at STR, SNP, and Indel loci. *S. Guha, R. Chakraborty.* Ctr. Genome Information, Univ Cincinnati, Cincinnati, OH.

The impact of variability of pattern and rates of mutations of genomic markers in understanding the history of evolution of modern human is not well-understood. High intra-population gene diversity and multiple measures of genetic variability at the STR loci, compared to the SNP and Indel loci, make them useful in inferring past evolutionary history. But, STR loci, categorized by their repeat motif size, differ in a number of aspects, requiring their separate analyses. We analyzed 1,306 genomic markers (783 STRs, 210 Indels, and 313 SNPs) in 36 worldwide populations to study genome diversity in the present human populations. The loci were grouped by their type and analyzed for each population group separately. At a global level, STRs exhibit lower F_{ST} between geographic groups of populations and higher intra-group diversity compared with SNPs and Indels. When each geographic group was considered separately, small isolated populations (e.g., Native Americans) exhibited similar F_{ST} values for all types of loci, in spite of STRs having higher gene diversity than the SNPs and Indels. Likewise, in Europeans, though gene diversities for all types of STRs are high (about 70%), the low F_{ST} values for all types of loci are suggestive of extensive gene flow among them. Genetic variation defined by gene diversity and allele size variance shows different trends of variation across four types of STRs; namely, little variation of gene diversity, but decreased allele size variance with increasing repeat motifs. While mutation rate decreasing with motif size can explain the trend in allele size variance, a poor correlation of gene diversity and allele size variance across loci in all groups for the di-STRs is probably caused by presence of allele size gaps. In contrast, allele size variance, gene diversity, and number of alleles are strongly correlated for tri- and tetra-STRs. The positive correlation of allele size variance and presence of gaps within the range of allele sizes in the di-STRs alone explains these observations. Unexpected high imbalance index (β) at the di-STRs due to high allele size variance also supports this assertion.

1346/T

Haplotype and nucleotide diversities in two hypervariable regions of mtDNA in world populations and their forensic implications. *W. Niu[1], N. Wang[2], B. Budowle[3], R. Chakraborty[1].* 1) Ctr. Genome Information, Univ. Cincinnati, Cincinnati, OH; 2) Div. Allergy and Human Genetics, Cincinnati Children's Hospital Med. Ctr., Cincinnati, OH; 3) Lab. Div., FBI Academy, Quantico, VA.

Sequence data from hypervariable regions HV1 and HV2 of mtDNA are used in DNA forensics. MtDNA sequence match arises from three scenarios: 1) the two samples are of a single origin, 2) donors of the two samples are of the same maternal lineage, or 3) the observed match is coincidental. The match probability is generally obtained by the counting method (i.e., relative frequency of the target sequence in a database). Merging of populations in such a database requires evaluation of mtDNA diversity within and between populations. We addressed this by using HV1 and HV2 sequence data from 5,944 individuals belonging to 17 populations encompassing 638 nucleotide sites. Elimination of sequences with ambiguous sites resulted in 5,295 sequences, spanning both HV1 and HV2. Two measures of diversity can be defined for mtDNA: haplotype diversity (in which each distinct haplotype is treated as an allele, irrespective of their sequence dissimilarity) and nucleotide diversity (extent of nucleotide mismatches between sequences). The former is directly relevant to forensic inference. A comparison of these two measures of diversity shows that the within-population diversity at nucleotide level varies more widely across world-wide populations (observed mean mismatch 6.6 in Greece to 15.9 in Kenya) than that at haplotype level (haplotype diversity of 0.9126 in Apache Indians to 0.9992 in Spain and China). Consequently, the coefficient of nucleotide diversity among the 6 population groups of the world is considerably larger (N_{ST}=8.9%) than the coefficient of haplotype diversity (G_{ST}=0.95%). G_{ST} between populations within group is even smaller (G_{ST} almost zero for Caucasians to 1.8% between the two populations of Native Americans). Comparative data on autosomal STRs yield larger G_{ST} values, suggesting that a broader merging of populations may be enough for mtDNA database to get a count-based estimate of rarity of any specific haplotype observed in forensic case work. (Research supported by NIH grant GM 41399).

1347/T

Genealogical relations and genetic distance between an Otomi-speaking community and a Tepehua-speaking community in the state of Hidalgo, Mexico. *A. Sanchez-Boiso[1], R. Peñaloza[3], R. Sanchez-Urbina[1], E. Castro-Sierra[2], R.I. Ortiz de Luna[1], L. Buentello[4], F. Salamanca[3], M.P. Flores[6], J. Aguirre-Hernandez[5], V. Moran-Barroso[1].* 1) Department of Genetics, Hospital Infantil de Mexico Federico Gomez (HIMFG), Mexico City, Mexico; 2) Laboratory of Psychoacoustics, HIMFG; 3) Medical Research Unit in Genetics, Hospital de Pediatria del CMNSXXI-IMSS; 4) Anthropological Research Institute, UNAM; 5) Department of Clinical Veterinary Medicine, University of Cambridge; 6) Department of Social Work, HIMFG.

Mitochondrial DNA (mtDNA) of native Mexican populations has been studied in the context of the settling of the American continent, analyzing the frequencies of the four Amerindian haplogroups: A, B, C and D. mtDNA haplogroup frequency was determined in 2 Mexican populations: an Otomi-speaking (Otomanguean; San Antonio el Grande) population and a Tepehua-speaking (Penutian; Huehuetla) population living within 3 miles from each other in Eastern Mexico. IRB approval and informed consent were obtained. Blood samples for mtDNA analysis were taken from 38 unrelated subjects in San Antonio el Grande and 36 unrelated subjects in Huehuetla. DNA extraction was carried out using commercial kits. Amplification of four Amerindian mtDNA haplogroups was achieved using previously reported primers. PCR products of haplogroups A, C and D were digested with specific restriction enzymes. Haplogroup B was analyzed using polyacrylamide gels. San Antonio el Grande: highest frequency (percentage) was 39 for haplogroups A and C, 11 for B and 3 for D. Huehuetla: highest frequency was 39 for B, 33 for A, 14 for C and 6 for D. In both populations, 8 percent of samples did not correspond to any of the analyzed haplogroups and were classified as "other". Haplogroups A and C predominated in San Antonio el Grande, the latter haplogroup with a frequency similar to one reported for a Raramuri population (Uto-Aztecan) in Northwestern Mexico. In Huehuetla, predominant haplogroup was B followed by haplogroup A. Results reveal distribution of B and C haplogroups differing between both populations, in agreement with their belonging to different language stocks, despite living within close proximity from each other.

1348/T

A 2-D graphic clustering model for reconstructing migration routes of human populations. *F. Xue[1,2], L. Jin[1,3].* 1) Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, China; 2) School of Public Health, Shandong University, Shandong, China; 3) CAS-MPG Partner Institute for Computational Biology, SIBS, CAS, Shanghai, China.

The classical clustering methods are less appropriate in reflecting spatial or geographic information including physical distance and spatial connectivity of the populations. We present a novel clustering approach, 2-D graphic clustering model (2-D GCM) which is based on three matrices of populations: genetics distance *Fst* matrix, spatial location matrix, and spatial adjoining matrix. Using different genetic markers (Y-chromosome haplogroups, mtDNA haplogroups, microsatellites and HLA-A), we will show a general spatial pattern of East Asian populations and their migration routes which are in accordance with the historical records. The results demonstrate that 2-D GCM can be used to reveal spatial genetic structure of populations, spatial genetic relationship between populations, and their migration routes.

1349/T

Gene-language correlations in the Luo population of Kenya. *J. Hirbo[1], F. Reed[1], S. Omar[2], M. Ibrahim[3], S. Tishkoff[1].* 1) Dept Biol, Univ Maryland, College Park, MD; 2) Kenya Medical Research Institute, Centre for Biotechnology Research and Development, Nairobi, Kenya; 3) Dept. of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

The Luo speak an Eastern Sudanic (Nilotic) language and are found predominantly in the Western part of Kenya (East Africa), as well as in parts of Western Uganda and the upper tip of Tanzania. The eastern Sudanic (Nilotic) language is a sub-family of the Nilo-Saharan language family, typically spoken by pastoralist populations of eastern Africa. Although the Luo speak a Nilo-saharan language, previous blood group studies done in East African populations found no significant difference in allele frequencies between the Luo and populations from Kenya and Uganda that speak a Bantu language (classified as part of the Niger-Kordofanian language family). In addition, preliminary results from a larger study of African population structure using a panel of ~1200 autosomal microsatellite and indel polymorphisms show that the Luo are genetically similar to eastern African Bantu-speaking populations. To determine both the maternal and paternal contribution to the observed profiles, we genotyped 50 unique event polymorphisms and 16 microsatellites on the Y chromosome, and sequenced the entire mitochondrial D-loop region, in a total of 300 individuals from the Luo and seven other populations living in the vicinity of the Luo populations, who speak eastern Sudanic or Bantu languages. Although the maternal mtDNA lineages among the Luo are similar to the lineages from Bantu-speakers, the paternal Y chromosome lineages are most similar to lineages present in Nilotic-speaking populations. The discordance between gene-language correlations for the mtDNA and Y chromosome lineages is most likely due to differential male and female gene flow into the Luo population.

1350/T

Comparison of genetic distance measures. *O. Libiger[1,2], C.M. Nievergelt[3], N.J. Schork[1,2,3].* 1) Scripps Genomic Medicine, Scripps Health, La Jolla, CA, USA; 2) The Scripps Research Institute, La Jolla, CA, USA; 3) 3The Center for Human Genetics and Genomics, University of California, La Jolla, CA, USA.

Many genetic research initiatives utilize information about the genetic distance between pairs of populations. Recently, such information became instrumental in assessing population substructure in genetic association studies. Many numerical measures have been proposed that indicate the degree of genetic distance using differences in allele frequencies between the populations. With the recent influx of genetic data from various populations, it is important that results of analyses performed on different datasets are comparable. However, it is not clear whether genetic distances between the same pairs of populations calculated with different formulas yield the same results. Additionally, genetic distance measures are often assumed to follow certain properties, e.g., have values between zero and one. Some of these properties have not been fully explored. In this study, we used simulation to generate different sets of allele frequencies at biallelic markers that modeled pairs of populations with various degrees of genetic distance and used these data to compare six widely used genetic distance measures (Weir and Cockerham's Fst statistics, Weir's genetic distance, Nei's standard genetic distance and Takezaki and Nei's distance measures). We assessed the measures' range, their sensitivity, and the correlations among the measures. Our results show perfect correlation between all pairs of measures with the exception of correlations that involved Nei's genetic distance. Also, the range of Nei's genetic distance differed significantly from the ranges of the other distance measures studied (0.003 - 4.0 vs. -0.005 - 1.0). Finally, Nei's genetic distance exhibited comparatively lower sensitivity for similar populations but greater sensitivity for more distant populations. The results of this study suggest that while the values of some genetic distance measures can be directly compared, the values of others, e.g., distances of Weir and Nei, cannot. Thus, specifying the genetic distance measure that was used to assess population structure is highly recommended.

1351/T

Admixture Analysis in Mestizos from Pacific and Atlantic Mexican coasts. *J.M. Oliva-Ortiz[1,2], J. Becerra-Contreras[1,2], J.R. Padilla-Gutiérrez[1,2], L.B. López-Hernández[1,2], K.R. Morales-González[1,2], M.T. Magaña-Torres[1,2], F. Rivas[1,2], L. Sandoval-Ramírez[1,2].* 1) División de Genética, Centro de Investigación Biomédica de Occidente, CMNO, IMSS, Guadalajara, Jalisco, México; 2) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México.

The admixture among Europeans, Africans and Amerindians after Mexican colonization was not homogeneous throughout the country. Study of markers with blood groups, HLA, nuclear and mitochondrial polymorphisms showed great diversity in the admixture levels depending on the analyzed geographic region of Mexico (Lisker et al. 1996; Rangel-Villalobos et al. 2000; Gorodezky et al. 2001; Cerda-Flores et al. 2002; Green et al. 2002; Magaña et al. 2002). In this study, samples of 201 mestizos from Pacific and 72 from Atlantic coasts were genotyped in order to estimate the admixture proportion. Four mtDNA haplogroups (A, B, C, and D) which characterized most Native American lineages, and one (L) that define African lineage were analyzed. For the Y-chromosome markers we studied; DYS287, DYS199, DYS19, DYS390, DYS447 and DYS458 that define some populations groups. The mtDNA origin of individuals from Atlantic coast was 98.6% Amerindian and 1.4% African; while in Pacific coast was 95.5% Amerindian and 4.5% African. Analysis of Y-chromosome markers showed that 28.2% of the alleles from Pacific coast were African and 37.5% Amerindian. In the Atlantic coast 10.2% of alleles had African origin and 39.7% Amerindian. The Pacific Costa Chica's population showed a higher admixture proportion. The results showed a different contribution of male and female gene pools in these admixtures Mexican populations.

1352/T

A Test for Hardy-Weinberg populations using just two individuals. *N.M. Scott, J.C Long.* Dept. of Human Genetics, Univ. of Michigan, Ann Arbor, MI.

Human Genetic studies often use location, race, or isolate status as a proxy for a population in Hardy Weinberg Equilibrium (HWE). Even so, cryptic population structure is a problem in many samples. Here we provide a multiple locus test to determine if two individuals represent random draws from the same HWE population. Our basic premise is that the proportion of homozygous loci in a single individual is a valid and unbiased estimator for the homozygosity in a HWE population. Therefore, homozygosity should not differ significantly between two individuals from the same HWE population. The comparison of two individuals is strengthened by considering their pseudo-homozygosity, which we define as the probability that, at a locus, a random allele chosen from the genotype of one individual is identical in state with a random allele chosen from the genotype of the other individual. For a pair of individuals from the same HWE population, the expectation of pseudo-homozygosity equals the population homozygosity. Thus, a pair of individuals provides three estimates of population homozygosity.

Here we derive the variances and covariances of these estimates for multiple unlinked SNP loci. We also derive a test statistic for homogeneity of the three estimates and use computer simulation to show that a chi-square distribution with three degrees of freedom fits the distribution of the test statistic. For the case that the homogeneity hypothesis is rejected, we develop linear contrasts to distinguish between two alternative hypotheses: high individual homozygosity between a pair of individuals in comparison to their pseudo-homozygosity, and low individual homozygosity between a pair of individuals in comparison to their pseudo-homozygosity. Biologically, high pseudo-homozygosity is consistent with relatives sampled from the same HWE population, whereas, low pseudo-homozygosity is consistent with sampling individuals from different HWE populations and/or inbreeding. To prove our principles, we apply this method to HapMap population and family trio data sets, and show analytically that it is easy to identify population misplacement and cryptic family structure with genome-wide SNP data. Supported by NIH grant T32-HG00040.

1353/T

Noncoding sequence variation in human populations. *J.D. Wall[1], M.P. Cox[2], A. Woerner[2], M.F. Hammer[2].* 1) Institute for Human Genetics, UCSF, San Francisco, CA; 2) ARL Division of Biotechnology, University of Arizona, Tucson, AZ.

We conducted a large resequencing study of nuclear noncoding DNA sequence variation in a diverse collection of six human populations. Our study design allows us to obtain a more complete view of human genetic diversity, and only 20% of the SNPs that we found were contained in the HapMap. As in previous studies, non-African populations have less variation, fewer rare variants and more linkage disequilibrium than sub-Saharan African populations. Strikingly, however, levels of differentiation between populations are higher than previously reported, especially on the X chromosome.

1354/T

Whole-genome association studies have substantially increased power in admixed populations. *A.L. Price[1,2], S.R. Myers[2], N. Patterson[2], D. Reich[1,2].* 1) Harvard Medical School, Boston, MA; 2) The Broad Institute, Cambridge, MA.

Whole-genome association studies (WGAS) are a powerful way to identify common variants conferring disease risk. WGAS have been carried out almost exclusively in populations of European ancestry, with little or no representation of underserved populations such as Latinos and African Americans. This may be due in part to the technical challenges posed by admixed populations, but we and others have now developed methods that enable fully powered WGAS in these populations (see Myers et al. abstract). We set out to investigate the power of WGAS in admixed populations, and found that there is a major gain in power and efficiency. These populations offer more power on average because (a) multiple ancestral populations provide increased genetic variation, and (b) there is no noise introduced from controls in the admixture association component of the overall signal.

Using the empirical distribution of ancestry proportions in Latino Americans and the empirical joint distribution of European and Native American allele frequencies, we calculated how many Latino samples would be required to achieve power comparable to genotyping 1,000 European cases and 1,000 European controls in a whole-genome scan. We found that 10% fewer samples, or 900 cases and 900 controls, were required for random markers. For markers in the top 10% of frequency differentiation between Europeans and Native Americans, which might drive differences in prevalence of diseases such as type 2 diabetes, only 660 cases and 660 controls were required. (These calculations assume that the causal variant has been genotyped, and do not account for the advantage of increased linkage disequilibrium within chromosomal segments of Native American ancestry.) Sample for sample, our results indicate that admixed populations are substantially more powerful for identifying disease variants, even for variants with only average differences in frequency across populations. For these reasons we suggest that researchers should specifically choose to study admixed populations - in preference to unadmixed populations - for any WGAS for which samples from both admixed and unadmixed populations are available.

1355/T

Population Stratification in the Quebec Founder Population. *J. Raelson, V. Pinchuk, E. Hardy, L. Nadeau, T.V. Nguyen, B. Stojkovic, V. Perepetchai, P. Croteau, A. Belouchi, P. Van Eerdewegh, T. Keith.* Genizon BioSciences, St-Laurent, QC, Canada.

The Quebec Founder Population (QFP) is a population isolate with demographic characteristics that make it a valuable resource for genome wide association studies (GWAS) of complex diseases. During the course of our GWAS we have observed stratification between distinct regional sub-populations of Quebec. Using a 374K chip data (Human Hap300 from Illumina supplemented with 57K SNPs based on LD in the QFP) for a sample of about 1300 QFP control subjects, we have observed differences in allele frequency for tens of thousands of SNPs and relatively high values of lambda for Genomic Control when comparing regional sub-samples. Such regional differences can seriously confound case-control studies where regional mismatch exists between cases and controls producing many false positives. Accordingly, we have developed algorithms to detect the presence of stratification and to build data sets in which cases and controls are closely matched for geographic origin. One approach is genealogical, based upon the use of grandparental birth location. We have developed an algorithm that weights both quality of available information and extent of grandparental region matching that routinely produces optimally matched sets of cases and controls with corresponding lambda values reduced to 1.00 from 1.3-1.4 in input data sets. A second algorithm matches case and control samples using genotype information. Existing methods such as Genomic Control and EIGENSTRAT correct single marker p-values. These methods are not adapted to genome-wide haplotype analyses which we routinely use. Our algorithm, based upon multivariate correspondence analysis, matches cases to controls according to chi-squared distances computed over all markers. Paired individuals are then sorted by this statistic in increasing order and a cut-off is applied at a matching distance corresponding to a lambda value close to 1.00. Both algorithms successfully remove the problem of population stratification. The usefulness of the methodologies will be illustrated with data from GWAS in the QFP.

1356/T

Measures of population structure. *H. Xu, V. George.* Department of Biostatistics, Medical College of Georgia, Augusta, GA.

Large-scale genome-wide association studies are promising for unraveling the genetic basis of complex diseases. Population structure is a potential problem, the effects of which on genetic association studies are controversial. The first step to systematically quantify the effects of population structure is to choose an appropriate measure of population structure for human data. The commonly used measure is *Wright's Fst*, which measures the genetic variance between subpopulations as a fraction of the total genetic variance. For a set of subpopulations it is generally assumed to be one value of *Fst*, even though it could be different for distinct loci. Recently, a new *c parameter* was proposed for SNP data, which was assumed to be subpopulation-specific and common for all loci. In this study, we performed extensive coalescent-based simulations of samples with varying levels of population structure to investigate the properties and relationships of both measures. It is found that the two measures generally agree well when the subpopulations have similar levels of differentiation, but may differ otherwise. Based on the comparison results, we propose an *adjusted c parameter* based on the effective population size of each subpopulation. It has the advantage of easy interpretation as one measure of population structure and yet can also assess population differentiation.

1357/T

The history of Amerindian mtDNA lineages in the Caribbean. *J.C. Martínez-Cruzado[1], A. Feliciano-Vélez[1], E. González-Bonilla[1], P. Valencia-Rivera[1], E. Gómez-Sánchez[1], V. Reyes-Ortiz[1], M. Rivera-Vega[1], A. Águlvarez-Serrano[2], A. Román-Colón[1], J.S. Ramírez-Lugo[1].* 1) Department of Biology, University of Puerto Rico at Mayagüez, Mayagüez, PR; 2) Museo Arqueológico Regional Altos de Chavón, La Romana, Dominican Republic.

Christopher Columbus described a people of Arawak culture predominating both in Puerto Rico and Hispaniola, plus other more geographically constrained peoples in the latter island. We aim to learn about the pre-Columbian migrations to the Caribbean that gave rise to these populations through control region sequencing of Amerindian mtDNAs and partial restriction typing of the coding region. Median network analysis of the data obtained from a subset of 122 Amerindian mtDNAs selected from a sample set representative of the Puerto Rico population suggests the presence of 19 maternal lineages, 10 of which account for 89% of all Amerindian mtDNAs on the island. The most frequent lineage, C-I, accounts for 21% and displays a star-like phylogeny, suggesting a demographic expansion soon after its arrival, the time of which is estimated at 2500 YBP. In addition, only and all members of this lineage display a rare 7013 *Rsa*I site loss found only in the Amazon, birthplace of the Arawak culture. Its estimated time of arrival is consistent with that of the South American Saladoids, regarded as the first agricultural society of the Caribbean. Four other lineages, accounting for 36% of all Amerindian mtDNAs, show higher diversity, non-star-like phylogenies, and may stem from Archaic cultures. They all show particular signature polymorphisms not found elsewhere in the continent or the Dominican Republic. Thus, barring *in situ* evolution, different continental origins may be proposed for the peoples who first populated Hispaniola and Puerto Rico. In the Dominican Republic, lineage C-I accounts for only 1.9% of all Amerindian mtDNAs. Hence, female-mediated gene flow between the two islands seems to have been restricted. Haplogroup A accounts for 65% of all Amerindian mtDNAs in the Dominican Republic. A median network constructed from control region sequences of 32 haplogroup A mtDNAs suggests geographic partition within this country.

1358/T

The ancestry of Y-chromosomes in Puerto Rico. *K. Ocasio-González, A. Díaz-Lameiro, K. Martínez-Vargas, M. López-Muñoz, J.C. Nazario-Borges, J.C. Martínez-Cruzado.* Department of Biology, University of Puerto Rico at Mayagüez, Mayagüez, PR.

Most Latin American populations show asymmetric ancestry, with European ancestry predominating in the paternal lineages and Amerindian or African ancestry in the maternal lineages. In consistency with this pattern, the female ancestry of Puerto Ricans has recently been shown to be predominantly Amerindian, followed by African and West Eurasian ancestries in that order. In a progressing program of research, we have identified the haplogroup of 202 Puerto Rican Y-chromosomes through biallelic marker typing and estimated that 151 (74.8%) are of West Eurasian origin, 48 (23.8%) Sub-Saharan African, and 3 (1.5%) Amerindian. In addition, 17 Y-STR loci of 35 samples have been typed, producing 32 haplotypes, the haplogroup of 18 of which have been identified through biallelic markers. The Y-STR data was used to construct a median-joining network that produced five clusters of 18, 4, 3, 3, and 3 haplotypes each. One haplotype of haplogroup still unknown remained separated from all five clusters. Y-STR-haplogroup matching analysis strongly suggested that all Y-chromosomes belonging to each Y-STR cluster shared the same continental origin. For example, all 11 haplotypes for which the haplogroup was known in the 18-haplotype cluster belonged to West Eurasian haplogroup R1b. In that way, two clusters totaling 21 haplotypes, were identified as West Eurasian in origin, and two clusters totaling 7 haplotypes as Sub-Saharan African. The haplogroups of none of the three haplotypes of the remaining cluster have yet been identified. A search in the Y-chromosome haplotype reference database produced results consistent with the deduced origin of the clusters, and suggested that the origin of the remaining cluster is Asian or Native American. It did not suggest an origin for the isolated haplotype. We conclude that, as in other Latin American populations, the continental origin frequency of Y-chromosomes in Puerto Rico is inverted relative to that of mtDNA. We further conclude that Y-STR haplotypes are excellent predictors of haplogroup identity.

1359/T

Evaluation of linkage disequilibrium in the Azores Islands and mainland Portugal. *C.C. Branco[1,2], E. Cabrol[1], M. São Bento[1], C.T. Gomes[1], R. Cabral[1,2], A.M. Vicente[2,3], P.R. Pacheco[1,2], L. Mota-Vieira[1,2].* 1) Dept Molec Gen, Pathology Unit, Hosp Divino Espirito Santo, Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Centro de Biopatologia, Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal.

The study of the extent of LD and population structure is a good starting point for the investigation of complex traits. Here, we characterize the LD extent in the Azores and mainland Portugal populations. The sample distribution per Azorean group and island was the following: Eastern group, 207 (São Miguel 185; Santa Maria 22); Central group, 150 (Terceira 54; Pico 29; São Jorge 23; Faial 25; Graciosa 19) and the Western group, 75 (Flores 59; Corvo 16). In addition, 97 individuals from mainland Portugal were analysed. LD was evaluated in the Xq13.3 region by genotyping eight STR markers. Pairwise LD calculation demonstrates higher number of significant associations in the Western group (10 out of 28 comparisons), in contrast to the 3 found in the Central and Eastern groups. Additionally, D' analysis indicates that the Western group presents higher values when compared with the other two groups. However, all islands groups show values of D' lower than 0.33, suggesting no extensive LD in these populations. As expected, the highest D' values are found for shorter distances for all populations. Taken together, the data show that the Azorean population presents a lower average D' (0.142) compared with mainland Portugal (0.226). These results suggest that the identification of identical by descent (IBD) regions surrounding disease susceptibility or other complex trait loci in the Azorean, as well as in the mainland populations, would require a very high density of markers. On the other hand, the easy reconstruction of large pedigrees in the Azorean population is a valuable resource for the fine mapping of disease genes.

1360/T

Genetic studies on an isolate region of Sardinia unravels history and evolution of its population. *G. Pistis[1], C. Fraumene[2], N. Pirastu[2], E. Mocci[2], M.T. Manias[2], V. Cabras[2], R. Stradoni[1], M. Cosso[2], D. Farris[2], F. Marras[2], R. Atzeni[2], A. Angius[1,2], M. Pirastu[1,2].* 1) Inst Population Genetics, Alghero, Italy; 2) Shardna Lifesciences, Cagliari, Italy.

In Sardinia the mountainous secluded area of Ogliastra has always been described as "an Island within an Island" implying a high genetic homogeneity. We studied 9 different villages (15000 inhabitants) within this region characterized by high endogamy (from 70 to 90%), low immigration, remote origin and 400 years of genealogical records. In each village, we reconstructed all genealogical maternal lineages representing more than 90% of the present day population. The Dloop region of mtDNA was sequenced in 885 samples (the entire mtDNA in a subset of them) chosen to analyze the different branches in each individual pedigree. Haplogroups analysis shows that in each village there is a limited number of maternal founders and only a few of them shared between the different isolates. Multiple correspondence analysis on the haplogroups reflects the geography of the region and shows that the two closest villages (Talana and Urzulei) seem to have a different origin than the other ones. We calculated LD genome wide (D', r2, LDU) using 500k SNP on 50 samples from each of the 6 villages. We found that the isolates farthest from the sea have a higher LD probably due to an higher isolation. Talana, which is highest on the mountains, showed 298 LDU while Baunei, close to the sea, 566 LDU even thought they are only few miles away. Looking at the LDU map it is clear that there is a common underling pattern of cold and hot recombination spots. This is even more clear if we use those markers which are informative in both our population and the CEU from HapMap, probably showing the effect of evolution on the genome. Preliminary data on LD blocks structure similarities suggest that this depends more on the environment the populations live in, than phylogenic relations between them. The understanding of the genetic makeup of these different populations will be instrumental to better approach the study of common diseases, considering the interaction between history, evolution and changes in today life style.

1361/T

African-American gender biased gene flow revealed by mtDNA haplotypes. *N. Wang[1], W. Niu[2], B. Budowle[3], R. Chakraborty[2].* 1) Div. Allergy and Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) Center for Genome Information, Univ. of Cincinnati, Cincinnati, OH; 3) Laboratory Division, FBI Academy, Quantico, VA.

As an admixed population, African-Americans are the results of gene flow between Africans and Americans of European descent within the last 20 generations. Such admixture histories have advantage in uncovering the genes underlying human complex diseases that have different prevalence in the parental populations (also called admixture mapping). Understanding the gene flow to African-Americans from the parental populations is critical especially for diseases caused by imprinting genes (i.e., genes with parent-of-origin effect). In the present study, sequence data on mitochondrial hyper-variable regions I and II (HV1 and HV2) from 240 Africans (45 from Egypt, 98 from Kenya, and 97 from Sierra Leone), 1303 African-Americans, and 1794 Caucasians (95 from Austria, 107 from France, 43 from Greece, 158 from Spain, and 1391 from the USA) were extensively analyzed to study the gene flow to African-Americans from maternal lineage. Our results show that the haplotype diversities are 0.9979, 0.9984, and 0.9967 for the Africans, African-Americans, and Caucasians, respectively, suggesting that the extent of mtDNA haplotype difference at individual level is large. However, within each population, the mismatch distribution in the African-Americans (observed mean 14.4) is close to that in the Africans (observed mean 14.8), but significantly higher than that in the Caucasians (observed mean 8.0). In addition, our results reveal that common African-American haplotypes cluster closely with haplotypes found in the Africans, strongly suggesting gender-biased gene flow to African-Americans with nearly exclusive African female contributions. Taken together, the results obtained herein should provide important guidelines for studying diseases related to imprinting genes and diseases linked to mitochondrial DNA in the African-Americans. (Research supported by NIH grant GM 41399 to RC).

1362/T

Genetic variation in the Iban population of Sarawak. *T. Simonson[1], B. Mowry[2, 3], R. Barrett[4], E. Jerah[2], W.S. Watkins[1], Y. Zhang[1], L.B. Jorde[1].* 1) Dept of Human Genetics, University of Utah; 2) Queensland Centre for Mental Health Research; 3) Dept of Psychiatry, University of Queensland; 4) (deceased) Dept of Psychiatry, University of Adelaide.

Genetically isolated human populations provide unique opportunities for molecular investigations of complex diseases. The Iban of Sarawak are an isolated branch of the Dayak peoples residing in Northwestern Borneo. No previous study has examined genetic diversity within this population, and their relationships with other populations remain poorly understood, making the Iban an ideal population for population genetic investigations. Thirty-eight neutral autosomal STRs, in concert with Y chromosome SNPs and HVS1 mitochondrial sequence, were assayed in 94 Iban males. We found high expected (0.660) and slightly lower observed (0.653) STR heterozygosity in the Iban (within the ranges of most outbred populations), suggesting they have not experienced the putative lack of gene flow and strong bottleneck suspected from their traditional cultural practices and exodus into Sarawak. In addition to the reduction in observed heterozygosity, STR allele size variance is slightly decreased compared to various Southeast Asians and, although minor, may prove beneficial for mapping genes related to complex disease. Maternally inherited Iban HVS1 diversity estimates (0.019) are greater than continental populations studied (0.008-0.016), with the exception of highly diverse Africa populations (0.025). Y chromosome SNPs indicate a high frequency of haplogroup O (f_{O3}= 0.38, $f_{O1/O2}$= 0.49) and a low frequency of haplogroup C (f_C=0.04), consistent with other male Asian populations. Genetic distance estimates indicate the Iban are most closely related to East Asians followed by Indian, European, and African continental groups. Autosomal genetic distances between the Iban and the Cambodian, Vietnamese, Japanese, and Chinese are 0.027, 0.030, 0.038, and 0.042, respectively, suggesting the Iban are most genetically similar to Cambodian and Vietnamese populations. Supported by NIH Grant GM-59290 and NSF Grant BCS-0218370.

1363/T

Haplotypes 5' and 3' of the β globin cluster genes in two Mexican Amerindian populations. *M. Casas-Castañeda[1], M.T. Magaña[2,3], L. Sandoval[2,3], A.R. Villalobos-Arambula[4], F.J. Perea[2,3], B. Ibarra[2,3].* 1) Instituto de Ciencias Biologicas, Universidad Autonoma de Guadalajara, Guadalajara, Guadalajara, Mexico; 2) Division de Genética Centro de Investigacion Biomedica Occidente Inst Mex del Seguro Social Guadalajara Mexico; 3) Doctorado en Genetica Humana Universidad de Guadalajara Guadalajara, Mexico; 4) Centro Universitario de Ciencias Biologicas y Agropecuarias Universidad de Guadalajara Guadalajara Mexico.

In this work we analyzed ten polymorphisms of the β-globin genes, in two Mexican native populations, Purepechas and Tarahumaras, with the aim of determining the 5'and 3'haplotypes (Hps) and the relationship with the reported populations. Five sites (Hinc II-ε, Hind III-Gγ, Hind III-Aγ, Hinc II-5'and Hinc II-3'ψβ) to the 5' Hp and five (Exon 1 nucleotide 16, intron 1 nucleotides 46, 74 and 81, and Hinf I-β) to the 3' Hp. Six sites were identified by RFLP's and four by sequencing. The results were compared with the previously studied populations. 5' Hp: In Purepechas we found 8 different Hps, the three most common were 1 (72.3%), 11 (10.3%) and 2 (8.3%). In Tarahumaras we observed 7 distinct haplotypes, with 1 (79.4%), 14 (8.7%) and 5 (5.1%) having the highest frequencies. The genetic and nucleotide diversity in both populations showed mean values respect to 32 reported populations. In the genetic distances, only Tarahumaras did not show significant differences with Huichols and Koreans. 3' Hp: The Purepechas revealed 7 different Hp and 6 the Tarahumaras; C, A and B1 were the more frequent in both populations. We found three news 3'Hps, CTGCT in both populations, GTTCT in Purepechas and GTGCA in Tarahumaras. The diversity values are similar in the 10 analyzed populations, in agreement with the location of the 5 studied polymorphisms, since they are intragenic and then more conserved. Both populations did not display differences with Asian populations (Sumatra and Mongolia); in addition Tarahumaras also was similar to Nuu-Chah-Nult Amerindians.

1364/T

COALESCENT SIMULATION OF GENOME WIDE ASSOCIATION DATA IN ADMIXED POPULATIONS. *G. Chen[1], S. Kim[1], R. Varma[2,3], P. Marjoram[2], J. Wall[1].* 1) Institute for Human Genetics, University of California, San Francisco, CA; 2) Department of Preventive Medicine, Keck School of Medicine, USC, Los Angeles, CA; 3) Doheny Eye Institute and Department of Ophthalmology, Keck School of Medicine, USC, Los Angeles, CA.

Genome wide association (GWA) studies have quickly become a popular approach for elucidating the genetic variants that contribute to the susceptibility of common diseases. Issues regarding the design of GWA studies such as the appropriate sample size or marker density for a desired level of power and specificity in a target population can be addressed by analysis of simulated data reflecting assumptions about that population's history. A widely used model-based approach for simulations is the coalescent, which models the genealogy of a set of sampled chromosomes from the present time back to their most common recent ancestor. Current implementations of the coalescent that precisely incorporate recombination are limited to simulating only short stretches of DNA due to memory constraints. We present an implementation which can more efficiently simulate SNP data across entire chromosomes by providing a close approximation to the coalescent. The simulator also provides the user with the flexibility of modeling population events such as growth (i.e. instantaneous or exponential), population splitting, migration between sub-populations, and sub-population merging, which may be relevant for modeling admixed study populations. We compare summary statistics (e.g. LD decay, haplotype diversity) of data generated from our program to those of real genotype data generated from Affymetrix 500k genotyping arrays in a study of age related macular degeneration in Latinos, a population known to share European and Native American ancestry.

1365/T

On the edge of the Bantu expansions: patterns of mtDNA and Y-chromosome variation in southwestern Angola. *M. Coelho[1,2], J. Rocha[1,2], S. Beleza[1].* 1) IPATIMUP, Porto, Portugal; 2) Department of Zoology and Anthropology, Faculty of Sciences, University of Porto, Porto, Portugal.

Among the complex series of demographic movements that have shaped the patterns of human genetic variation in sub-Saharan Africa, the massive dispersal of Bantu-speaking farmers across subequatorial regions in the last 4000-5000 years remains one of the most impressive examples of large population movements in our species. In spite of the relative recentness of these dispersals, several important questions about the tempo and mode of demographic events undergone by the ancestors of present-day Bantu farmers still persist. Current studies on Bantu expansions are hampered by poor sampling within the vast region encompassing sub-equatorial Africa. For example, the region of Angola remains a persistent gap in studies of African genetic variation, and only scarce information is available from the broad area beyond the Cuanza River, crucial for understanding the dramatic push of Bantu peoples towards the arid steppes and deserts of Southwest Africa. Here, we present a preliminary analysis of the patterns of Y-chromosome and mtDNA genetic variation in a panel of 359 individuals from different ethno-linguistic groups from the region of the Namibe desert in Southwestern Angola: Kimbundo, Umbundo, Nkhumbi, Mwila, Nyaneka, Nyemba and Herero/Kuvale. Patterns of Y chromosome microsatellite variation were found to be relatively homogeneous across the different Angolan groups and, like in most Bantu populations, were dominated by a small number of E3a* haplotypes with intermediate frequencies. As a whole, mtDNA variation was consistent with the general pattern previously observed in populations located along the western stream of the Bantu expansion. However, we found that, within the southern Angola context, the Mwila and Herero pastoral groups were clear outliers due to the assimilation of significant amounts of L0d/K lineages (15-23%) that are typical of southern Khoisans. These results provide evidence for substantial female driven Khoisan-Bantu interactions that may have important implications in the understanding of the current distribution of pastoralism in Southwest Africa area.

1366/T

Long range haplotype diversity analysis and Haplotype sharing between the Mexican Mestizo and the European, Asian and African Populations. *A. Hidalgo, J. Estrada-Gil, L. Uribe-Figueroa, I. Silva-Zolezzi, A. Contreras, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Given the history of the Mexican Mestizo population, resulting from the admixure of Amerindian, Spaniards and to a lesser extent Africans, we are evaluating genomic variability in our population and comparing it with other populations. Phase I of the Mexican Genome Diversity Project (MGDP), genotyped 300 Mestizos from six states of the country (Guanajuato, Guerrero, Sonora, Veracruz, Yucatan and Zacatecas) using the Affymetrix 100K array. Long range haplotype diversity (LRHD) and haplotype sharing (HS) were compared between the Mestizos and populations of the International HapMap. Data was phased using FastPhase v1.1.4, LRHD was calculated in 1 Mb windows, and the frequency of the haplotypes was averaged and compared to the percentage of chromosomes represented by those haplotypes. HS was assessed by comparing haplotypes frequencies of five SNPs(~100 kb). LRHD analysis showed that 67.8 haplotypes per megabase capture 95% of the chromosomes in the Mexicans, while 92.6, 82.2 and 69.4 are necessary to achieve this coverage in the YRI, CEU and JPT-CHB populations, respectively. Haplotype sharing indicated that 64% of the haplotypes (5% minor allele frequency) from the YRI are present in the Mexicans, as well as 74% from the JPT-CHB and 80% from the CEU. The Asian-CEU combination shared 93% of the haplotypes and 96% of the combined haplotypes from the four HapMap populations were present in the Mexicans. Our results indicate that data from the International HapMap can be used in the Mexican population. However the highest power can only be obtained using the combination of the four populations. The remaining 4% of the haplotypes, not represented in the HapMap data, might be derived from the Amerindian component in the admixture that led to the modern Mexican Mestizos. To increase genomic resolution in our population, we are increasing coverage to over 1 million SNPs. In addition, and we are including Amerindian samples in our study. These results will contribute to strengthen resources for association studies in Mexican populations.

1367/T

Comparison of background relatedness by analysis of the distribution of homozygous segments in the four ethnic groups of the HapMap Phase II dataset. *T.A. Johnson[1,2], T. Tsunoda[1].* 1) Laboratory for Medical Informatics, SNP Research Center, RIKEN Yokohama Institute, Yokohama, Japan; 2) Tokyo Medical and Dental University, Department of Bioinformatics, Medical Research Institute, Tokyo, Japan.

Relatedness exists as a continuum from that observed between family members, to that which distinguishes geographically isolated populations, to that seen between members of a particular ethnic group, and on to that which makes us all human. Mapping of loci identitical-by-descent in closely related individuals has been extremely important for interrogation of rare but highly penetrant Mendelian diseases, while toward the middle of the relatedness spectrum, that seen within ethnic populations, such phenomena as linkage disequilibrium and haplotype-block structure has provided researchers with some of the tools to examine common disease on a population-wide basis. One extreme example of this background relatedness has been the discovery of extended runs of homozygous loci, some on the order of megabases, in population genetic data. We explored this on a finer scale using release 22 of the HapMap Phase II dataset by detecting all homozygous segments across the 270 individuals sampled from the Yoruba (YRI), Caucasian (CEU), Chinese (CHB), and Japanese (JPT) populations. To filter out uninformative segments while allowing for inter-population comparison, we calculated a homozygosity probability score (HPS), which is the product of the lowest observed homozygosity from the four ethnic groups of each locus in a detected segment. For segments with HPS<=0.01, the average total basepair length of segments on autosomes was 0.85E+09, 1.13E+09, 1.23E+09, and 1.24E+09, for individuals from the YRI, CEU, CHB, and JPT groups, respectively. To examine background relatedness coming from relatively more recent ancestors, we examined segments >130 kb which showed the average total basepair length of segments on autosomes was 1.34E+08, 3.54E+08, 4.46E+08, and 4.62E+08, for individuals from the YRI, CEU, CHB, and JPT groups, respectively. To further compare levels of background relatedness, we will provide maps of the genome-wide binned coverage of extended homozygosity for each population.

1368/T

Haplotype-based population tree for 45 human population samples. *K.K. Kidd, A.J. Pakstis, W.C. Speed, S. Gu, N. Mukherjee, M.P. Donnelly, J.R. Kidd.* Dept Genetics, Yale U. Sch Med, New Haven, CT.

While enormous SNP and STRP datasets are emerging for a handful of human populations, studies of genetic relationships of human populations from around the world to date have used the HGDP-CEPH panel of ~1,000 individuals. Our study of 2,345 individuals from 45 populations includes supersets for 15 of the 51 HGDP-CEPH groups. Our larger sample sizes per population allow more accurate estimation of haplotype frequencies. Geographic regions represented include: Africa(10 pops), S.W. Asia(4), Europe(9), N. Asia/Siberia(3), E. Asia(9), Pacific Is.(2), N.America(4), S. America(4). We analyzed 506 multi-SNP haplotyped loci from 2,556 SNPs distributed across 17 autosomes (~6.2 million genotypes). Regions with multiple SNPs were analyzed for LD (HAPLOT3) to identify relatively independent SNP clusters with strong inter-SNP LD. Haplotype frequencies were calculated via EM algorithm(HAPLO). The use of frequencies of multi-SNP haplotypes helps overcome the diverse ascertainment biases associated with individual SNPs. Avg heterozygosities for 506 haplotypes range from .4 to .7 among the groups studied with Africans highest (avg ~0.65) and groups farther from Africa trending lower; S.American groups avg ~0.48. The first 3 PCA factors account for ~90% of the genetic variation; pop. samples cluster in clear geographical patterns. Over 500 alternative trees were evaluated with the Least Squares search program (LSSEARCH) to find the best tree. PHYLIP programs were used to generate 1,000 replicate data sets and generate bootstrap values for the consensus tree. Bootstrap values of 100% occur 11 times for various population groupings with the African branch accounting for 6 while the rest either divide major tree segments separating continental groupings or particular population clusters. Several boostrap values in the 90-99% range were found for groupings within continental regions. While major improvements in the robustness of various population groupings were achieved compared to earlier studies with smaller marker sets, larger datasets are needed to get high bootstrap values within continental groupings where populations are closely related. Supported by NIH GM57672.

1369/T

Population structure in Sweden - A Y-chromosomal and mitochondrial DNA analysis. *T. Lappalainen[1], U. Hannelius[2], E. Salmela[1,6], C.M. Lindgren[3], K. Huoponen[4], M.-L. Savontaus[4], J. Kere[2,5,6], P. Lahermo[1].* 1) Finnish Genome Center, Institute for Molecular Mecidine Finland, University of Helsinki, Helsinki, Finland; 2) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 3) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK; 4) Department of Medical Genetics, University of Turku, Turku, Finland; 5) Clinical Research Centre, Karolinska University Hospital, Stockholm, Sweden; 6) Department of Medical Genetics, University of Helsinki, Helsinki, Finland.

A population sample representing the current Swedish population was analyzed for both maternally and paternally inherited markers with the aim of characterizing the genetic variation and structure of a modern North European population. We genotyped 12 Y-chromosomal and 27 mitochondrial DNA SNPs from DNA extracted and amplified from Guthrie cards of all the children born in Sweden during one week in 2003. The sample set consisted of 1914 samples (960 males) grouped according to place of birth. The ancient migration patterns are reflected in the clear north-south gradients in several palaeolithic and neolithic haplogroups in the mtDNA (U5, I, K, T, X) and the Y chromosome (R1b, N3). The haplogroup frequencies of the counties closest to Finland and Norway showed clear associations to the neighboring populations, resulting from the formation of the nations during the past millennium. Moreover, the recent immigration waves of the 20th century are visible both maternally and paternally, and have led to increased diversity and divergence from the main population in the major cities. Unfavorable population development in the ancient or recent past can be detected in several remote counties with low diversities and other signs of low population size and/or population crises. In conclusion, our study yielded valuable information about the various factors affecting the structure of the modern Swedish population that is vital for the use of the population in large population-based studies. Our sampling strategy, nonselective on the current population rather than stratified according to ancestry, represents the future of genetic studies in the increasingly panmictic populations of the world.

1370/T

Relic Distribution of Y-Chromosome Haplogroup D Suggests Ancient Paleolithic Migration of Modern Humans in Eastern Asia. *H. Shi[1], H. Zhong[2], Y. Peng[1], Y.L. Dong[3], X.B. Qi[1], F. Zhang[4], L.F. Liu[5], S.J. Tan[3], R.L. Ma[2], C.J. Xiao[3], L. Jin[4], B. Su[1].* 1) Kunming Institute of Zoology and Kunming Primate Research Centre, Chinese Academy of Sciences, Kunming, PR China; 2) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, PR China; 3) Human Genetics Centre, Yunnan University, Kunming, PR China; 4) State Key Laboratory of Genetic Engineering and Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, PR China; 5) Huaihua Medical College, Huaihua, Hunan, PR China.

The Y chromosome haplogroup D is East Asian specific and prevalent in Tibetan and Japanese populations (30%-40%), but rare in other East Asian populations (<5%). We analyzed 5,174 Y chromosomes from 74 East Asian populations by typing haplogroup D related SNPs and eight Y chromosome microsatellite loci. We identified six sublineages under haplogroup D, and their distribution across East Asia suggested an ancient Paleolithic south-to-north migration, which likely predates the previously proposed northward diaspora of modern humans (reflected by the dominant occurrence of O3-M122 in East Asians) resulting in current relic distribution of haplogroup D in East Asia.

1371/T

Population Structure in the Britain. *D. Davison, C. Spencer, J. Marchini, P. Donnelly.* Department of Statistics, University of Oxford, Oxford, United Kingdom.

Population structure is of interest in its own right, and for the light it can shed on population and demographic history. It is also well known to be a confounding effect in genetic association studies, although to date only limited empirical data have been available to assess this effect in some geographical locations. The Wellcome Trust Case-Control Consortium genotyped over 16,000 British individuals at 500,000 SNPs. The data provides an unprecedented opportunity to assess genetic population structure in this context. Thirteen genomic regions were shown to exhibit extensive geographical variation across Britain. For some, including Lactase, natural selection has previously been implicated in generating these differences. We assess the role of natural selection at the novel loci, and describe choices of ancestry informative markers for association studies. We also develop and apply other statistical methods for understanding the much less extensive population structure throughout most of the genome.

1372/T

The genetic structure of Pacific Islanders. *J.S. Friedlaender[1], F.R. Friedlaender[2], F.A. Reed[3], K.K Kidd[4], J.R. Kidd[4], G. Chambers[5], R. Lea[5], J.H. Loo[6], G. Koki[7], J.A. Hodgson[8], D.A. Merriwether[9], J.L. Weber[10].* 1) Anthropology,Temple University, Philadelphia, PA; 2) Independent Researcher, Philadelphia, PA; 3) Biology,University of Maryland, College Park, MD; 4) Genetics,Yale University, New Haven, CT; 5) Biological Sciences,Victoria University, Wellington, NZ; 6) Transfusion Medicine, Mackay Memorial Hospital, Taipei, Taiwan; 7) I.M.R., Goroka,Papua New Guinea; 8) Anthropology, New York University, NY; 9) Anthropology, Binghamton University, Binghamton, NY; 10) Marshfield Clinic Research Foundation, Marshfield, WI.

Human genetic diversity in the Pacific has not been adequately sampled. As a result, population relationships there have been open to debate. A genome scan of 687 autosomal microsatellites and 203 insertion/deletions on 936 individuals from 40 populations now shows the remarkable nature of Melanesian variation, and allows for a more complete comparison of Pacific populations with groups from other regions. While genetic diversity within individual Pacific populations is shown to be low, the diversity among Melanesian groups is very high. There is considerably more variation among groups in the island of New Britain than among East Asian or European populations.Melanesian diversity varies with island size and topographical complexity. The greatest distinctions are among the isolated groups in large island interiors. The pattern also follows language distinctions. Papuan-speaking groups are the most distinctive, and Austronesian groups, which tend to live along the coastlines, are more intermixed.In contrast, the Polynesian, Taiwan Aboriginal, and Micronesian groups are similar to each other and to East Asian populations. They have weak associations with Melanesians. An “Austronesian” genetic signature exists in less than half the Melanesian groups that speak Austronesian languages. This signature was not detected in any Papuan-speaking group. These findings provide a resolution to the debates over Polynesian origins and interactions with Melanesians; the debates had heavily relied on the evidence from single locus mitochondrial DNA or Y chromosome variation.

1373/T

Unsupervised clustering of individuals into HLA genetic clusters using Hardy-Weinberg Deviation. *L. Gragert[1], M. Maiers[1], W. Klitz[2].* 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) Public Health Institute, Oakland and University of California, Berkeley, CA.

The Hardy-Weinberg equilibrium (HWE) of genotypes describes the random combination of male and female gametes in a randomly mating population. In HLA studies deviation from HWE has been used to infer typing inaccuracies, possible selection and population stratification. Here we show that in large and consistently typed samples of bone marrow donor registries, HWE deviations can be used to demonstrate not just the existence of stratification among major world populations but to actually identify population subgroups. We have created a novel distance measure for clustering human populations using distortions of genotypic proportions from HWE. The individuals are grouped without using any prior knowledge of population haplotype frequency or self-described race/ethnicity of the genotypes. This method is applied in the case of the HLA system, which exhibits both extremely high polymorphism and privacy of HLA haplotypes to specific ancestral populations. A cohort of 880,000 individuals typed using consistent methods for the HLA-A, B, and DRB1 loci from several racial/ethnic backgrounds represented in the National Marrow Donor Program (NMDP) adult donor registry was analyzed. To illustrate the effectiveness of the algorithm, the top 200 haplotypes from an equally mixed sample of European-Americans and African-Americans were clustered with 97.5% accuracy based on comparisons with haplotype frequency distributions from known populations. This clustering method can be used for labeling donors who do not supply race/ethnicity information, identifying and analyzing subpopulations within broader race/ethnic classifications, and estimating the level of admixture between populations.

1374/T

Deducing source population HLA composition in US ethnic groups. *W. Klitz[1,2], L. Gragert[3], M. Maiers[3].* 1) Sch Public Health, Univ California, Berkeley, CA; 2) Public Health Institute, Oakland, CA; 3) National Marrow Donor Program, Minneapolis, MN.

The USA is indeed a nation of immigrants, ranging from the first prehistoric arrivals from Asia to the sea, land and air additions of more recent times. Despite admixture since arrival, the unique genetic record of the source populations of US ethnic groups awaits revelation. We uncover the parental population contributions of two major admixed US ethnic groups, African Americans and Hispanics, utilizing HLA (Human Leukocyte Antigen) variation from the A, B and DRB1 loci. HLA typing of donor samples from the National Marrow Donor Program was used to estimate three locus haplotypes with more than 90,000 samples in each group, including European Americans as representative of one of the parental populations of each of the admixed groups. The results showed that Europeans contributed to both the African Americans and Hispanics, as is well known from demographic history. Each of the admixed populations had less than 40% haplotype similarity to the European Americans. Remarkably, HLA haplotypes demonstrated nearly complete separation according to continental source (Africa, America and Europe) with nearly all haplotypes being discretely assignable to place of origin. This work recovers the original HLA types of the founding populations, populations which typically are no longer coherent and available for study after the original founder events often centuries ago. We conclude that minimal overlap in HLA haplotypes is present between populations having different continental origins. This is unlike other genetic systems, including genomic surveys of SNPs and microsatellites in which summaries of contrast between human populations reveal only statistical tendencies of population differentiation. In contrast, HLA haplotypic variation has evolved rapidly among human populations as they spread across the globe, producing the nearly complete separation among groups.

1375/T

HLA frequencies and genetic distances between Ashkenazi and non-Ashkenazi Israeli Jews. *M. Maiers[1], L. Gragert[1], M. Fernandez-Vina[2], W. Klitz[3], I. Haviv[4], S. Israel[4], C. Brautbar[4].* 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) Laboratory Medicine, MD Anderson Cancer Center, Houston, TX; 3) Public Health Institute, Oakland, CA; 4) Hadassah Medical Center, Jerusalem, Israel.

We have analyzed the HLA allele and haplotype frequenices of a cohort of Israeli Jewish individuals in 2 ethnic categories:10,078 Ashkenazi and 5,360 Non-Ashkenazi. 2-digit HLA Haplotype frequency analysis was performed using the EM algorithm on HLA-A, -B and -DRB1 typing results obtained by a combination of serologic and DNA-based methods. A set of 3 US cohorts were analyzed for comparison: 433,901 US_European, 103,382 US_African, 8,753 US_Japanese. Genetic distance computations were performed on equal-sized cohorts of 5,000 individuals selected at random from each group. The similarity index (Renkonen s If) was highest between the Ashkenazi and non-Ashkenazi groups (0.331). The similarity index between US_European and Ashkenazi was 0.291 and between US_European and non-Ashkenazi was 0.282. By way of comparison the similarity index between US_European and US_African was 0.268 and between US_European and US_Japanese was 0.160. Several other genetic distance measures were computed (Fst, Wn, Nei) on the A-B-DRB1 2-digit haplotype frequencies. In all cases, Ashkenazi and non-Ashkenazi were found to be the most similar. Genetic diversity within each group was evaluated based on the sum of the top 3 haplotypes and the non-Ashkenazi group was found to be more diverse with only 0.049 compared to 0.128 for Ashkenazi. We have also performed a sub-analysis by country of origin which includes samples from countries with very little published HLA information (non-Ashkenazi: Morocco, Iraq, Yemen, Iran, Tunisia, Libya, Egypt, Algeria, Syria and India, Ashkenazi: Russia, Romania, and Poland).

1376/T

Culture creates genetic structure in Daghestan. *E. Marchani[1], W.S. Watkins[2], K. Bulayeva[3], H.C. Harpending[1], L.B. Jorde[2].* 1) Dept Anthropology, Univ Utah; 2) Eccles Inst of Human Genetics, Univ Utah; 3) N.I. Vavilov Inst of General Genetics, Russian Academy of Sciences.

We investigate the effect of mating practices on genetic structure in Daghestan by comparing the frequency of 24 mitochondrial DNA (mtDNA) and 22 Y chromosome (NRY) haplotype-defining single nucleotide polymorphisms in three highland (Avar, Dargin, Kubachi) and two lowland (Kumik, Nogai) populations from Daghestan. AMOVA analysis reveals three times the amount of genetic structure in the NRY data than in the mtDNA data (mtDNA Φ_{ST}=10.3%, NRY Φ_{ST}=31.5%). The same comparison in a series of European and East Asian populations produces nearly-equal values for both sets of markers (mtDNA Φ_{ST}=46.1%; NRY Φ_{ST}=42.9%). This is consistent with the ethnographic record of patrilocality among highland Daghestani populations.

AMOVA between highland and lowland pooled populations (Φ_{ST}=42.7%, p<0.001) reveals NRY genetic structure nearly as great as that between European and East Asian populations posted above. The highland-lowland structure is not nearly as strong in the mtDNA data (Φ_{ST}= 3.9%, p<0.01), and weaker than that observed when all Daghestani populations are considered independently (Φ_{ST}=10.3%, p<0.001). Reduced NRY haplogroup diversity among highland populations (*h*: 0.00-0.47) when compared to lowland populations (*h*: 0.82-0.93) suggests drift caused by isolation and patrilocality.

The pairwise Φ_{ST} between the Nogai and Europe (21.0%) exceeds that (13.2%) between the Nogai and East Asia, consistent with their Mongolian origin. This unique observation is opposite the relationship observed between the Nogai and Europe and East Asia in the NRY data (Europe Φ_{ST}=18.8%; East Asia Φ_{ST}=45.1%), even though they are the only Daghestani population to exhibit Asian Y haplogroups D and Z. These results demonstrate that population history, isolation, and patrilocality have all left distinctive signatures on the genetic structure of Daghestan's populations. Supported by NIH Grant GM-59290 and NSF Grant BCS-0218370.

1377/T

Genetic Substructure in New Hampshire. *C. Sloan, A. Andrew, E. Duell, M. Karagas, J.H. Moore.* Dartmouth Medical School, Lebanon, NH.

The impact of geography and ecology on the genetic architecture of common human diseases is largely unknown. Understanding the geographic distribution of genetic background is likely to improve our ability to identify both genetic and environmental risk factors. The goal of the present study was to characterize genetic structure in the population of New Hampshire as a first step toward ecogeographic genetic epidemiology of spatially distributed common diseases in the United States. We sampled 865 control subjects for an epidemiologic study of cancer from across the state of New Hampshire. We measured 1474 SNPs from approximately 500 cancer susceptibility genes and used Bayesian clustering implemented in the Structure program to identify genetic substructure in this spatially extended sample of subjects. Clusters were evaluated using fixation index (Fst) and admixture statistics along with a novel Hamming distance metric. The Bayesian clustering results suggest four distinct genetic subgroups within New Hampshire (FSTs= 0.0699, 0.0798, 0.0466, 0.0204). The observed genetic structure may arise from the state's unique ethnic composition and history that includes a large proportion of Caucasian individuals with French and French Canadian descent as well as Caucasian individuals of English-Irish descent. The identification of genetic substructure among a largely Caucasian population of European descent in the state of New Hampshire is consistent with recent genetic structure results from studies in Europe including countries such as Iceland. These results, in combination with spatial information about environmental exposure, will play an important role in helping to explain regional differences in incidence of common human diseases.

1378/T

Identification and application of European substructure ancestry information and markers. *C. Tian[1], R.M. Plenge[2], P.K. Gregersen[3], M.F. Seldin[1].* 1) Rowe Program, UC Davis, Davis, CA; 2) Broad Institute, Cambridge, MA; 3) North Shore-LIJ Res Inst, Manhasset, NY.

European population genetic substructure was examined in >1000 subjects of European descent, each genotyped with >300K SNPs. Both STRUCTURE and principle component (PC) analyses showed the largest division/vector differentiated northern from southern European ancestry. A second vector using PC further separated Italian, Spanish and Greek subjects from those of Ashkenazi Jewish ancestry as well as distinguishing among northern European populations. In separate analyses of "northern" European subjects other substructure relationships were discerned e.g. Irish subjects were distinguished from those of eastern and northern European descent. [Eigen values (mean +/- SD) for 4 grandparent defined subjects showed: Eastern European, -.096 +/- .013; Swedish, -.054 +/- .017; German, -.040 +/- .016; United Kingdom, .016 +/- .025; Irish, .055 +/- .013] Additional studies defined European substructure ancestry informative markers (ESAIMs). A robust set of 1400 ESAIMs for identifying north/south European ancestry was developed using selected subject subsets. The STRUCTURE results (K = 2) from subjects with 4 grandparental data (not used for ESAIMs selection) showed clear separation of self-identified subjects Ashkenazi Jewish heritage (mean 83% south; median, 87%) from 37 subjects of Western, Northern or Central heritage belonging to the "northern" group (mean 4% south; median, 3%), and 51 subjects of Greek, Italian, or Spanish origin were intermediate (mean south, 41%; median, 42%). The mean individual 90% Bayesian confidence interval (CI) using these 1400 ESAIMs was 12.7%. Smaller ESAIMs sets showed strong correlations with the 1400 set e.g. 384 ESAIMs, r2 = .970, CI = 17.2%. Additional studies are defining ESAIMs to ascertain and control for other differences in European substructure. The results provide further insight into European population genetic substructure and also demonstrate that ESAIMS can be used for improving type 1 and type 2 error rates in association testing of candidate genes and in replication studies of WGA scans including examples from rheumatoid arthritis studies.

1379/T

Geospatial variation in the Human Leukocyte Antigen (HLA) system in the United States. *E.P. Williams, M. Maiers, L. Gragert.* Bioinformatics, National Marrow Donor Program, Minneapolis, MN.

We examined geospatial variation in HLA alleles, haplotypes, and phenotypes in the United States from a sample of 3.8 million volunteer donors in the National Marrow Donor Program (NMDP) registry. Each of the donors was typed for HLA-A, B, and DR at 2-digit resolution and linked with provided zip code and self-identified race and ethnicity (SIRE) information. Within the broad SIRE categories, variation in many HLA alleles and haplotypes is both significant and measurable, indicating a differing ancestral makeup in different US regions. We used the coefficient of variance statistic across regions of the United States to identify the strongest genetic clines in frequency and identify the areas that are the most unique genetically. Combining several genetic clines for a set of alleles and haplotypes can identify US regions of strong genetic similarity. For populations not native to the North America (European-Americans, Hispanics, Asian-Americans, African-Americans), geospatially-variable immigration trends to the US may be derived in the future with the aid of geospatial HLA frequency patterns from their ancestral countries. Signatures of substructure within the indigenous North American population especially can be seen. For example, the HLA-DR14 type has a coefficient of variation of 0.814 across states in Native Americans with a strong negative gradient eastward over the Rocky Mountains, while the coefficient of variation is 0.072 for European-Americans, 0.305 for Hispanics, 0.128 in African-Americans, and 0.138 in Asian-Americans.

1380/F

A large extended Newfoundland family with nonsyndromic sensorineural hearing loss is a third family found to be linked to Xp21.2 (DFN4). *N. Merner, K. Richardson, E. Ives, A. Griffin, T.L. Young.* Discipline of Genetics, Memorial University, St. John's, NL, Canada.

X-linked deafness is rare accounting for only 1% of all hereditary deafness cases. To date there have been four loci identified, namely DFN2, DFN3, DFN4 and DFN6. Of these, only one gene, POU3F4 in DFN3 has been identified. Two extensive Newfoundland families with nonsyndromic sensorineural hearing loss show an x-linked pattern of inheritance. Affected individuals experience a progressive type of sloping hearing loss that is moderate to profound in severity. Obligate female carriers show variable expression. Both families trace back to an isolated area on the province's north-east coast and share several surnames, thus we believe that they share a common ancestor. Combined there are 50 affected individuals over 6 generations with several consanguinity loops. The entire X chromosome was genotyped using 18 markers from panel 28 of the ABI Genome Wide Linkage Kit (v2.5). Hearing loss was linked to DXS1214 (Xp21.2), the DFN4 locus. After fine mapping, a deafness-associated haplotype was identified in affected individuals spanning 1.3 Mbp, from markers DXS992-DXS1219. Within this region there were 3 annotated genes, namely TAB3, FTHL17 and DMD. All coding exons and intron-exon boundaries were sequenced using ABI BigDye Terminator v3.1 cycling sequencing kit. The centromeric boundary had a crossover in the middle of DMD between markers DXS997 and DXS1219 therefore only exons 49-79 of the largest DMD isoform and the non-overlapping exons of isoforms Dp71 and Dp116 were sequenced. As well, because intragenic rearrangements account for mutations in the DMD gene, MLPA was performed to detect potential duplications and/or deletions. After analysis no pathogenic variants were found. However, the critical region was narrowed to approximately 800Kb. The most likely candidate is the DMD gene because two other families have been previously reported to be linked to the DMD locus and the mdx mouse model has been shown to have auditory dysfunction.

1381/F

Goldsurfer2: A comprehensive tool for the analysis and visualization of whole genome association studies. *F. Pettersson[1], A.P. Morris[1], M.R. Barnes[2], L.R. Cardon[1].* 1) Dept Bioinformatics, Wellcome Trust Centre, Oxford, United Kingdom; 2) Molecular Discovery Informatics, GlaxoSmithKline Pharmaceuticals, Harlow, Essex, United Kingdom.

With recent advances in the efficiency of high-throughput single nucleotide polymorphism (SNP) genotyping technology, genome-wide association studies are now routinely undertaken with the sample sizes necessary to detect the modest genetic effects we expect for complex diseases. There is now a clear demand for efficient analysis tools that can deal with the sheer volume of data generated by these experiments. To meet these demands, we have developed Goldsurfer2, an interactive and user-friendly graphical application to be used in all steps of WGA projects from initial data QC and analysis to biological evaluation and validation of results. The program is implemented in Java and can be used on all platforms. Basic statistical calculations, such as simple tests of SNP association with disease, genotyping failure rates, allele frequencies and Hardy-Weinberg disequilibrium are built in. However, methodology for more complex analysis techniques can be added to the platform. Recently added methods include multivariate statistics such as principal component analysis applied for investigating population stratification and higher-level LD structures. Evaluation of results may involve further statistical analysis, such as reviewing raw genotyping intensities for potential genotyping errors. Finally significant associations need to be prioritised using functional and biological interpretation methods, browsing available biological annotation, pathway information and patterns of linkage disequilibrium. For these tasks there are built in functionalities for importing data, performing calculations and comparative visualisation of results. The interface has been updated for easy switching between a gene centric and SNP centric view of data. For example genes can be selected based on their functional annotation and can be further used for prioritising SNPs and vice versa. For eQTL studies normalized gene expression data can be imported and analysed. The software can be downloaded from www.well.ox.ac.uk/gs2.

1382/F

Suggestive linkage of Brachydactyly Type A3 to 7q36. *K.D. Williams[1], J. Blangero[2], C.R. Cottom[1], S. Lawrence[1], J. Subedi[3], B. Jha[3], T. Dyer[2], J.L. VandeBerg[2], S. Williams-Blangero[2], B. Towne[1].* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Miami University, Oxford, OH; 4) Tribhuvan University Institute of Medicine, Kathmandu, Nepal.

Brachydactyly Type A3 (BDA3) is characterized by a short and broad middle phalanx of the fifth digit. A high prevalence of BDA3 has been observed among children in the Jiri Growth Study, a genetic epidemiological study of child health conducted in the endogamous Jirel ethnic group of eastern Nepal. A hand-wrist x-ray is taken annually of each child to assess skeletal development. The most recent radiographs of 1,357 Jirel children, adolescents, and young adults (676 boys; 681 girls) aged 3-20 years were examined for the presence or absence of BDA3. The overall prevalence of BDA3 in this sample was 10.5% (12.9% of the males and 8.9% of the females were classified as BDA3 affected). An initial whole genome linkage scan was performed on a subset of 426 individuals who each have been genotyped for ~400 autosomal markers. A variance components-based linkage analysis method was used to analyze these data and obtain multipoint LOD scores. The additive genetic heritability of BDA3 was highly statistically significant in this sample ($h^2 \pm SE = 0.87 \pm 0.16$, $p<0.0001$). Suggestive linkage was found of BDA3 to markers on chromosome 7q at 177 cM between 7q36.2-7q36.3 (LOD score = 2.00). A possible positional candidate gene near this region is Sonic Hedgehog (*SHH*), which has an important role in embryo formation. Mutations of *SHH* in mouse models have produced limb and digit dysmorphologies, and in humans, developmental disorders that include a short and broad middle fifth phalanx of the fifth digit as part of their etiology have been mapped to the 7q36 region. This study is the first to report linkage results for BDA3, and the first to suggest that either *SHH*, or genes near *SHH* working individually or in concert with it (or with other genes in the region), may contribute to this specific skeletal anomaly. Supported by NIH grants F32HD053206, R01HD40377, R01AI37091, R01AI44406, and R37MH59490.

1383/F

Mapping a new candidate locus for autosomal dominant partial epilepsy with auditory features. *F.R. Torres[1], E. Bilevicius[2], R. Secolin[1], N.F. Santos[1], E. Kobayashi[2], L.A.C. Sardinha[2], F. Cendes[2], I. Lopes-Cendes[1].* 1) Depatment of Medical Genetics, UNICAMP, Campinas, São Paulo, Brazil; 2) Department of Neurology, UNICAMP, Campinas, São Paulo, Brazil.

Mutations in the leucine-rich glioma inactivated 1 gene (*LGI1*) have been indentifed in only 50% of the families with autosomal dominant partial epilepsy with auditory features (ADPEAF). The objective of this study was to search for the alternative locus for ADPEAF in a family with no mutations in *LGI1*. We studied one large family with 23 affected individuals. Clinical evaluation with neurological exam, electroencephalogram (EEG) and magnetic resonance imaging (MRI) studies were performed. We genotyped 45 individuals of this for 350 microsatellites markers from the ABI PRISM®Linkage Mapping Sets v2.5 kit. We assumed an autosomal dominant inheritance with 80% penetrance for the parametric linkage analysis. Characteristic auditory auras were observed in 18/23 (78%) patients. *Déjà-vu* phenomena was identified in 13/18 (72%) patients. Isolated *déjà-vu* episodes were detected in three individuals and generalized tonic-clonic seizures were reported by two subjects. No MRI abnormalities were found in this family. Genome scan yield a maximum two-point lod score for a microsatellite marker localized on chromosome 1p36. There is an over-representation of the *déjà-vu* phenomena in this family, a clinical characteristic unusual in families with LGI1 mutation. The description of a new locus for ADPEAF confirms genetic and phenotypic heterogeneity in this syndrome.

1384/F

Genomewide-scan analysis of a multigenerational French Canadian family affected with intracranial aneurysms. *D.J. Verlaan, R. Gillis, J. St-Onge, A. Desjarlais, A. Noreau, G.A. Rouleau.* Centre de Recherche du CHUM, Universite de Montreal, Montreal, PQ, Canada.

Background: Intracranial aneurysms (IA) are dilations of intracranial arteries that occur most commonly at arterial bifurcations. Unruptured IAs are present in approximately 3-6% of the population aged older than 30 years old. Aneurysms are only rarely symptomatic unless they rupture, which typically results in a subarachnoid haemorrhage that is associated with high morbidity and mortality. The purpose of our study is to map a gene that predisposes to IA.

Methods: A multi-generational French Canadian family containing 11 affected individuals with IA was identified. Six affected cases (2 are reconstructable) and 8 unaffected individuals were sent for an 8 cM genomewide scan at deCODE (Reykjavik, Iceland). The disease segregation within the family was compatible with a Mendelian inheritance pattern, and a parametric LOD score approach was used to test for linkage. Multipoint LOD scores of the autosomes were calculated using GENEHUNTER version 2.1_r5 beta and two-point linkage for the X chromosome was calculated using MLINK from the FASTLINK 3.0P package. An affecteds-only approach was performed, using an autosomal dominant model, a phenocopy frequency of 0.01, a penetrance of 0.8, a disease allele frequency of 0.001 and deCODE allele frequencies.

Results: Preliminary multipoint analyses suggest six possible regions of linkage on chromosomes 3p26.3-1, 4p16.3-1, 5p14.3-q11.2, 7p22.3-21.3, 8p23.1-3, and 18q21.33-22.3. Except for the 5p14.3-q11.2, where an association with the *versican* gene was identified, none of these regions have previously been implicated in the pathogenesis of IA.

Conclusion: Further genotyping and collection of additional individuals will permit us to determine the inclusion or exclusion of these positive regions. Genotyping of additional FC families may also help us determine where this susceptibility locus lies.

1385/F

Interaction of blood pressure and LOC387715 SNP for risk of age-related macular degeneration. *K.E. Lee[1], R. Klein[1], B.E.K. Klein[1], C.L. Thompson[2], J. Capriotti[2], T. Joshi[2], S.K. Iyengar[2].* 1) Dept of Ophthalmology & Visual Science, Univ of WI Medical School, Madison, WI; 2) Dept of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Two genes, CFH and LOC387715, are known to cause age-related macular degeneration (AMD). High blood pressure (BP) independently increases risk for AMD. We examined a potential gene-environment interaction between BP and the A69S (G→T) variant of the LOC387715 SNP (LOC) for risk of incident late AMD and of incident retinal pigment epithelium depigmentation (RPEdepig), an early lesion of AMD. Family members within the population-based Beaver Dam Eye Study had 8 SNPs, including LOC, in the 10q26 region genotyped using a TaqMan assay (N=2230). Assessment of AMD was done using the Wisconsin ARM grading system. The mean of the two systolic BP measures from a random-zero sphygmomanometer (according to the Hypertension Detection and Follow-up Program protocol) will be used for this report. Discrete linear logistic regression was used to asses the 15-year cumulative incidence associated with a 20 mmHg increase in systolic blood pressure (SBP) from the baseline examination. Results are reported as odds ratio (95% confidence interval) from age-adjusted models. Ignoring genotype, the SBP20 risk for the incidence of late AMD is 1.21 (1.01, 1.46) and for the incidence of RPEdepig is 1.19 (1.04, 1.37). These associations are higher among those with the high risk genotype (T/T) for LOC. Risk of late AMD for SBP20 is 1.34 (0.75,2.40) among the 1012 with the nonrisk genotype (G/G), 1.35 (0.95,1.91) among the 601 heterozygote (G/T) and 2.74 (1.28,5.86) among the 81 T/T variants. The p-value for interaction is 0.20. Risk of RPEdepig for SBP20 is 0.83 (0.61,1.15) among those with G/G, 1.34 (1.00,1.81) among those with G/T and 2.94 (1.37,6.30) among those with T/T variants. The p-value for interaction is 0.005. These results remain after adjustment for smoking and body mass index. These data suggest that blood pressure control may have a larger benefit for reducing the risk of incident AMD among persons with theA69S (G→T) variant of the LOC387715 SNP.

1386/F

Analysis of T2D-Associated SNPs Identified from Whole Genome Association Studies in the IRAS Family Study: Replication Studies and Quantitative Trait Analysis. *N.D. Palmer[1], C. Langefeld[1], J. Ziegler[1], M. Goodarzi[2], J. Norris[3], S. Haffner[4], M. Bryer-Ash[5], R. Bergman[6], K. Taylor[2], J. Rotter[2], D. Bowden[1].* 1) Wake Forest Univ., Winston-Salem, NC; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) Univ. of Colorado, Denver, CO; 4) Univ. of Texas Health, San Antonio, TX; 5) University of California, Los Angeles, CA; 6) University of Southern California, Los Angeles, CA.

Recent advances have facilitated genome-wide association (GWA) studies that systematically search the genome for disease susceptibility loci. In this study we tested the most significant associations with type 2 diabetes (T2D) from 4 recent GWA studies and using quantitative trait analysis, assessed β-cell function (acute insulin response; AIR and disposition index; DI) and insulin sensitivity (S_I). Seventeen SNPs in 11 loci were genotyped on 1417 Hispanic Americans (HA) and 605 African Americans (AA) from the IRAS Family Study and analyzed for association with T2D and measures of glucose homeostasis from the FSIGT/MINMOD using SOLAR and QPDT. Association with T2D was observed in two regions: EXT2/ALX4 (chr. 11) (HA P<0.016) also associated with insulin secretion (AIR P<0.008), and IDE/KIF11/HHEX (chr. 10) (AA P<0.04) also associated with insulin secretion (AIR P<0.02). In HA, though not associated with T2D, a SNP in IGF2BP2 (chr. 3) was associated with the disposition index (P<0.004), fasting glucose (P<0.04); the two SNPs in CDKAL1 (chr. 6) were associated with AIR (P<0.005) and the SNP in LOC387761 (chr. 11) was associated with AIR (P<0.005) and fasting glucose (P<0.01). Little evidence of association was observed in AA. Loci with little association evidence with the traits examined include: PKN2 (chr. 1), FLJ39370 (chr. 4), SLC30A8 (chr. 8), CDKN2B/A (chr. 9), an intragenic region of chr. 11 and FTO (chr. 16). These results suggest some T2D susceptibility loci in the HA population that modulate T2D risk through variation in insulin secretion, but provide little support for association with T2D or glucose homeostasis in AA. Extensions of these initial studies are needed to definitively evaluate ethnic-specific susceptibility variants in these populations.

1387/F

A two stage association study on chromosome 2q34-37 and fibronectin 1 (FN1) gene in European American case-parent trios with nonsyndromic oral clefts. *J.W. Park[1], I. McIntosh[2], J.B. Hetmanski[3], E.W. Jabs[4], C.A. Vander Kolk[5], S.S. Chong[6], M.D. Fallin[3], R. Ingersoll[4], A.F. Scott[4], T.H. Beaty[3].* 1) Dept. Molecular & Cellular Biology, Sungkyunkwan School of Medicine, Suwon, Korea; 2) Dept. Medical Genetics, American Univ. of the Caribbean, St. Maarten, Netherlands Antilles; 3) Dept. Epidemiology, Johns Hopkins Univ., Baltimore, MD; 4) Dept. Genetic Medicine, Johns Hopkins Univ., Baltimore, MD; 5) Dept. Surgery, Johns Hopkins Univ., Baltimore, MD; 6) Dept. Pediatrics, National Univ. of Singapore, Singapore.

New candidate genes involved in nonsyndromic oral clefts, a common but complex group of birth defects, can be identified through a two stage design that combines fine-mapping of specific chromosomal regions and the subsequent study of candidate genes. This approach may be more cost-effective compared to genome wide screens. We identified a number of suggestive regions showing positive evidence for linkage and disequilibrium with fine mapping panels of 490, 229, 157 and 121 single nucleotide polymorphism (SNP) markers located in each of chromosomal regions: 2q34-37, 3p25-26, 5q31 and 5q35-qter, respectively, in 58 European-American case-parent trios from Maryland. As a second stage of study, a panel of 40 SNPs located near or in the fibronectin 1 gene (FN1 on 2q34) which showed the highest statistical evidence ($p=9x10^{-5}$) through the fine mapping on the 2q34-37 region was evaluated in 97 European-American trios using the transmission disequilibrium test (TDT). Evidence for transmission distortion was observed from either individual markers or sliding windows of haplotypes consisting of 2 to 5 SNPs (the lowest p=0.002). Intronic SNPs, rs12052402 (p=0.022) and rs724617 (P=0.005) in the FN1 and LOC646324 (2q35) genes, respectively, yielded the strongest statistical evidence among 73 trios with nonsyndromic cleft lip with or without palate (CL/P) and among 24 trios with cleft palate only (CP), respectively. While these results are consistent with the complex etiologic heterogeneity of nonsyndromic oral clefts, the contribution of FN1 to susceptibility of oral clefts will require further confirmatory studies.

1388/F

Further Fine-Mapping of 11 Candidate Genes for Schizophrenia in the Ashkenazim. *A.E. Pulver[1], V.K. Lasseter[1], M.D. Fallin[2], D. Avramopoulos[1], J.A. McGrath[1], P.S. Wolyniec[1], G. Nestadt[1], K.Y. Liang[3], P.-L. Chen[4], Y. Liu[4], D. Valle[4].* 1) Department of Psychiatry & Behavioral Sciences, The Johns Hopkins School of Medicine, Baltimore, MD; 2) Department of Epidemiology, the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Biostatistics, the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) Institute of Genetic Medicine, The Johns Hopkins School of Medicine, Baltimore, MD.

Previously,we reported family-based association analyses of 64 candidate genes for schizophrenia (SZ) and/or bipolar disorder (BPI) using Ashkenazi Jewish (AJ) parent/child triads (Fallin et al. 2005). With an average intermarker density of 11.9 Kb, 274 parent/child triads revealed 6 genes (RGS4, SCA1, GRM4, DPYSL2, NOS1, GRID1) with genotype relative risks significant at the p<.01 for SZ and 6 genes(DPYSL2, DTNBP1, G30/G72, GRID1, GRM4, NOS1) with overlapping suggestive evidence (p<.05) in both SZ and BPI triads. We report here follow-up SNP genotyping in a subset of 11 of the original 64 genes (CABIN1, DAOA, DPYSL2, DTNBP1, GFRA1, GRM4, KIF13A, NOS1, PNOC, RGS4, and SCA1) using an expanded sample of 409 AJ SZ cases and 395 screened AJ controls. We genotyped 1044 SNPs covering 2.2 Mb of sequence at an intermarker density of 2.1 Kb (5.6 times the original SNP density). Statistical comparisons of allelic and genotypic frequencies between cases and controls revealed nominal p<.01 for DPYSL2, SCA1, KIF13A, GRM4, GFRA1, and NOS1. The most significant findings are for the dihydropyrimidinase-related protein 2 gene (DPYSL2) on chromosome 8p21. Haplotype-based analyses using both LD-block-based haplotypes (p<.002) and sliding windows of 2-6 contiguous SNPs (p<.001) across the gene further characterize this susceptibility locus. These studies contribute to our understanding of genetic suceptibility for schizophrenia in the AJ population. Reference: Fallin MD, Lasseter VK, Avramopoulos D, Nicodemus KK, Wolyniec PS, McGrath JA, Steel G, Nestadt G, Liang KY, Huganir RL, Valle D, Pulver AE (2005) *Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am J Hum Genet 77:918-936* .

1389/F

Autosomal dominant multiple familial trichoepithelioma in a large consanguineous Bedouin family: linkage to chromosome 16q12-13. *H. Romi[1], A. Zvulunov[3], R. Ofir[1], K. Elbedour[2], O.S. Birk[1,2].* 1) The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva , Israele; 2) Genetics Institute, Soroka Medical Center, Beer-Sheva, Israel; 3) Schneider Children's Medical Center, Petah Tikva, Israel.

Multiple familial trichoepithelioma (MFT) is an autosomal dominant skin disease characterized by the presence of many small benign epithelial tumors with pilar differentiation predominantly on the face. The appearance of the lesions produces significant cosmetic distortion and causes much discomfort to the patients. A candidate MTF locus has been mapped to chromosome 9p21 in three north American families. Recently, mutations in the disease gene for familial cylindromatosis, the CYLD gene located on chromosome 16q12-13, have been shown to underlie MFT in four Chinese families. In order to identify the genetic defect causing autosomal dominant MFT in a large consanguineous Bedouin family in southern Israel, we initially performed linkage analysis with microsatellite markers from 9p21, ruling out linkage to this locus. Using microsatellite markers spanning the CYLD gene locus at 16q12-13, we genotyped all available individuals. Our results demonstrate linkage association to this region. Sequencing of the CYLD gene in the Bedouin patients is underway. These findings imply that CYLD defects are the cause of MFT in populations other than the Chinese.

1390/F

Analysis of Candidate Genes for Age-related Macular Degeneration on Chromosome 16p. *K.L. Spencer[1], L.M. Olson[1], P. Gallins[3], M.A. Hauser[2], S. Schmidt[2], W.K. Scott[3], N. Schnetz-Boutaud[1], A. Agarwal[1], E.A. Postel[2], M.A. Pericak-Vance[3], J.L. Haines[1].* 1) Ctr for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Ctr for Human Genetics, Duke University, Durham, NC; 3) Institute for Human Genomics, University of Miami, Miami, FL.

Age-related macular degeneration(AMD) is a complex disease of the central retina caused by both genetic and environmental risk factors. The association of Y402H in the complement factor H gene on chromosome (chr) 1, A69S in LOC387715 on chr 10 , and R32W of complement factor B on chr 6 with AMD have been well-documented. While Y402H and A69S increase risk for AMD, R32W provides protection from disease. Consistent with the results of several genomic screens, we also have evidence of another AMD susceptibility locus that resides on chr.16p. We genotyped 312 SNPs across chr. 16 in a dataset of 127 multiplex families as part of a genome-wide screen. In an independent dataset of 584 cases and 248 unrelated controls we genotyped a subset of 137 SNPs that are concentrated between 10-31 Mb to follow up on linkage results in this region. Based on these data and gene expression, we selected 4 candidate genes for further consideration: CACNG3, HS3ST4, IL4R, and Q7Z6F8. We genotyped ~10 additional SNPs per gene, and tested these SNPs for association in the families using "Association in the Presence of Linkage" (APL) and chi-square tests in the case-control dataset. Variants in CACNG3 and Q7Z6F8 were associated with AMD in both the family-based and case control datasets (smallest CACNG3 APL p=0.007, p=0.01 case-control; smallest Q7Z6F8 APL p=0.02, p=0.006 case-control). In addition to being associated with AMD in both datasets, rs757200 in CACNG3 was also strongly linked to disease (APL haplotype p=0.01, allelic p=0.046 in individuals who carry at least one risk allele at Y402H and A69S, two-point nonparametric LOD=3.34). Variants in CACNG3 and Q7Z6F8 may be associated with AMD, but confirmation of these findings in independent datasets will be necessary.

1391/F

Association of FOXP2 genetic markers with Procedural Learning and Language. *J.B. Tomblin[1], M.H. Christiansen[2], J.B. Bjork[3], S.K. Iyengar[4], J.C. Murray[3].* 1) Dept Speech Pathology and Audiology, U of Iowa, Iowa City, IA; 2) Dept Psychology, Cornell University, Ithaca, NY; 3) Dept Pediatrics, U of Iowa, Iowa City, IA; 4) Dept Epidemiology and Biostatistics, Iowa City, IA.

Procedural learning is an implicit mechanism for acquiring skills, unconsciously setting into memory task-oriented procedures without explicit knowledge of how the skill was acquired. Both Liegeous (2003) and Ullman and Pierpont (2005) have proposed that products of the FOXP2 gene influence procedural learning. These claims are based on language and neuroimaging data from members of the KE family, who have a point mutation in the DNA binding domain of FOXP2. To date, studies of FOXP2 have been limited to a few families. Further, studies using quantitative traits and other direct measures of procedural learning have not been associated with FOXP2. We used a Serial Recall Task (SRT), generally regarded as a measure of procedural learning and associated learning rates, to examine allelic variation among SNP markers within FOXP2. The participants were eighth-grade students (N=123). Stimuli comprised sequences of images presented in both random and predictable order. Participant response was measured by reaction time, and learning was reflected in decreased reaction time on the patterned trials. Genomic DNA from the subjects was used to evaluate a set of six SNPs selected to provide coverage of the principal haplotype blocks within the FOXP2 gene. A significant association was found between SNP variants and SRT learning rate for SNPs rs1916988 F (2, 821)=6.37, p<0.001, and rs7785701 F (2, 565)=3.32, p=0.037. In both cases the individuals with the CC genotype demonstrated poorer learning than those with the TT or heterozygote forms. Additionally, one other SNP (rs1005958) approached significance F (2, 925)=2.10, p=0.123. The rs1916988 SNP lies in the promoter region of FOXP2 and rs7785701 and rs1005958 are intronic SNPs. These results provide evidence that FOXP2 genotypic variants are associated with individual differences in the procedural learning system as measured by the SRT task. These results provide the first direct evidence of an association between FOXP2 and procedural learning.

1392/F

Portability of HapMap tagSNPs in 70 Asian Populations. *S. Xu[1,2], L. Jin[1,2].* 1) CAS-MPG Partner Institute of Computational Biology, SIBS, CAS, Shanghai 200031, China; 2) MOE Key Laboratory of Contemporary Anthropology and Laboratory of Theoretical Systems Biology, Institute of Genetics, School of Life Science, Fudan University, Shanghai 200433, China.

The International HapMap Project provides a database of SNP genotypes in four populations from which tag SNPs could be hopefully chosen to apply for linkage disequilibrium-based association studies in other populations. In PanAsian SNP Project, we generated genotype data in more than 70 Asian populations for 58,960 SNPs which distribute on 22 autosomes and X chromosome. We selected 180 regions where inter-marker distance are less than 6 kb/SNP and evaluated the portability of tag SNPs picked from HapMap samples to 70 Asian populations. In East Asian populations, the portability of common SNPs (MAF >0.05) selected from CHB and JPT (r^2>0.8) is generally more than 82%, but it can be as small as 70% in some of Southeast Asian populations. For Indian populations, the portability of common SNPs selected from CHB and JPT is less than 80%, but it can be increased to 85% or larger by using the tags from CEU. Our results indicate that HapMap data of CHB and JPT samples can be used to select tags for genome-wide association studies in many East Asian populations, but for some Southeast Asian populations, this strategy may not be sufficient.

1393/F

Familial Interstitial Pneumonia (FIP) is linked to Chromosomes 10, 11 and 12. *M.C. Speer[1], L.H. Burch[2], M.P. Steele[1,2], A. Herron[1], J.E. Loyd[3], K.K. Brown[4], J.A. Phillips III[3], A. Wise[2], S.H. Slifer[1], C.F. Potocky[1], M.I. Schwarz[4], D.A. Schwartz[1,2].* 1) Duke Univ Medical Ctr, Durham, NC; 2) National Institute of Environmental Health Sciences, Research Triangle Park, NC; 3) Vanderbilt University School of Medicine, Nashville, TN; 4) National Jewish Medical and Research Center and University of Colorado Health Science Center, Denver, CO.

The idiopathic interstitial pneumonias (IIP) are a clinically heterogeneous group of fibrosing interstitial lung diseases that lead to hypoxemic respiratory insufficiency with both genetic and environmental contributions to etiology. The most common IIP is usual interstitial pneumonia (UIP), the underlying histology of idiopathic pulmonary fibrosis(IPF). Typically, IPF (OMIM178500) presents in late life and is lethal within 4-5 years of diagnosis. Treatment options, apart from lung transplantation, are limited and do not appear to prolong survival. Identifying the genetic basis for this condition will lead to earlier identification and enhanced interventions. We performed a genomic screen using 890 microsatellite repeat markers spaced at an average 4.1 cM including 82 families with ≥ 2 members with probable/definite IIP. We identified a maximum multipoint lod score of 3.03 at D11S1318, incorporating a 16.4 cM region bounded by D11S4046 and D11S4149. A second linkage peak spans 15 cM and is bounded by D10S1751 and D10S1664 (maximum multipoint LOD score of 2.27 at D10S1649). Families with fewer than 67% smokers among affected individuals contributed significantly to evidence for linkage at 11pter (p=0.01; maximum lod = 4.58). The 82 families were subdivided into those with only IPF-type disease [homogeneous familial interstitial pneumonia - FIP] and families with ≥ 1 case of IPF and one other type of IIP [heterogenous FIP]. When considered alone, the homogeneous families identified a region of interest at D12S368 (maximum multipoint lod score 1.89). In summary, we identified regions on chromosomes 10, 11, and 12 that likely contain genes contributing to FIP. Moreover, our findings indicate that linkage on chromosome 11 is influenced by cigarette smoking and that linkage on chromosome 12 is influenced by disease phenotype.

1394/F

THE FIRST LATINO WHOLE GENOME ADMIXTURE SCAN, FOCUSING ON COLOMBIANS WITH TYPE 2 DIABETES. *F. Yu[1, 2], A. Price[1, 2], N. Patterson[1, 2], A. Waliszewska[1, 2], C. Schirmer[1, 2], J. Neubauer[1, 2], G. Bedoya[3], C. Duque[3], A. Villegas[3], A. Ruiz-Linares[3, 4], D. Reich[1, 2].* 1) Dept Genetics, Harvard Med Sch, Boston, MA; 2) Broad Inst. of MIT & Harvard; 3) Laboratorio de Genética Molecular, Universidad de Antioquia, Medellín, Colombia; 4) The Galton Laboratory, Dept. of Biology, Univ. College London.

The prevalence of type 2 diabetes (T2D) in Latino populations is much higher compared with populations of European ancestry. The history of recently admixed ancestries from different continents in Latinos makes "admixture mapping" a potentially powerful technology for finding T2D susceptibility loci in this population. With the recent success of admixture scans in African Americans for multiple sclerosis, prostate cancer, and markers of inflammation, the likelihood of success of whole-genome admixture scans in Latinos is all the greater. However, the application of admixture mapping to find genes in Latinos was not feasible until recently, when three groups including our own simultaneously published genome-wide admixture mapping SNP panels for Latinos (Mao et al. 2007, Price et al. 2007, and Tian et al. 2007). We took advantage of this opportunity and embarked on, to our knowledge, the first Latino whole genome admixture scan. Based on the three independent mapping panels, we selected a panel of 1536 SNP markers across the genome. Here, we will report the results of genotyping these SNPs in >1,000 Colombian T2D cases to screen for T2D disease genes. We will report the results of analyses, using our ANCESTRYMAP software, to search for genomic segments with increased ancestry from one of the ancestral populations, which can indicate the position of a disease locus. If there is a positive signal, a second stage fine mapping will be used for follow-up analysis.

1395/F

An international collaborative SNP-based whole genome linkage screen for high myopia. *Y-J. Li[1], A. Bulusu[1], R. Metlapally[1], F. Malecaze[2], P. Calvas[2], J.A. Guggenheim[3], D. Mackey[4], T. Rosenberg[5], S. Paget[2], P. Holmans[3], T.L. Young[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr,USA; 2) Toulouse Univ. Hospital, France; 3) School of Optometry and Vision Sciences, Cardiff Univ., UK; 4) Dept. of Ophthalmology, Univ. of Melbourne, Australia; 5) Gordon Norrie Ctr., Kennedy Inst. Nat'l Eye Clinic, Hellerup, Denmark.

Introduction: Myopia (nearsightedness) is a common complex disorder, and severe forms have implications for blindness due to increased risk of premature cataracts, glaucoma, retinal detachment, and chorioretinal degeneration. Multiple studies support a strong genetic component for myopic development. Several non-syndromic high-grade myopia loci have been mapped. The purpose of this study was to map new loci, refine existing locus intervals, and to identify associated genes for high myopia. **Methods:** A total of 6008 SNPs distributed genome-wide from the Illumina Linkage Panel IVb were genotyped. After screening for Mendelian and family relationship errors by PEDCHECK, RELPAIR and PREST programs, a collaborative 5-site international dataset of 249 multiplex high myopia families and 5880 SNPs was compiled. FASTLINK and MERLIN were used for 2-point and multipoint linkage analysis, respectively, for high myopia. Overall and center-specific datasets were evaluated. **Results:** FASTLINK revealed 15 SNPs on chromosomes 2, 5, and 12, with 2-point LOD scores > 2.0. The highest 2-point LOD score was 3.18 for rs581642 on chromosome 12. Parametric multipoint analysis showed a 23.5cM linkage region on chromosome 12 with all marker HLOD scores ≥ 2 (peak HLOD=3.48). This interval was also supported by center-specific analysis. Other significant multipoint linkage regions were found on chromosome 2 (with a peak HLOD= 2.25), and chromosomes 5, 9, and X with HLOD scores > 1.0. **Conclusion:** The present results confirmed previously reported high myopia mapped loci on chromosomes 2 (MYP12), 12 (MYP3), and X (MYP1), with potential new linkage regions found on chromosomes 5 and 9. Linkage analyses for refractive error and other stratified datasets are underway. This is the largest linkage screen to date for mapping risk loci for high myopia.

1396/F

STRs Markers DXS7424 and DXS101 are Useful on Carriers Female Disease Fabry in Colombian Families. *A. Lopez[1], S. Ospina[1,2], P. Paez[1], C. Duran[1], J.C. Prieto[1,3].* 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Cundinamarca, Colombia; 2) Universidad del Rosario, Bogota, Colombia; 3) Hospital la Victoria, Secretaria Distrital de Salud, Bogota, Colombia.

Fabry disease is an X-linked recessive disorder caused by the deficiency of the lysosomal hydrolase alpha-galactosidase A. Affected males are characterized by the clinic presentation of acroparesthesias, corneal opacities, angiokeratoma, hypohidrosis, renal and cardiac alteration. Its diagnosis is confirmed trough the demonstration of a deficiency of alpha-galactosidase activity in plasma and leukocytes. Carrier females can present some minor symptoms or being asymptomatic and the level of GALA are not correlated with the state of the carrier. The objective of this study was to detect of carriers making a haplotype analysis, applying STR flanking the GALA gene determining allelic frequencies and heterozigosity of the used markers then to compare with other ethnics groups. Methodology: We described 2 Colombian families that have diagnosis of this disease. For the population studied were utilized the markers DXS7424 and DXS101 which flank the GALA gene. Results: There were identified 12 alleles for marker DXS101 with a heterocigocity of 92.16%, PIC 0,884.; meanwhile for marker DXS7424 were identified 9 alleles, with a heterozygosity of 98%, PIC 0,780. Haplotipification of affected males, carriers females and relatives evidenced that affected males share the same haplotype of obligate carrier females. Discusion: Haplotype analyses trough the use of STRs, is a good alternative to identify the carriers where the enzymatic levels are normal. The markers DXS101 and DXS7424 are highly informative markers to the disease, because they present a high polymorphism and heterozygosity in the general population.

1397/F

Evidence that a high myopia locus maps to chromosome 12q. *C.P. Pang, C.Y. Lam, D.S.P. Fan, P.O.S. Tam, D.S.C. Lam.* Ophthalmology & Visual Sci, Chinese Univ Hong Kong, Hong Kong, HKSAR, China.

High myopia is defined as refractive error ≤-6.00 D. It affects more than 10% adult population in Hong Kong. Heredity is a major contributing factor of high myopia. While no myopia gene is known yet, 14 chromosomal loci were mapped and two candidate genes were suggested. The aim of this study was designed to evaluate the genetic component of Chinese high myopia pedigrees originating from Hong Kong. Whole genome scan was performed on 14 participants from a 3 generations autosomal dominant Hong Kong family by using the ABI MD-10 marker set with an average spacing of 10cM. Regions containing markers that yielded LOD scores > 1.0 were further analyzed by fine mapping in which additional microsatellite markers flanking the particular marker with the highest LOD score. Mutation screening of candidate gene was performed by direct sequencing of the gene. From whole genome scan, two point LOD score > 1 were observed on chromosomes 12. Region was further analyzed by additional microsatellite markers flanking the particular markers. A Maximum two point LOD score of 2.11 was obtained at marker D12S88 and suggested linkage region was narrowed at 12q22.2 by haplotype analysis in one pedigree. Lumican, which is located within this region, was screened and no segregation of polymorphism was observed within the pedigree. The mapped high myopia locus on chromosome 12 in this study overlapped with the reported MYP3 locus but with a smaller interval than the one reported. Lumican was excluded to be a candidate gene of high myopia. The results give evidence that unidentified genes will underlie high myopia in our Hong Kong Chinese pedigrees.

1398/F

Evidence and characterisation of a colorectal cancer susceptibility locus on chromosome 3q22 from a high-density SNP genome-wide linkage scan. *E. Papaemmanuil[1], Z. Kemp[2], E. Webb[1], L. Carvajal-Carmona[2], W. Wood[1], E. Barclay[2], M. Gorman[2], I. Tomlinson[2], R. Houlston[1].* 1) Molecular population genetics, The Institute of Cancer Research, Sutton, Surrey, United Kingdom; 2) Molecular and Population Genetics, London Research Institute, Cancer Research UK, London, United Kingdom.

Germline mutations in *APC*, DNA mis-match repair genes, *MutYH, SMAD4, ALK3* and *STK11* contribute to inherited susceptibility to colorectal cancer (CRC). Colorectal tumor families which show evidence against linkage to known loci and from kindreds who fulfil the clinical (Amsterdam) criteria for Hereditary non polyposis colorectal cancer (HNPCC) but whose CRCs do not show microsatellite instability (MSI), provide evidence for the existence of uncharacterized high/moderate-penetrance CRC genes. Such observations strongly support the continued search for novel CRC predisposition genes through genome-wide linkage searches.

To identify novel colorectal cancer susceptibility genes through linkage analyses we have been analyzing CRC families that segregate microsatellite stable (MSS) cancers in which involvement of known susceptibility genes has been excluded. Our analysis is being based on the use of high-density SNP arrays that provide maximal power to detect linkage and allow the incorporation of genotyping information from additional families when they become available. Based on the analysis of 69 families we have shown evidence for a new susceptibility locus on chromosome 3q22. To clarify the impact of this locus on CRC susceptibility we have analyzed an additional series of 32 families. Data on a combined analysis will be presented.

1399/F

NPL analysis in 3-generation Brazilian families multipli-affected with aggressive and chronic periodontitis. *G.E. Rapp[1], A. McQuillan[2], B. North[3], P. Brett[2], M.S. Tonetti[4].* 1) Clinical Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil; 2) University College of London, United Kingdom; 3) Imperial College of London, United Kingdom; 4) European Research Group on Periodontology, Italy.

Background: Periodontitis is a multifactorial inflammatory disease, presenting a rare aggressive (AgP) and a more common chronic form (CP). Both forms compromise few or several teeth and may lead to tooth loss. A genetic susceptibility has been shown for various disease phenotypes in several populations around the world. The aim was to test linkage of polymorphisms in candidate genes to periodontitis on a set of 3-generation Brazilian families. Material and Methods: Three large pedigrees were selected after confirmed diagnoses of AgP in the proband. A 6 site/tooth full-mouth probing was performed by a calibrated examiner in the 58 pedigree members (all non-smokers). The phenotype was defined based on questionnaire (total edentulisms) and clinical attachment loss ≥ 4mm due to pocketing in at least 4 sites of different teeth, what includes CP. In total, 17 were considered affected, 26 non-affected and 15 with unknown diagnosis because of low age (≤ 14 years). SLINK simulation method (AD; E=0; F= 0.98, 0.75, 0.5; P=0, 0.02) showed maximum expected lod scores of 9.67 in all pedigrees. A multipoint NPL to D1S1595, FcG3A, FcG3B65, FcG3B36, D1S1679, D7S1802, IL6-1750, IL6-1363, IL6-572, IL6-174, D7S1802, IL13954, VDR-312 was tested using Simwalk 2.91. Results: The most significant values were found for D1S1595 (p=0.0157) and D1S1679 (p=0.0124). Conclusions: Bearing in mind the unknown underlying genetic model of periodontitis, the findings provided interesting positional information on human chromosome 1 for future additional genomic screening. A possible common genetic background of both studied clinical forms of periodontitis may exist. Ministry of Education of Brazil CAPES 2622/03-3.

1400/F

The Future is Now. Will the Real Disease Gene Please Stand Up? *M. Schmidt, E. Martin.* Human Genomics, University of Miami, Miami, FL.

In 1996, Risch and Merikangas touted the transmission/disequilibrium test (TDT) as the future of gene mapping for complex diseases. They suggested a million-marker screen affected sibpair families (ASPs), demonstrating that the TDT was a more powerful test of linkage than traditional linkage tests based on allele-sharing when the marker is also associated with the disease locus. While the future of genotyping has arrived, successes in family-based association studies have been modest. This is often attributed to excessive false positives in candidate gene studies. This problem is only exacerbated by the increasing numbers of whole genome association (WGA) screens. When applied in ASPs, the TDT statistic, which assumes transmissions to siblings are independent, is not expected to have a constant variance in the presence of linkage. This results in more extreme statistics which further aggravates the problem of having high levels of type I error. So an important question is how many positive TDT results will show up on a chromosome containing a disease gene due only to linkage, and will they obfuscate the true disease location. To answer this question we combined theory and computer simulations. Our studies show that in ASPs the normal version of the TDT statistic has a mean of 0 and a variance of 1 in unlinked regions, but has a variance larger than 1 in linked regions. The PDT statistic adjusts for correlation between siblings due to linkage and maintains a constant variance of 1 at unassociated markers irrespective of linkage. The TDT statistic is generally larger than the PDT statistic across linked regions for unassociated as well as associated markers. As fair comparison the scores of both statistics were ranked. TDT did a slightly better job placing the associated marker near the top. Though, strictly speaking, the TDT in ASPs should be interpreted as a test of linkage and not a test of association, there is a good chance that if a marker stands out, the marker is associated as well as linked. In conclusion, our results suggest that TDT is an effective screening tool for WGA, especially in multiplex families.

1401/F

Progress towards the genetic characterization of psoriasis and atopic dermatitis susceptibility within the EDC. *C. Sinibaldi[1], N. Paolillo[1], E. Giardina[1], C. Peconi[1], T. Lepre[1], S. Nisticò[2], G. Novelli[1,3].* 1) Department of Biopathology,Tor Vergata University of Rome,Italy; 2) Department of Dermatology, Tor Vergata University, Rome, Italy; 3) Department of Cardiovascular Medicine, University of Arkansas for Medical Sciences, Little Rock, AR,USA.

Atopic dermatitis (ATOD) and psoriasis (PS) represent common chronic inflammatory skin diseases triggered by both genetic and environmental factors. Linkage studies performed in PS/ATOD families revealed a significant linkage to the epidermal differentiation complex (EDC)locus on chromosome 1q21.We refined the susceptibility region for PS (PSORS4) and ATOD (ATOD2) to a 42 kb interval. Recently, it has been reported that loss-of-function mutations of an independent gene (FLG) located in the EDC are associated with ATOD in many populations. On the basis of these findings we performed an association study both to identify the susceptibility variant/haplotype in PSORS4/ATOD2 locus and in order to disclose a potential contribution of FLG mutations to genetic susceptibility of PS and ATOD in Italian populations. Data generated in two cohorts of 100 PS and 80 ATOD Italian trios, revealed three distinct associated haplotypes within the PSORS4-ATOD region: Hap1 region (9.7 kb),generated significant association in both the diseases (PS p=0.0229; ATOD p=0.0077), the second, Hap2 (8.8 kb) showed association in the ATOD cohort (p=0.0257); the third, Hap3 (38.5 kb) generated significant p-value in the PS cohort (p=0.0270). On the basis of these findings we selected an additional panel of 15 SNPs spanning the three associated regions and typed a newly recruited cohort of 310 PS patients, 210 healthy controls and 130 ATOD trios. Preliminary data confirm the association observed in our first run of genotyping and suggest the existence of a master susceptibility locus for both the diseases. Genotyping of FLG mutations (R501X and 2281del4) failed to reveal evidence of association in ATOD and PS patients. These results support the independence of PSORS4 and ATOD2 genetic susceptibility from known FLG mutations in Italian patients. This work was supported by A.DI.PSO (Italian Association for the Defense of Psoriatic Patients).

1402/F

Linkage of cross-sectional and longitudinal measures of cystic fibrosis lung disease severity to chromosome 5q. *L.L. Vanscoy[1], S.M. Blackman[1], J.M. Collaco[1], L. Bremer[1], R. Dorfman[2], P. Durie[2], J. Zielenski[2], K. Naughton[1], A. Bowers[1], G.R. Cutting[1].* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Hospital for Sick Children, Toronto, Canada.

Affected twins and siblings demonstrate that modifier genes are major contributors to variation in lung disease severity in cystic fibrosis (CF). To identify regions encompassing these modifiers, we performed genome wide linkage analysis on 683 siblings with CF (360 families). Lung disease severity was defined using forced expiratory volume in 1 second (FEV1), a quantitative measure highly correlated with survival. To facilitate comparison of patients, lung function measures were converted to disease-specific percentiles. The best CF-specific %ile for FEV1 within the last year of available data (MaxFEV1CF%ile) was used as a cross-sectional measure. The lifetime average CF-specific %ile for FEV1 (AvgFEV1CF%ile) and the estimated percent-predicted FEV1 at age 20 (EstFEV1%pred@20yrs) were used as longitudinal measures. Short tandem repeat markers were typed in all affected individuals and their parents (Marshfield Genotyping Center: 402 markers or DeCode: 1030 markers). Two-point and multipoint linkage analyses using Sequential Oligogenic Linkage Analysis Routines (SOLAR) revealed linkage of all 3 lung phenotypes to chromosome 5. Peak multipoint LOD scores on chromosome 5 occurred at 196 cM for MaxFEV1CF%ile and AvgFEV1CF%ile (LOD 3.0 and LOD 3.4, respectively) and at 191 cM for EstFEV1%pred@20yrs (LOD 2.8). Single point LOD scores on chromosome 5 peaked at marker AAT072 (3.3 for MaxFEV1CF%, 3.4 for AvgFEV1CF%, and 1.88 for EstFEV1%pred@20yrs). The region of linkage encompasses approximately 6 megabases near the telomere of chromosome 5q. Linkage to one or two lung phenotypes was observed on chromosome 1 (GATA12A07N, LOD 4.5 for EstFEV1%pred@20yrs) and chromosome 14 (GGAA30H04ZP, LOD 2.33 and GGAA4A12, LOD 2.13 for MaxFEV1CF% and EstFEV1%pred@20yrs, respectively). Linkage of the 3 quantitative pulmonary traits to chromosome 5 suggests that this region encompasses genetic modifiers of CF lung disease severity.

1403/F

Identification of a novel autosomal dominant limb-girdle muscular dystrophy. *N.H. Wang[1], Y.W. Yang[2], H.W. Chen[1], C.H. Chen[1], Y.T. Chen[1], J.Y. Wu[1].* 1) IBMS, Academia Sinica, Taipei, Taiwan, Taiwan; 2) Dept of Neurology, China Medical University Hospital, Taichung, Taiwan.

The limb-girdle muscular dystrophies (LGMD) comprise a clinically and genetically heterogeneous group of muscle disorders with shoulder and pelvic girdle muscles weakness. Clinical course in this heterogeneous group has great variability, ranging from severe forms with various onset age and variable rate of progression. Onset age of LGMD occurs from the late first decade to middle. Early symptoms include difficulty walking, running, standing up from a squatting position, raising arms above the head, and carrying heavy things. The weakness of limb girdle muscles is progressive and the rate of progression is greatly variable. Variation seen in the LGMDs is caused by the mutations in different genes or the different changes within the same gene. These differences can lead to more severe or milder forms of LGMD. During the past decade, through molecular genetic discoveries and improved clinical criteria, more than 14 genes/loci responsible for limb girdle muscular dystrophy (LGMD) have been mapped, including eight types of autosomal recessive LGMD (AR-LGMD) and six types of autosomal dominant LGMD (AD-LGMD). A four-generation family with AD-LGMD was identified in Taiwan. Characteristic muscle weakness predominantly involving the pelvic and shoulder girdle proximal muscles was shown in 11 individuals. Age at onset ranges from 10-40. Cardiac involvement, calf hypertrophy, and contractures are not observed in these affected individuals. Linkage analysis to chromosomes 5q31, 1q11, 3p25, 6q23, and 7q demonstrated that this disease is not allelic to LGMD forms 1A, 1B, 1C, 1D, and 1E. Genome wide linkage-analysis using 384 markers was performed and no marker with LOD score larger than 2 was identified. A 10K SNP oligoarray was also used for linkage analysis. Two chromosome regions (about 8 Mbp and 3.6 Mbp, respectively) with LOD score larger than 4 were identified. Those two regions were not one of the previous loci identified to be either autosomal dominant or autosomal recessive LGMD. A new gene is probably involved in the autosomal dominant limb girdle muscular dystrophy.

1404/F

Replication and refinement of the chromosome 12q MYP3 locus in an international high myopia family cohort. *T.L. Young[1], A. Bulusu[1], R. Metlapally[1], F. Malecaze[2], P. Calvas[2], J.A. Guggenheim[3], D. Mackey[4], T. Rosenberg[5], S. Paget[2], P. Holmans[1], Y-J. Li[1].* 1) Center for Human Genetics, Duke U. Med. Ctr., USA; 2) Toulouse U., France; 3) School of Optometry and Vision Sciences, Cardiff U., Wales; 4) Dept. of Ophthalmology, U. of Melbourne, Australia; 5) Gordon Norrie Ctr., Kennedy Inst. Nat'l Eye Clinic, Hellerup, Denmark.

Introduction: Severe myopia (nearsightedness of > -6 diopters) predisposes individuals to retinal detachment, chorioretinal degeneration, cataract, and glaucoma. Multiple myopia-risk genetic loci have been reported. This is a whole genome linkage study of a large high myopia family dataset focused on the chromosome 12q MYP3 locus. Methods: A 5-site international collaboration of 249 multiplex high myopia families (at least 2 affected individuals per family) dataset was compiled. Whole genome SNP genotyping was performed using 6008 SNPs. Assuming a dominant affected-only model, 2-pt and multipoint linkage analyses were performed using the FASTLINK and MERLIN programs, respectively. Analyses were performed on overall and center-specific datasets. Results: The most significant linkage region was found on chromosome 12 with 2-point LOD scores > 3.0 for markers rs581642 (53.89cM) and rs1849929 (112.37cM). Parametric multipoint analysis revealed a tighter significant linkage interval of 23.5cM centered at marker rs337663 (101.97cM, HLOD=3.48). Non-parametric linkage analysis showed four suggestive linkage regions with LOD scores > 1.0 (peaks at 56.41cM, 73.95cM, 103.49cM, and 138cM). The parametric linkage region (101.97cM) overlaps with the 3rd non-parametric linkage region (103.49cM), and the Duke and Hellerup family sets are primary contributors to this interval. The Toulouse and Cardiff family sets primarily contribute to the 2nd and 4th non-parametric linkage peaks, respectively. Conclusion: This is the first SNP-based whole genome linkage screen using a large, international family dataset to determine loci for severe myopia. Heterogeneity exists among centers, and the best consensus linkage region is near 101.97cM, which replicates previous MYP3 locus microsatellite analyses. The MYP3 locus was contracted to a 23.5cM region.

1405/F

Association between IBD and the TL1A/DR3 ligand/receptor pair. *L. Mei[1], X. Su[1], K. Taylor[1], S. Targan[2], J. Rotter[1].* 1) Dept Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) IBD Center, Cedars-Sinai Medical Ctr, Los Angeles, CA.

Background: The TNF-like cytokine, TL1A, binds to the death domain receptor (DR3), and induces NFKB1 expression in Th1 cells. Up-regulation of TL1Aand DR3 is related to the gut inflammation characteristic of Crohn's disease (CD). Since TL1A was recently identified as a CD susceptibility gene by genome-wide association and confirmed by our group, our aim was to investigate whether a genetic interaction between TL1A and DR3 contributed to CD. Method: Eight DR3 and 5 TL1A SNPs were genotyped in 763 CD, 351 ulcerative colitis (UC) and 254 controls. Haplotype blocks were constructed by Haploview; individual haplotypes were assigned by PHASE and ordered by frequency; associations were tested by chi-square and permutation. Gene-gene interaction was tested by logistic regression. Results: Two major haplotypes of DR3 were associated with CD. In non-Jews, CD patients had a lower frequency of homozygotes of H1 (66.2% vs. 76.7%, p=0.007) and a higher frequency of H2 carriers (13.1% vs. 7.5%, p=0.035) when compared with controls; however, this association was absent in Jewish CD. In non-Jewish UC, a similar trend of association for H1 and H2 was also observed, though it was not statistically significant. H2 of TL1A has been reported to be negatively associated with CD (39% vs. 50%) and UC (37.3% vs. 50%) recently by our group, and this effect was also seen only in non-Jews. When analyzing DR3 and TL1A together, a significant dose-effect was observed among protective factors (DR3 H1 and TL1A H2) in non-Jewish IBD (p trend <0.0001), odds ratio ranging from 1 to 0.47 (1 protective factor) to 0.19 (both protective factors). No statistical interaction was detected between these two genes. Conclusion: The DR3 association observed supports that idea that the TL1A/DR3 interaction contributes to CD pathogenesis. "Hits" from genome-wide association studies will identify pathways that may contain other genetic determinants of complex traits.

1406/F

"Hits" from a whole genome associations lead to pathways with substantial genetic contribution to complex traits. *K. Taylor[1,2], S. Targan[2], L. Mei[1], X. Su[1], A. Ippoliti[2], E. Mengesha[1], L. King[1], K. Papadakis[2], J. Rotter[1,2].* 1) Medical Genetics Institute, Cedars Sinai Medical Ctr, Los Angeles, CA; 2) Inflammatory Bowel Disease Center, Cedars Sinai Medical Ctr, Los Angeles, CA.

A genome-wide association study identified IL23R as a Crohn's disease (CD) susceptibility gene and recent evidence supports a role for the IL17-IL23 pathway, and Th17 cells, in immune disorders including CD. Our aim was to examine the genetic contribution of this pathway to CD susceptibility. **Methods:** 763 CD subjects and 254 controls were genotyped for SNPs in IL17-IL23 pathway genes: IL23A, IL23R, IL17A, IL17RA, IL12B, and IL12RB1; haplotypes were assigned using PhaseV2; and association was tested by chi square and permutation. Synergy, defined as CD risk in excess of that of each individual gene, was tested by logistic regression. **Results:** IL23R, IL17A, IL17RA, and IL12RB1 "risk" and "protective" haplotypes contribute substantially to CD risk as shown by association and by high population attributable risk (PAR; IL23R, 19%; IL17A, 15%; IL17RA, 10%; IL12RB1, 40%). The OR for CD increased with the number of "risk" haplotypes (OR =1 for 0-1 "risk" haplotype, 1.3 for 2, 2.5 for 3, and 4.0 for 4, p<0.0001). Synergy was observed between IL23R and IL17A and between IL23R and IL17RA (OR=1 for the IL23R or IL17A "risk" haplotype alone, 2.4 for both, p=0.047 for interaction; OR ~1.1 for IL23R or IL17RA "risk" alone, ~3.0 for both, p=0.036). In contrast no synergy was observed with other CD susceptibility variants (CARD15, ATG16L1, PHOX2B, OctN, 10q, FAM92B or NCF4). **Discussion:** Substantial CD susceptibility was contributed by genes of the IL17-IL23 pathway together, beyond that identified by genome-wide association. Furthermore, IL23R CD susceptibility required the presence of a "risk" haplotype from either IL17A or IL17RA. The lack of a synergistic interaction with common CARD15 mutations supports the hypothesis that the IL17-IL23 pathway and CARD15 act separately to increase CD risk. These observations further suggest that "hits" from genome-wide association studies will lead to pathways harboring genes that, in combination and in interaction, substantially contribute to human complex traits.

1407/F

Complete Genomic Screen in Familial Parkinson Disease. *G.M. Mayhew[1], Y. Liu[2], M.A. Hauser[2], Y.J. Li[2], R. Jewett[1], J. Stajich[2], E.R. Martin[1], J.M. Vance[1], W.K. Scott[1].* 1) Miami Inst Human Genomics, Miller School of Med, Univ of Miami, Miami, FL; 2) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Many whole genome screens (WGS) for loci linked or associated with Parkinson disease (PD) have been performed, with only a small amount of agreement across studies. Despite that, most confirmed gene associations in PD lie within these linkage regions. Therefore, to attempt to further identify genes important to PD, we expanded our initial WGS performed in 2001 (174 families, 356 microsatellite markers) with a second WGS in an augmented dataset of 302 multiplex families (2 or more sampled individuals with PD) using a denser map of 6008 single nucleotide polymorphisms (SNPs; average spacing 0.62 cM). The families contained 1505 sampled members (669 affected), 248 sampled affected sibling pairs and 175 other sampled affected relative pairs. Mean age at onset was 59.7±13.2 years. Linkage analysis using dominant and recessive affecteds-only models identified two novel regions. In addition to having two-point MLOD scores greater than 2, one of these regions (on chromosome 3) generated a multipoint MLOD score greater than 2, making it the most interesting region in the screen. A second novel region of strong interest (on chromosome 18) had markers with both two-point and multipoint MLOD scores greater than 1.5. These results implicate two additional genomic regions for follow-up studies and extend the picture of genetic heterogeneity that characterizes studies of the late-onset, complex form of PD.

1408/F

Admixture Genome Scan for Loci Involved in Cleft Lip. *L.M. Moreno[1], E.W. Pugh[2], M. Moreno[3], M. Arcos-Burgos[4], C. Valencia-Ramirez[3], M.L. Marazita[5], J.C. Murray[1], A.C Lidral[1].* 1) Univ of Iowa, Iowa City, IA; 2) CIDR, Baltimore, MD; 3) U. of Antioquia, Medellin, Colombia; 4) NIH,Bethesda, MD; 5) U of Pittsburgh, Pittsburgh, PA.

Introduction: The prevalence of nonsyndromic cleft lip with or without cleft palate (CL/P) varies by ancestry and is highest among Amerindians and Asians followed by Caucasian and African populations. Admixture mapping can identify genomic areas containing disease loci that are linked to ancestry markers. Purpose: We performed the first genome wide admixture mapping to identify disease loci for CL/P. Methods: 162 affected probands and 52 controls from North West Colombia were genotyped by the Center for Inherited Disease Research for 385 STRPs. 86 individuals from the Human Diversity Panel representing the founding populations were genotyped for over 400 markers by the Mammalian Genotyping Service Center. Alleles were aligned for 254 markers by using CEPH controls genotyped in both labs to adjust for allele size differences. The software STRUCTURE was used to compare Amerindian ancestry at each marker to the genome average of Amerindian ancestry in a two-sided hypothesis approach to evaluate for departures above and below the genome average. Results: Areas of excess Amerindian ancestry in cases compared to controls were observed at 1p22-p33 with markers D1S728, (109 cM, Z score 2.0) and D1S551 (114cM, Z score 1.6). On the contrary, an area with a significant excess of Amerindian ancestry was found among the controls at 17q11-q21 with markers spanning a region of 45-67cM (D17S219-D17S975-D17S188-D17S129, Z scores 4.0-7.0). Both 1p22-p33 and 17q11-q21 have been previously identified as susceptibility areas for CL/P. Conclusions: The opposite differences in Amerindian ancestry at these two regions provide further support for the multifactorial etiology of clefting and imply that disease risk in this Colombian population is the result of admixture of susceptibility loci from two different founding populations. The study highlights the utility of admixture mapping in complex traits.

1409/F

Genome-wide linkage study for INSULIN RESISTANCE in an isolated population of Mongolia. *H. Park[1], Y.S. Ju[1], J.S. Seo[1,2].* 1) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, 28 Yongon-Dong, Chongno-Gu, Seoul 110-799, KoreaDept Biochemistry, Seoul National Univ, Seoul, Korea; 2) Macrogen Inc., Korea.

As metabolic syndrome has been spotlighted in the field of medical science recently, insulin resistance has become one of the most interesting subjects. Metabolic syndrome is known as major risk factor of cardiovascular disease, which is very prevalent not only in western society, but also in developing countries. Metabolic syndrome includes type 2 diabetes mellitus, hypertension, hyperlipidemia, obesity and other abnormalities. These disorders coexist frequently, and it is suggested that there is common pathophysiological background among them, which is insulin resistance.

We have analyzed data with Mongolian individuals from large extended families in genetically isolated population. A total of 1029 individuals (441 males and 588 females) from 196 families were enrolled. After genotyping by use of 389 microsatellite markers, we performed a genome-wide linkage search with variance component analysis. We calculated Homeostasis Model Assessment II(HOMA II) index as an indicator of insulin resistance.

Variance component analysis provided estimates of heritability of insulin resistance, revealing insulin resistance was under significant genetic influences. The overall heritability of the insulin resistance cholesterol was 0.40. We found several significant quantitative locus of traits. Among them, the locus with highest LOD score was on chromosome 14q11-12 with LOD score 2.29.

Further analysis of these positive regions by fine mapping and association analysis is warranted to identify specific genes. To our knowledge, this study represents the first genome-wide linkage scan for insulin resistance in an Asian in the region of Asia.

1410/F

Novel linkage for tuberculosis susceptibility: The household contact study in Kampala, Uganda. *C.M. Stein[1,2], S. Zalwango[2,3], D.V. Leontiev[1], W.H. Boom[2], S.K. Iyengar[1], R.C. Elston[1], R.D. Mugerwa[2,3], C.C. Whalen[1,2].* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Tuberculosis Research Unit, Case Western Reserve Univ, Cleveland, OH; 3) Clinical Epidemiology Unit, Makerere University School of Medicine, Kampala, Uganda.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is an enduring public health problem globally and several studies suggest a role of host genetic susceptibility in increased TB risk. As part of a household contact study in Kampala, Uganda, we have taken a unique approach to the study of genetic susceptibility to TB, by studying three phenotypes: culture confirmed TB disease, tumor necrosis factor-alpha (TNF) expression in response to Mtb culture filtrate, and resistance to Mtb infection in the face of continuous exposure as evidenced by a persistently negative tuberculin skin test (PTST). We conducted a full microsatellite genome scan, using genotypes generated by the Center for Medical Genetics at Marshfield. Multipoint model-free linkage analysis was conducted using a recent extension in the implementation of the Haseman-Elston regression model that includes half sibling pairs, and HIV status was included as a covariate in the model. We analyzed individuals from 184 pedigrees, comprising 266 full sibling pairs and 185 half sibling pairs. Preliminary results demonstrate linkage within 15 Mb of a number of candidate gene regions, including IL-6 (TB p=0.0003), SLC11A1 (TNF p=0.05, PTST p=0.02), IL-1 complex (TB p=0.01), IL12BR2 (TNF p=0.05), IL12A (TB p=0.02) and IFNGR2 (TNF p=0.001). We have previously shown association of Mtb-induced TNF expression and TB with IFNGR1, TNFR1 and IL-10 in this sample, therefore other genes in the cascade are logical candidates. In addition, we detected suggestive linkage to three novel regions, one with all three phenotypes, another with only TB as the phenotype, and the third with PTST as the phenotype. These results further illustrate the role of the TNF intermediate phenotype in genetic susceptibility to TB. Further work is needed to identify candidate genes in these novel regions and conduct fine mapping studies.

1411/F

Genome-wide association study identifies new susceptibility genes for obesity. *Y.J. Liu[1], X.G. Liu[2], L. Wang[2], J. Liu[1], L.J. Zhao[1], H. Yan[2], D.H. Xiong[3], J.L. Li[1], R.R. Recker[3], C. Papasian[1], H.W. Deng[1,2,3,4].* 1) Basic Medical Sciences, University of Missouri - Kansa, Kansas city, MO; 2) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an , Shaanxi 710049, P. R. China; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, NE 68131, USA; 4) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China.

Obesity is a serious health problem that causes or exacerbates several common diseases. Obesity has strong genetic determination. Extensive candidate gene association studies and whole genome linkage scans have been performed but have achieved limited success in identification of obesity susceptibility genes. The recent advance in introduction of technological platforms for whole-genome association (WGA) studies provides an opportunity to identify obesity genes with modest effects. We report results of a WGA study in 1000 unrelated Caucasian subjects using Affymetrix 500K SNP arrays. Quantitative phenotypes used in this study are BMI (body mass index) and body fat mass measured by DXA (dual X-ray absorptometry). We performed association analyses for single SNPs as well as haplotypes with sliding windows of various sizes. For BMI, the most significant association (P= 9.4 x 10^{-10})was found on locus 10q25 (rs1385092) near the SORCS3 (SORCS RECEPTOR 3) gene. Interestingly, additional 10 SNPs around rs1385092 within the SORCS3 gene achieved P values of less than 2.0 x 10^{-9}. For fat mass, the most significant association (P= 2.2 x 10^{-8}) was also observed on 10q25. The results of haplotype analyses of different sliding window sizes further confirmed the significant association on 10q25 (P values of ~10^{-9} - 10^{-8}). Notably, linkage of the locus 10q25 to obesity and related phenotypes has been repeatedly reported in earlier studies of various populations (including ours), further supporting the importance of the gene(s) at 10q25 on development of obesity.

1412/F

Large-scale in Silico Mapping of Complex Quantitative Traits in Inbred Mice. *P.Y. Liu, H. Vikis, Y. Lu, D. Wang, M. You.* Department of Surgery and the Alvin J. Siteman Cancer Center, Washington University, St Louis, MO.

Understanding the genetic basis of common disease and disease-related quantitative traits will aid in the development of diagnostics and therapeutics. The processs of gene discovery can be sped up by rapid and effective integration of well-defined mouse genome and phenome data resources. We describe here an *in silico* gene-discovery strategy through genome-wide association (GWA) scans in inbred mice with a wide range of genetic variation. We identified 937 quantitative trait loci (QTLs) from a survey of 173 mouse phenotypes, which include models of human disease (atherosclerosis, cardiovascular disease, cancer and obesity) as well as behavioral, hematological, immunological, metabolic, and neurological traits. 67% of QTLs were refined into genomic regions <0.5 Mb with ~40-fold increase in mapping precision as compared with classical linkage analysis. This makes for more efficient identification of the genes that underlie disease. We have identified two QTL genes, Adam12 and Cdh2, as causal genetic variants for atherogenic diet-induced obesity. Our findings demonstrate that GWA analysis in mice has the potential to resolve multiple tightly linked QTLs and achieve single-gene resolution. These high-resolution QTL data can serve as a primary resource for positional cloning and gene identification in the research community.

1413/F

A family-based genome-wide association study of childhood asthma and airway hyperresponsiveness. *B. Raby[1], J. Lasky-Su[1,2], A. Murphy[1], R. Lazarus[1], J. Ziniti[1], B. Klanderman[1], J. Sylvia[1], A. Patel[1], C. Lange[2], E. Silverman[1], S. Weiss[1].* 1) Channing Laboratory, Brigham & Women's Hosp, Boston, MA; 2) Harvard School of Public Health, Boston MA.

We performed a family-based genome-wide association study for asthma and airway hyperresponsiveness (AHR - an cardinal intermediate asthma phenotype) in 422 nuclear families (n= 1215) ascertained through asthmatic probands 5-12 years old with mild-to-moderate asthma participating in a clinical trial. AHR, as measured by methacholine PC_{20}, was recorded annually for 4 years during the clinical trial. Single nucleotide polymorphism (SNP) genotyping was performed using Illumina HumanHap 550v3 BeadChip. Data management and genotype quality control was performed using PLINK. Of 561,466 SNPs on the arrays, 2.46% were removed during data cleaning due to genotype completion rates <90%, parental-offspring genotype incompatibilities, MAF=0, or because the assay sequence could not be reliably aligned to one genomic locus. Genotype data was inadequate for 43 subjects (3.5%). Thus, data from 403 asthmatic probands and their family members were analyzed. We performed family-based association testing on the 534,290 reliable autosomal markers using PBAT (for asthma) and FBAT-PC (for repeated measures analysis of log-transformed methacholine PC_{20}) under additive genetic models. We applied the conditional power screening approach (Van Steen 2005) to adjust for multiple comparisons. For asthma, 6 SNPs clustering in 2 distinct regions on chromosomes 5p ($p=4x10^{-6}$) and 6p ($p=10^{-4}$-10^{-6}) demonstrated suggestive evidence of genome-wide association. Linkage of the 5p locus with asthma has been reported in previous linkage studies (CSGA 1997). The chromosome 6p locus harbors 15 genes - none have been previously implicated in asthma. 4 SNPs on chromosomes 22q ($p=10^{-3}$-10^{-5}) and 3q (p=0.03) were associated with $logPC_{20}$. Linkage to asthma or AHR has not been demonstrated previously with these regions. Together, these data provide novel insights into the pathobiology of asthma, and warrant further testing of the associated variants in additional asthma cohorts. Funding: NIH/NHLBI grants U01 HL065899, P01 HL083069, K08 HL74193.

1414/F

Identification of loci for body height by genome-wide association (GWA): a comparison of microarray platforms. *F. Rivadeneira[1,2], M.J. Moorhouse[1,2], J.M. van Meurs[1], P. Arp[1], M. Jhamai[1], A. Hofman[2], H.A. Pols[1,2], M.H. Kayser[3], A.G. Uitterlinden[1,2].* 1) Internal Medicine; 2) Epidemiology & Biostatistics; 3) Forensic Medicine, Erasmus MC Rotterdam, Netherlands.

Introduction: Body height is a highly heritable complex trait. GWA is a hypothesis-free design we used to identify loci influencing height variation.

Methods: 490 non-diseased Dutch Caucasian women (age 65-75 years) were selected for a pilot-study using the Affymetrix(AFFY) Mapping500K dual array. Of these, 433 were also genotyped for the Illumina (ILLU) HumanHap550 array as part of a large GWA effort in a population-based cohort (n=10,000). 393 women were analysed after excluding 15 missing one AFFY array and 26 with X-ray diagnosed vertebral fractures. Height was measured with stadometer. Allele calling inclusion thresholds were 95%(AFFY-BRLMM) and 98%(ILLU). PLINK was used for QC (*IBS clustering, HWE<0.001, and MAF<0.01 filtering*) and association testing. Loci were ranked based on significance in the total set, effect-consistency across 2 random sets (n=196 each) and after adaptive permutation of selected SNPs.

Results: Average call-rates were 0.985 for the remaining 417,464 AFFY SNPs and 0.995 for the 532,202 ILLU SNPs. P_{unadj} ranged between $9x10^{-7}$ to $9x10^{-4}$ for 299 SNPs/142 loci(L) still significant after permutation (all p_{emp}<0.002) : *AFFY*: 146/75L, *ILLU*: 153/102L and *BOTH*: 9/35L. The top five hits included loci on:

Chr20: 4 SNPs(3 AFFY/ 1 ILLU), MAF=0.04, B=5.0 cm, gene region YES;
Chr02: 2 SNPs(0 AFFY/ 2 ILLU), MAF=0.47, B=1.9 cm, gene region YES;
Chr06: 1 SNP (0 AFFY/ 1 ILLU), MAF=0.44, B=-2.0cm, gene region NO;
Chr05: 1 SNP (1 AFFY/ 0 ILLU), MAF=0.12, B=-3.1cm, gene region NO;
Chr11: 1 SNP (1 AFFY/ 0 ILLU), MAF=0.12, B=3.1 cm, gene region NO.

Ignoring presence of vertebral fractures diluted most associations.

Conclusion: In this pilot study we identified multiple loci influencing height that warrant replication in different cohorts to establish consistency and true effect size. Both genotyping platforms have modest overlap in the loci identified (25%) thus, seem complementary.

1415/F

Identification of novel genes for early age-related macular degeneration (AMD): genome-wide association results from the Los Angeles Latino eye study (LALES). *C. Shtir[1, 2], H. Volk[3], P. Marjoram[3], T. Triche[4, 6, 7], D. Hinton[2, 4, 5], R. Varma[1, 2].* 1) Doheny Eye Institute, University of Southern California, Los Angeles, CA; 2) Ophthalmology, University of Southern California, Los Angeles, CA; 3) Preventive Medicine,University of Southern California, Los Angeles, CA; 4) Pathology, University of Southern California, Los Angeles, CA; 5) Neurosurgery, University of Southern California, Los Angeles, CA; 6) Cancer Biology, University of Southern California, Los Angeles, CA; 7) Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA.

Many genetic and environmental risk factors have been strongly associated with the development of both early and advanced AMD (Klein et al., 2005). Advanced AMD is a leading cause of blindness and its prevalence is significantly higher in Caucasians compared to Latinos (Varma et al., 2004). However, early AMD remains very common among Latinos (Varma, 2004). This study seeks to identify new genes associated with early AMD among Latinos through the use of a genome wide association study based on a 500K Affymetrix chip data set. Prevalent early AMD cases (n=101) and control subjects (n=202) were ascertained from the Los Angeles Latino Eye Study (LALES). Early AMD cases were identified by the presence of intermediate to large soft drusen in both eyes by masked grading of fundus photographs. Using single allelic tests, we identify 26 significant SNPs distributed among 14 chromosomes, all of which survive Bonferroni correction for multiple genome-wide comparisons, 19 of which are intragenic while 7 are intergenic. We then use haplotype association and haplotype trend regression analyses to further explore the disequilibrium structures neighboring these SNPs and to detect situations when single marker effects do not carry enough information to describe the underlying genetic variation. Our results suggest that different genetic factors play a role in AMD etiology among Latinos, which may uniquely protect Latinos from progressing to advanced AMD and its associated blindness.

1416/F

Whole genome association analysis in anencephaly. *D. Stamm[1, 2], C.S. Haynes[1], D. Siegel[1], L. Mehltretter[1], K. Soldano[1], A. Trott[1], J. Rimmler[1], A. Dellinger[1], J.R. Gilbert[3], M.C. Speer[1], NTD Collaborative Group.* 1) Duke University Medical Center, Durham, NC; 2) University of North Carolina, Chapel Hill, NC; 3) The Institute for Human Genomics, University of Miami, Miami, FL.

Neural tube defects (NTDs) are common birth defects and are considered complex in etiology, with both genetic and environmental factors implicated. Cranial NTD defects include anencephaly, acrania and encephalocele. Since anencephaly is the most severe form of cranial level NTDs, we hypothesize that the underlying genetic burden is higher in anencephaly than in other NTDs. To capitalize on this hypothesized higher genetic burden among anencephalics than other types of NTDs, we performed a whole genome association analysis in 49 NTD families with cranial defects (174 individuals) using Illumina's 317K SNP chip. Plink was used to calculate p-values for tests of departure from HWE and TDT for family-based association analysis. Two phenotypic classifications were used. The broad classification included families with a cranial NTD (n=49) in the analysis whereas the narrow classification included only families with anencephaly (n =44). The TDT showed 7 and 15 SNPs with p-values between 10^{-4} to 10^{-5} for the narrow and broad phenotypic classifications. Some of these SNPs mapped to the following genes: *INADL*, *LRP1B*, *STK39*, *RAD51L1*, *PAK7*, and *ACTN2*. Two SNPs are in *INADL* including rs1134767 (non-synonymous) and rs6697273 (intronic), and are in strong LD (r^2 =0.90) with one another. Interestingly, *INADL* has higher expression in Carnegie Stage C12 (neural tube closing) than Carnegie Stage 13 (neural tube closed; p <.03) in a comparison of SAGE libraries of human fetal neural tube tissue (see abstracts by Xu and Dellinger at this meeting). *INADL* regulates the frizzled-dependent planar cell polarity pathway (PCP) in the *Drosophila* eye; PCP is involved in appropriate neural tube closure. Together, these data suggest *INADL* may be a novel NTD candidate gene. Microarray expression analysis comparing gene expression differences between amniocytes from anencephalic fetuses vs. control fetusus, currently in progress, may illuminate the role of *INADL* and other genes identified from this study in NTD risk.

1417/F

Gene-centric Association Mapping of Chromosome 3p implicates potential role of GPX1 in Crohn's Disease. *J. Rioux[1,11], A. Ng[2], C. Lefebvre[1], M. Stewart[2], A. Latiano[3], S. Brant[4], J. Cho[5], R. Duerr[6], M. Silverberg[7], K. Taylor[8], G. Aumais[9], C. Deslandres[10], G. Jobin[9], V. Annese[3], M. Daly[11,12], R. Xavier[2,12], P. Goyette[1].* 1) Montreal Heart Institute, Montreal, PQ, Canada; 2) CCIB, Harvard, Boston, MA, USA; 3) CSSIRCCS Hospital, San Giovanni Rotondo, Italy; 4) Johns Hopkins University, Baltimore, MD , USA; 5) Yale University, New Haven, CT, USA; 6) University of Pittsburgh, Pittsburgh, PA, USA; 7) Mount Sinai Hospital, Toronto, Ontario, Canada; 8) Cedars-Sinai Medical Center, Los Angeles, CA, USA; 9) Hôpital Maisonneuve-Rosemont, Montreal, PQ, Canada; 10) Hôpital Sainte-Justine, Montreal, Quebec, Canada; 11) Broad Institute of MIT & Harvard, Cambridge, MA, USA; 12) Massachusetts General Hospital, Boston, MA, USA.

Genome-wide linkage studies of Crohn's Disease (CD), followed by association mapping have led to the discovery of the NOD2 and IBD5 susceptibility loci. Recent genome-wide association studies have identified multiple other CD risk genes (eg. IL23R, ATG16L1, PTGER4, IRGM) but together these only explain a fraction of the genetic susceptibility to CD. We have therefore been pursuing a known CD linkage region on chr. 3p21-22 using a gene-centric association mapping approach. Specifically, within the linked region we identified functional candidate genes with strong prior probability by searching for literature co-citations with relevant keywords and by searching publicly available datasets for gene expression patterns consistent with genes having a role in immune and/or intestinal tissues. We then performed a two-stage association study, composed of a screening phase where SNPs tagging the common variation across the different candidates were evaluated in 1062 patients with IBD, and then a follow-up independent replication phase in 1960 patients with IBD from the NIDDK IBD Genetics Consortium. Significant evidence of association (pval=0.006) and replication (pvalue=0.003; combined pval<0.00001) and logistic regression analyses suggest a role for the glutathione peroxidase gene GPX1, a gene implicated in mouse models of mucosal inflammation. Differential gene expression and network analyses suggest that GPX1 acts via a TLR-mediated disease mechanism.

1418/F

Sequence variants in two novel genes and two intergenic regions within a QTL on human chromosome 7q36 alter plasma triglyceride levels in the human metabolic syndrome. *E.M. Smith[1], L. Martin[2], J. Charlesworth[3], J. Blangero[3], A.H. Kissebah[1], M. Olivier[1].* 1) HMGC, Medical College of Wisconsin, Milwaukee, WI; 2) Children's Hospital, Cincinnati, OH; 3) Southwest Foundation for Biomedical Research, San Antonio, TX.

We have previously identified a quantitative trait locus on human chromosome 7 (LOD = 3.7) linked to plasma triglyceride levels in an obese cohort of 2207 individuals of Northern European descent. The QTL interval spans a region of 5 Mb.

Single nucleotide polymorphisms were selected across the region based on the linkage disequilibrium (LD) patterns of the CEPH population of the HapMap. A total of 1,048 SNPs were genotyped using Molecular Inversion Probe technology (Affymetrix).

Of the 1,048 SNPs assayed, 109 (10.4%) displayed nominal significance (p<0.05) and nine were significantly associated with triglyceride levels after correction for multiple testing. These SNPs were located in six discrete regions of interest clustered in the center of the QTL, containing two genes (DPP6 and HTR5A) and two additional intergenic regions. Haplotype analysis of these regions suggests that each region of interest independently contributes to the overall effect. Haplotypes in high LD regions around DPP6, which spans 1.1Mb, collectively account for approximately 30% of the initially observed linkage. In addition, a haplotype covering the entire HTR5A gene region, spanning 13.6kb, accounts for 26% of the linkage. The two intergenic regions (46kb in total), both more than 85kb from the nearest gene or hypothetical protein, account for a further 18% of the linkage.

These results clearly prove that the initially observed linkage is caused by multiple causal loci each contributing to the observed effect. In addition to two genes, intergenic regions also significantly affect plasma triglyceride levels. However, the physiological mechanisms underlying the genic and non-genic effects remain to be elucidated.

1419/F

Translocations and inversions in Finland. *T. Varilo[1, 2], M. Pöyhönen[2, 3], R. Salonen[4], L. Peltonen[1, 2, 5].* 1) Dept of Mol Med, NPHI, Helsinki, Finland; 2) Dept of Med Genet, U of Helsinki; 3) Dept of Clinical Genet, Helsinki U Central Hospital; 4) Dept of Med Genet, Väestöliitto, Helsinki; 5) The Broad Institute at MIT and Harvard, Boston, MA.

Finland is known of its high standard of clinical medicine and unique founder populations exploited in the hunt of disease genes. What is perhaps not so well recognized is that Finland is probably the country with most comprehensive health registers and records. Information from all the hospitalizations, surgeries, chronic diseases, and prescriptions among many other health related information of the population has been filed for decades.

Relying this infrastructure we are collecting all some 3000 known reciprocal balanced translocations and inversions to a national database (www.fintransloc.org). By analyses of all the medical records and by searches of national medical registers, we are obtaining novel information of not only monogenic diseases with unidentified mutations, but also of multifactorial traits associated with any given chromosomal abnormality. Importantly, such a database will greatly assist genetic counseling efforts.

To date, we have gathered 494 carriers of translocations and inversions and linked them to 195 families. We are currently performing more detailed analyses if these families with the expectation that they could provide shortcuts in the identification of disease genes.

Examples: Fam 5, t(1;12), Specific delay in development, 6 carriers, 2 with specific delay, 1 with dyslexia, 2 with school difficulty. Fam 29, t(2;18), Dyspractic developmental speech disorder, 7 carriers, 3 with speech difficulty, 1 with dyslexia. Fam 45, t(2;22), Learning difficulty, 3 carriers, 1 with multiform learning difficulty. Fam 82, t(5;12), Fibroma molle in palate, 3 carriers, 3 with fibroma molle (plus 1 patient with chr. status unknown). Fam 107, inv 8, Borderline mental retardation, 16 carriers, 11 with borderline mental retardation. Fam 128, t(10;11), Aorta dilatation, 2 carriers, 1 aorta dilation (plus 3 patients with chr. status unknown).

1420/F

Association between a haplotype of the GATA3 gene and inflammatory bowel disease. *X. Su[1], KD. Taylor[1], L. Mei[1], SR. Targan[2], JI. Rotter[1].* 1) Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Inflammatory Bowel Disease Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Background and Aim: The GATA binding protein 3 (GATA3) gene codes for the GATA3 protein which is expressed in the T-lymphocyte lineage and is thought to participate in T-cell receptor gene activation through binding to enhancers. In addition, GATA3 may play an important role in the balance between Th1 and Th2 subsets in the immune response and is thus a candidate for the Th1/Th2 dysregulation characteristic of Crohn's disease (CD) and ulcerative colitis (UC). The aim of our study was to test the association of GATA3 variation with CD and UC. Methods: Seven GATA3 SNPs were genotyped in 763 CD, 351 UC and 254 controls; haplotype blocks were constructed by using haploview 3.3; haplotypes were assigned using PHASE 2.0; association tests were performed by using chi-square. Result: Two haplotype blocks with 3 haplotypes per block (freq>0.05) were observed. Block 2, haplotype 1 (H1:1111) was associated with CD (88.7% CD vs 82.3% controls, OR=1.7, 95%CI: 1.14-2.51, p=0.008). H1 was associated with UC with borderline statistical significance (87.7% UC, 82.3% control, p=0.06). This association was strongest in non-Jews (89.5% CD, 79.6% control, p= 0.001). Conclusion: The observation of an association between haplotype in GATA3 and CD supports the idea that GATA3 variation contributes to CD pathogenesis through possible effects on Th1/Th2 dysregulation.

1421/F

Genetic effects on the expression of genes implicated in human T-cell regulation of inflammation in pedigreed baboons. *A. Vinson[1], J.E. Curran[1], M.P. Johnson[1], T.D. Dyer[1], E.K. Moses[1], J. Blangero[1], S.A. Cole[1], L.A. Cox[1,2], J. Rogers[1,2], J.L. VandeBerg[1,2], C. Brugnara[3], O.S. Platt[3], M.C. Mahaney[1,2].* 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Southwest National Primate Research Center, San Antonio, TX; 3) Department of Laboratory Medicine, Harvard University Medical School, Cambridge, MA.

T-cell regulatory effects in inflammation are implicated in many complex human diseases, including atherosclerosis, osteoporosis, and autoimmune disease. T-cells regulate inflammation in part via activation and differentiation into T-helper cell subsets characterized by largely subset-specific cytokine production. The goal of this study was to evaluate the baboon (*Papio hamadryas*), a species with evolutionary proximity and physiological similarity to humans, as a model organism for studies of the genetics of human T-cell immunity. We addressed this goal by analyzing quantitative expression data measured in baboons for over 100 transcripts coded by genes implicated in human T-cell activation and differentiation. Expression data was generated from mRNA levels measured in lymphocytes from 499 pedigreed baboons using the Illumina Human Sentrix-6 BeadChip microarray system. Using a maximum likelihood variance decomposition approach, our analyses detected significant (p<0.05) additive genetic contributions to the variance in expression for 57 of these transcripts (h^2 estimates ranged from 0.09-0.79). Multipoint whole genome linkage screens detected significant evidence for QTLs influencing phenotypic variation in 14 of these 57 heritable transcripts (LOD score range 2.8-14.9, genome-wide P=0.036-9.71x10^{-15}). These results demonstrate that baboons display detectable genetic effects on the expression of multiple genes implicated in human T-cell activation and differentiation, and that QTLs influencing these processes may be localized to orthologous chromosomes in the human genome. Detection and localization of genes affecting quantitative variation in these transcripts to specific regions of the baboon genome suggests that studies dissecting the genetic architecture of T-cell activation and differentiation should be successful in this species.

1422/F

Refinement of the disease locus in Chinese families with TPTPS. *M. Sun, F. Ma, Q. Liu, X. Zhao, F. Wu, W.H.Y. Lo, X. Zhang.* Department of Medical Genetics, Peking Union Medical College, Beijing, China.

Triphalangeal thumb-polysyndactyly syndrome (TPTPS, MIM190605) is an autosomal dominant genetic disorder usually shows a duplicated triphalangeal thumb, normal index finger, and cutaneous syndactyly between fingers 3-5. The disease locus was linked to D7S550 on chromosome 7q36, with a maximum LOD score of 6.85 at $\theta = 0$. The entry of TPTPS in OMIM has been moved to PPD II in 2007 for the reason that they are being considered as the same disease. Preaxial polydactyly type II (PPD II, MIM 174500) is the PPD with opposable triphalangeal thumbs. The candidate locus has been refined to an interval of about 450 kb on chromosome 7q36. In PPD, mutations in the ZPA regulatory sequence (ZRS), a SHH enhancer, have been identified. However, no mutation of this ZRS was found in patients with TPTPS, indicating that TPTPS and PPD might not be the same limb malformation syndrome. We have performed linkage and molecular genetic analyses in four Chinese families with TPTPS. In two large families, we obtained LOD scores of >3 with markers at chromosome 7q36. Haplotype analysis using the same marker set showed haplotype sharing in the other two families. In one large family, we found a recombination event in one affected individual at D9S550. We also found another recombination event at D7S3161 in one individual in the other large family. These results refined the disease locus to the region between D9S550 and D7S3161, overlapping with the PPD locus. We are now sequencing all intergenic conserved elements to identify pathogenic mutations responsible for the TPTPS phenotype.

1423/F

Extent and distribution of linkage disequilibrium in the Old Order Amish. *A.M. Levin[1], E. Rampersaud[2], H. Shen[2], B.D. Mitchell[2], A.R. Shuldiner[2], J. O'Connell[2], J.A. Douglas[1].* 1) Dept Human Genetics, Univ Michigan, Ann Arbor, MI; 2) Dept Medicine, Univ Maryland, Baltimore, MD.

Knowledge of linkage disequilibrium patterns is useful in evaluating population structure and designing and interpreting genetic studies of complex traits and diseases. Because the demographic history of each population varies and is not accurately known, it is necessary to evaluate LD empirically in each population of inference. We conducted a genome-wide survey of LD with a high-density single nucleotide polymorphism (SNP) map (~400,000 markers with an average intermarker distance of ~7 kb) in a sample of 60 maximally unrelated individuals from the Old Order Amish (OOA) population of Lancaster County Pennsylvania, a closed, Caucasian population derived from a modest number of founders in the 1700's. We then compared the extent and distribution of LD in the OOA with the 60 founders from the HapMap CEU sample, an outbred, European-derived sample. Overall, LD patterns were remarkably similar between these two samples, presumably reflecting their recent shared demographic history. For example, for SNPs 20-50 kb apart with common alleles (minor allele frequency≥5%), $R^2 \geq 0.8$ for 7% and 6% of SNP pairs in the OOA and CEU samples, respectively. Most of this short-range LD presumably predates the founding of the OOA population and so differs little from the CEU source population. In contrast, for common alleles, long-range LD was ~2-fold higher in the OOA relative to the HapMap CEU sample. For example, for SNPs 0.5 to 1 Mb apart, D'=1 for ~14% of SNP pairs in the OOA sample and 7% of SNP pairs in the CEU sample. This difference for long-range LD presumably reflects the unique demographic history of the OOA population. These data are consistent with previous theoretical predictions (e.g., see Krugylak 1999) and recent empirical data from other founder populations (e.g., see Willer et al. 2006) that suggested minor differences in LD between isolated and mixed populations, particularly for common alleles and a modest number of founders. Our results have important implications for gene mapping in the OOA.

1424/F

Deletion of CFHL1 and CFHL3 Genes in Age-related Macular Degeneration. *L.M. Olson[1], K. Spencer[1], Y. Chen[2], P. Gallins[3], M.A. Hauser[2], S. Schmidt[2], W.K. Scott[3], N. Schnetz-Boutaud[1], A. Agarwal[1], E.A. Postel[2], M.A. Pericak-Vance[3], J.L. Haines[1].* 1) Dept Molec Phys & Biophysics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Ctr for Human Genetics, Duke University, Durham, NC; 3) Institute for Human Genomics, University of Miami, Miami, FL.

Age-related macular degeneration (AMD) distorts central vision and is the primary cause of blindness in the elderly in developed nations. Genetic risk factors for AMD include both susceptibility variants and protective haplotypes in the complement factor H (CFH) gene on chromosome (chr) 1, variants in the HTRA1/LOC387715 locus on chr 10, and the R32W polymorphism in complement factor B on chr. 6. Recently, deletion of the "CFH-like" genes CFHL1 and CFHL3 was found within a protective CFH haplotype, suggesting that these deletions may be protective for AMD (Hughes et al. 2006). We genotyped the deletion in 780 cases and 265 controls by PCR amplification with primers that amplify both a 325 bp product of CFH and a 381 bp product of CFHL1. We identified the deletion in 16 individuals, but the deletion does not segregate perfectly with the A allele of rs6677604, as suggested by Hughes et al. 2006. However, haplotype H4 (Hageman et al. 2005, 2007) had a frequency of ~47% in the deletion individuals, and the majority of these people are homozygous for the T allele of Y402H (14 TT Y402H homozygotes of 16 total deletion homozygotes). Overall, deletion homozygosity was significantly more frequent in controls than cases (2.6% controls, 0.8% cases, Fisher's exact p=0.025, OR=0.29, 95% CI 0.10-0.86). After controlling for age, Y402H, smoking, and A69S in LOC387715, the protective effect of the deletion was no longer statistically significant (OR=0.45, 95% CI 0.11-1.83, p=0.27). This may be caused by decreased power in a reduced sample of 469 cases and 190 controls with complete covariate data. Deletion of CFHL1 and CFHL3 may account for a small portion of the protection from AMD associated with particular haplotypes in CFH. The presence of protective haplotypes in CFH that do not carry the deletion (Hageman et al. 2005, 2007 and Spencer et al. 2007), suggest that other protective variants in this region have yet to be discovered.

1425/F

Shades of gray: A comparison of linkage disequilibrium (LD) between the CEPH and Hutterite populations. *E. Thompson[1], Y. Sun[1], D. Nicolae[1,2], C. Ober[1].* 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Departments of Statistics and Medicine, The University of Chicago, Chicago, IL.

Founder or isolated populations have advantages for genetic studies due to the decreased genetic and environmental heterogeneity that characterize these populations. Moreover, longer range LD in many of these populations should facilitate gene localization, but extensive LD may limit the utility of these populations with respect to gene identification. The North American Hutterite population is one of the best characterized young founder populations and this isolate has been the subject of our studies of complex traits, including asthma, allergy, and cardiovascular disease, for >10 years. Here, we assess the patterns and extent of global LD using SNP genotypes with minor allele frequencies (MAFs) >5% from the Affymetrix GeneChip® Mapping 500K array in 60 relatively unrelated Hutterites and 60 unrelated CEPH Caucasians (HapMap). We surveyed six 500kb genomic regions representing low recombination (12q), high recombination (22q), gene rich (18q), gene poor (21q), and Xp and Xq, as well as a long (chr 2q) and short (chr 21q) chromosome arm. Median (upper, lower quartiles) r^2 was 0.054 (0.147, 0.015) in Hutterites and 0.033 (0.099, 0.007) in CEPH on chromosome 2q and 0.042 (0.115, 0.011) in Hutterites and 0.023 (0.070, 0.005) in CEPH on chromosome 21q among SNP pairs within 500kb. Although LD among some marker pairs extend further in the Hutterites compared to CEPH, the pattern of LD and MAFs are remarkably similar in the two populations. These results indicate that 1) identifying disease genes should be no more difficult in the Hutterites than in outbred Caucasian populations, and 2) the same common disease alleles should be present in the Hutterites and outbred populations. On the other hand, the homogeneous environment that results from their communal lifestyle may enhance the effects of genes and facilitate gene discovery in the Hutterites.

1426/F

A candidate gene for autosomal dominant hereditary motor and sensory neuropathy with proximal dominancy (HMSN-P). *K. Maeda[1], R. Kaji[1], J. Jamiyansuren[1,2], K. Yasuno[3], H. Takashima[4], M. Nakagawa[5], S. Makino[2], G. Tamiya[2].* 1) Department of Neurology and Neuroscience, Tokushima University Graduate School of Medicine, Japan; 2) Division of Human Molecular Genetics, Department of Neurology and Neuroscience, Tokushima University Graduate School of Medicine, Japan; 3) Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Japan; 4) Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medicine, Japan; 5) Department of Neurology and Gerontology, Kyoto Prefectural University Graduate School of Medicine, Japan.

Hereditary motor and sensory neuropathy with proximal dominancy (HMSN-P; MIM 604484) is endemic to Okinawa Islands, the most southern part of Japan, which is characterized by autosomal dominant inheritance, slowly progressive proximal muscle atrophy and weakness, sensory disturbance such as paresthesia and vibration loss, leading to be bedridden. The disease locus of HMSN-P has been mapped to 3q13-14. Our linkage study in a newly-found large family with many members developed the similar symptoms of HMSN-P in a western part of Japan using 15 microsatellite markers around the HMSN-P locus identified a 7.3-Mb interval in 3p13 cosegregated with the disease (maximum two-point lod score of 8.44 at theta= 0.0). The candidate region was identical to the HMSN-P locus, but the disease haplotype in the large family was different from that previously reported, suggesting allelic heterogeneity. Through mutation search by extensive genomic sequencing and expression analysis of known-genes within the candidate region using RNA from a patient's lesioned tissues, we found new strong candidate genes of HMSN-P.

1427/F

Identification of a genomic locus associated with early onset familial Essential tremor. *A. Shatunov[1], Z. Mari[1], E. Peckham[1], R. Elble[2], J. Clarimon[1], N. Sambuughin[1], H.S. Lee[1], A.B. Singleton[1], D. Vojcic[1], M. Hallett[1], L.G. Goldfarb[1].* 1) National Institutes of Health, NINDS/NIA, Bethesda, MD; 2) Southern Illinois University School of Medicine, Springfield, IL.

Essential tremor (ET) is the most prevalent movement disorder showing evidence of non-random accumulation in some families. Late onset ET has previously been mapped to genetic loci on chromosomes 2p, 3q, and 6p, but no causative genes identified. We conducted a genomewide linkage screening of two North American and one Spanish family comprising a total of 52 genotyped individuals that included 19 patients diagnosed as definite ET. The average age of disease onset in each family was before 21 years. Genotyping was performed with Affymetrix GeneChip10K assay with 10,000 SNPs, and the region of interest additionally genotyped with 7 polymorphic microsatellite markers covering the area of 13 cM on chromosome 11p15. Linkage analysis was based on methodology implemented in Genehunter2 programs. The results indicate linkage to the 11p15 region with maximum cumulative LOD score 2.85 at marker D11S1984. The multipoint LOD score in the 13 cM region is 3.6. The multiple NPL score is 7.7 with p= 0.000038. Our findings provide evidence for linkage of ET to a novel susceptibility locus on chromosome 11p15.

1428/F

Reproducibility of pairwise linkage disequilibrium in genome wide association data: Comparisons of HapMap data with 2 large study samples. *R. Lazarus[1], W. Qiu[1], E.K. Silverman[1], B. Raby[1], P. Kraft[2], S. Chanock[3], D. Hunter[2], S.T. Weiss[1].* 1) Channing Laboratory, Boston, MA; 2) Harvard School of Public Health, Boston, MA; 3) CGEMS, NCI and NIH, Bethesda, MD.

Introduction: Small HapMap panels are used to design and evaluate products for efficient linkage disequilibrium (LD) based genome-wide association (GWA) studies. Pairwise LD estimated in small samples is biased upwards. We explored the practical effects of this bias by comparing LD in >550K genotypes in 2 study samples (n=793 and n=1197), with LD from corresponding genotypes for the 60 CEU HapMap founders. **Results:** The MAF distribution from HapMap genotypes was significantly different using the Kolmogorov-Smirnov (KS) test or a paired t-test from the two study samples (eg, KS p<1e-16, t-test p=0.002), but not significantly different between the 2 study samples (KS p= 0.64, t-test p=0.66). Pairwise LD as r^2 was estimated between each distinct pair formed by taking each SNP in turn with the 10 SNPs on each flank. Paired r^2 differences between the 2 study samples (μ=0.0034) were small but significant by paired t-test (p=0.006), with larger values in the smaller sample. HapMap r^2 values were significantly higher than either of the study samples by paired t-test (eg p<2e-16) although less than 1% (μ=0.009, σ=0.073) magnitude. 95% of all paired differences between HapMap and either study sample r^2 values were within +/- 0.14. If SNP tags are selected using 60 subjects and an r^2 threshold of 0.8, slightly less than half the pairs will have higher LD in study samples. Of those with lower LD, only a few percent will be below an r^2 of 0.66. r^2 determines effective sample size for LD mapping using a tag SNP, and power varies as the square root of the sample size. Where relative power for LD tagging is 1.0 for r^2=1.0, the lower 95th percentile r^2 value of 0.66 corresponds to a relative power of about 0.81 compared to 0.89 for r^2=0.8. **Conclusion:** Estimates of LD for SNP tagging based on the small HapMap samples are biased upwards, and are subject to substantial sampling variability. In practice the effect of this bias on GWA study power appears to be relatively small. **Support:**HG003646,HL065899,HL083069.

1429/F

Evaluation of association tests under models including multiple disease susceptibility variants. *X. Lou[1,2], S.S. Schmidt[1], E.R. Hauser[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Bioinformatics Research Center, North Carolina State University, Raleigh, NC.

Gene mapping of complex human diseases often results in the identification of several potential risk variants within a gene and/or in the identification of several genes within a linkage peak. These findings are at odds with the hypothesis that a single allele is responsible for a linkage peak. We are interested in understanding the behavior of several association analysis tests under single variant and multiple variant models within a wide linkage region. We have used two association methods that incorporate evidence for linkage in a family based association setting, APL and LAMP. Both tests work well for single marker analysis, but here we compare their performance under models with multiple susceptibility variants. We used the simulation program, SIMLA, to generate family data sets for use in linkage and association studies. SIMLA can generate two haplotypes associated with two distinct susceptibility variants (possibly with different disease risks) with up to 6 markers included in each haplotype. The disease model may also include an interaction between the two variants. We simulated a common disease with prevalence of 0.20 with two haplotypes independently associated with two recessively acting susceptibility variants, each with risk allele frequency of 0.15. We varied the variant-specific odds ratios from 2 to 4 and simulated complex LD structures. The power of APL to detect one or more associated marker allele(s) decreases as the effect size decreases, but APL maintains the nominal type I error rate under all situations. The type I error rate of both LAMP association tests is at the nominal level for single marker models. However, both LAMP-LE and LAMP-LD have high type I error rates (≈20%) when there are multiple disease-associated marker alleles. The LAMP-LE test maintains relatively high power even with lower effect sizes, while the power of the LAMP-LD test is at its type I error rate in the presence of multiple disease-associated marker alleles. The LAMP-LD test is difficult to interpret when there are multiple disease-associated markers in the linkage region.

1430/F

Searching for recessive alleles in Kosrae, an inbred island population. *J.K. Lowe[1,2,3], J.B. Maller[1,2], I. Pe'er[4], B.M. Neale[1,2], J. Salit[3], E. Kenny[3], M. Noel[3], R. Burkhardt[3], W. Ji[5], J.-N. Foo[5], R. Tewhey[1], P.E. Bonnen[3], N.P. Burtt[1], R.P. Lifton[5], J.L. Breslow[3], M.J. Daly[1,2], M. Stoffel[3], D.M. Altshuler[1,2,6], J.M. Friedman[3,7].* 1) Broad Institute of Harvard and MIT, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) The Rockefeller University, New York, NY; 4) Columbia University, New York, NY; 5) Yale University School of Medicine, New Haven, CT; 6) Harvard Medical School, Boston, MA; 7) Howard Hughes Medical Institute.

Geographic isolation and severe population bottlenecks produced an order of magnitude more extensive linkage disequilibrium in natives of the island of Kosrae, Federated States of Micronesia, than that seen in HapMap Asians or Caucasians. In screenings performed in 1994 and 2001, we ascertained 75% of the adult population of Kosrae (n≈3200 individuals) for traits related to Metabolic Syndrome, including obesity, dyslipidemia, and hypertension. Subjects were genotyped with the Affymetrix 100k and 500k SNP assays. We developed association methods to analyze this unique cohort in which 98% of genotyped subjects can be joined in a single extended pedigree and performed whole-genome association studies for BMI, plasma lipids, blood pressure, and other traits.

We also note that native Kosraens are dramatically more homozygous than HapMap Asian populations. Homozygous segments of ≥1 Mb make up an average of 5.2% of the Kosraen genome, compared to 0.6% in HapMap Asians. This unusual degree of homozygosity greatly facilitates the identification of recessive alleles contributing to global trait variation. We observe no correlation between an individual's % homozygosity and quantitative trait value. We attempted homozygosity mapping in the Kosraen cohort and report findings from locus-specific tests of association between homozygous segments and trait variation.

1431/F

Fine-mapping of chromosome 19 for total cholesterol levels in Pima Indians. *A. Malhotra[1,2], C.N. LeClair[2], H.C. Looker[1,3], K.A. Yeatts[2], W.C. Knowler[1], R.L. Hanson[1], J.K. Wolford[2].* 1) Diabetes Epidemiology and Clinical Research Section, NIDDK, Phoenix, AZ; 2) Diabetes, Cardiovascular & Metabolic Diseases Division, TGen, Phoenix, AZ; 3) Endocrinology, Diabetes and Bone Disease Division, MSSM, New York, NY.

Abnormal lipid levels are a major risk factor for coronary heart disease, which is highly prevalent in the general population. While environmental factors such as poor diet and lack of exercise contribute to abnormal lipid levels, genetic factors also play a role. In a previous study, a genome scan of lipid levels in 998 Pima Indian sibling pairs showed evidence for linkage for total cholesterol levels on chromosome 19 (peak LOD score=3.68 at 19.5 cM with a 1-LOD support interval spanning 17-41 cM). To fine-map the region of linkage where a gene affecting cholesterol levels might reside, we identified and genotyped 346 SNPs approximately 150 kb apart under the linkage peak. Upon removal of genotyping errors and creation of a linkage map using CRIMAP, we performed variance components linkage analysis as implemented in the program Merlin, for a combined map with both the original microsatellite markers and the SNPs. This analysis reduced the size of the linkage interval by 9 cM with a 1-LOD support interval spanning 28-43 cM and also showed a slight increase in peak LOD score (3.71 at 34.4 cM) when compared to the results from the original genome-scan (microsatellite markers only). These results provide finer localization of the locus on 19p affecting cholesterol levels. Furthermore, the results show the utility of using a dense map of SNP makers in substantially narrowing a region for linkage to be followed up for future gene-based studies.

1432/F

Detecting deletions in candidate genes for cleft lip and palate. *M. Shi[1], A. Jugessur[2], H. Gjessing[3], A.J. Wilcox[1], R.T. Lie[2], C.R. Weinberg[1], T.N. Trung[2], K. Christensen[4], J.C. Murray[5].* 1) Biostatistics Br, NIEHS, Res Triangle Park, NC; 2) University of Bergen, Bergen, Norway; 3) Norwegian Institute of Public Health, Oslo, Norway; 4) University of Southern Denmark, Odense, Denmark; 5) Department of Pediatrics, University of Iowa, Iowa City, IA.

Apparent deviations in family genotypes from Mendelian inheritance have routinely resulted in these datapoints being discarded as errors. With the realization of the importance and wide distribution of copy number variants in the human genome, methods have been developed to detect deletion events based on patterns of Mendelian inconsistencies in data collected from high-density SNP surveys. In this study, we analyzed genotype data from a large scale candidate gene study of isolated cleft lip and palate using a multi-national collection of samples to detect deletions as one component of an association study. Genotyping was performed by CIDR for over 1,200 SNPs in 340 candidate genes using 1,535 Norwegian and 1,397 Danish samples consisting mostly of caseparent triads. This dataset has a high duplicate reproducibility rate (99.98%) and low rates of missing data (0.55%), which provided a powerful opportunity for deletion detection. We detected ten potential deletion regions based on patterns of Mendelian failures. Four are located in the cleft candidate genes SUMO1, TGFBR2, FGF10, AP2, EDN1, and PVRL2, previously suggested to play a role in facial development. Overall 1.6% of families in this study had a suggested deletion suggesting a significant impact on genetic etiology by this mechanism. We are performing denser SNP genotyping, direct sequencing and quantitative analysis to confirm and characterize the size of these potential deletions.

1433/F

ARG analysis in the Genome Wide Association Scans of the Cancer Genetic Marker of Susceptibility Initiative. *Z. Wang[1,2], M. Yeager[1,2], K.B. Jacobs[3], M. Minichiello[4], N. Orr[6], R. Hoover[2], D.J. Hunter[2,5], S.J. Chanock[2,6], G. Thomas[2].* 1) SAIC-Frederick, MD; 2) DCEG, NCI, Bethesda, MD; 3) Bioinformed LLC, Gaithersburg, MD; 4) Wellcome Trust Sanger Institute, UK; 5) Harvard School of Public Health, Boston, MA; 6) Pediatric Oncology Branch, CCR, NCI, NIH, DHHS.

Although single marker association test is presently the main approach to the initial analysis of genome wide association studies (GWAS), multiple marker tests may better exploit the expected linkage disequilibrium between the typed SNPs and the untyped, but sought, functional polymorphism. We focused on an analytic tool based upon the inference of ancestral recombination graphs (ARGs), initially proposed by Minichiello and Durbin, that accounts for coalescent genealogy, to dissect two regions demonstrating promising association signals in the CGEMS GWAS. In the FGFR2 locus associated with post menopausal breast cancer, this approach revealed a single contiguous 20 Kb DNA segment in which all signals for association were high (p< 0.003), contrasting with the flanking regions, extending up to 100 Kb away in each direction, in which the signals were systematically low (p > 0.1). Thus is this case, the ARG analysis provided a precise information on the location of the functional polymorphism. In the 8q24 region associated with prostate cancer, two association signals were evidenced. The signals were located in regions separated by a hot spot of recombination suggesting that each region harbored an independent functional polymorphism. The ARG analysis further predicted that the mutational event responsible for the centrometic functional polymorphism may be ancient and possibly gave rise to the protective allele as the centromeric functional polymorphism appeared more recent and created the at-risk allele. We observed that, in regions with low linkage disequilibrium, the computational requirement for the ARG analysis is demanding. However, the computation may be implemented in parallel processing. The ARG analysis provides, for regions with strong association signals, a rapid and systematic approach to fine mapping. Its effectiveness in the analysis of GWAS is presently being assessed. Funded by NCI Contract N01-CO-12400.

1434/F

Replication of *FTO* variant with childhood obesity in Hong Kong Chinese. *C.H.T. Tam, M.C.Y. Ng, V.K.L. Lam, W.Y. So, R.C.W. Ma, J.C.N Chan.* Dept Medicine & Therapeutics, Chinese Univ of HK, Hong Kong, China.

Two recent studies in European populations suggest that *FTO* (fat mass and obesity associated) gene located on chromosome 16q12.2 are associated with both childhood and adult obesity, as well as type 2 diabetes. Each risk allele confers 31 to 47% increased risk for obesity. In this study, we aim to replicate the association at *FTO* with obesity using a proxy SNP rs8050136 in a random Chinese adolescent population from Hong Kong.

We genotyped rs8050136 at *FTO* in 976 adolescents [age mean ± SD = 15.3 ± 2 years, % males = 47] that participate in a health screening program. Associations of rs8050136 at additive model with metabolic traits including body mass index (BMI), waist circumference (WC), percentage body fat as measured by bioelectric impedance (FAT), systolic and diastolic blood pressure, lipids (total cholesterol, triglyceride, HDL, LDL), glucose at OGTT for 0, 60 and 120 min and fasting insulin were assessed by linear regression adjusted for covariates age and sex.

We found that the reported risk allele A of rs8050136 (P = 0.0013-0.011) was consistently and significantly associated with increased adiposity related traits (BMI: geometric mean (95% CI) = 20.6 (17.8-23.9) kg/m^2 for AA carriers, 20.2 (19.7-20.6) kg/m^2 for AC carriers, 19.5 (19.2-19.7) kg/m^2 for CC carriers; WC: geometric mean (95% CI) = 68.0 (60.7-76.1) cm for AA carriers, 69.0 (67.9-70.0) cm for AC carriers, 67.4 (66.8-68.0) cm for CC carriers; FAT: mean ± SD = 22.9 ± 7.9 % for AA carriers, 21.7 ± 7.5 % for AC carriers, 20.7 ± 7.0 % for CC carriers). However, we did not observe any association between rs8050136 and other metabolic traits. In summary, our study support *FTO* as a susceptibility locus influencing childhood obesity in Chinese population.

1435/F

Fine mapping of the chromosome 14 primary open angle glaucoma (POAG) region. *J.L. Wiggs[1], M.A. Hauser[2], R.R. Allingham[3], M.A. Pericak-Vance[4], J.L. Haines[5].* 1) Dept Ophthalmology, Harvard Medical Sch, MEEI, Boston, MA; 2) Center for Human Genetics, Duke University School of Medicine, Durham, NC; 3) Department of Ophthalmology, Duke University School of Medicine, Durham, NC; 4) University of Miami Miller School of Medicine, Miami, FL; 5) Center for Human Genetic Research, Vanderbilt Medical School, Nashville, TN.

Primary open angle glaucoma (POAG) is a genetically and phenotypically heterogeneous disorder that causes irreversible damage to the optic nerve and is a leading cause of blindness worldwide. Using a collection of 195 affected sibling pairs, we have previously completed a genome scan that provided evidence for POAG loci on chromosomes 2, 4, 14, 15, 17 and 19 (Wiggs et al., 2000). Model dependent and model free linkage analysis gave highest values for markers D14S264 and D15S165. Haplotype analysis performed with additional markers in the areas of peak linkage identified a 50 cM interval on chromosome 14q11-q22 extending from marker D14S72 to D14S274 shared by affected individuals in 53% of the pedigrees (n= 86) included in the analysis. SNP genotype data from a SNP-based genome scan (Illumina) using the same families was analyzed to further refine the region defined by the microsatellite haplotypes. 44 families had a shared haplotype among affected individuals that corresponded to the regions shared by the microsatellite markers. Of these families, the affected members of 23 families shared a portion of a single haplotype with the shared segment extending from rs1951085 to rs7160965, a region of 4.2 Mb that includes microsatellite marker D14S264. Candidate genes located within this reduced region will be evaluated for association with POAG using a case control cohort. These results suggest that a POAG susceptibility gene(s) located in this region is responsible for a significant portion of the genetic contribution to POAG in this population.

1436/W

Amino acid analysis in physiological fluids by liquid chromatography/mass spectrometry (LCMS): A fast, sensitive method for detection of disorders of amino acid metabolism and nutritional deficiencies. *S. Goldman, D. Salazar, J.A. Neidich, T. Lynn, C.M. Strom.* Biochemical Genetics Laboratory, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

We have developed a rapid, sensitive new method for amino acid analysis in plasma, urine, and cerebrospinal fluid that relies on liquid chromatography/mass spectrometry (LCMS). Accurate, quantitative amino acid testing is necessary for the diagnosis of a large number of disorders of amino acid metabolism, and is also used for the ongoing dietary monitoring required for patients once a diagnosis has been made. Our new method utilizes derivitization of terminal amino groups with phenylisothiocyanate (PITC), followed by separation of analytes on a C18 column, and allows for a rapid analysis time (25 minutes injection to injection) compared to traditional methods, while providing greater sensitivity than either ion-exchange chromatography (by amino acid analyzer) or HPLC alone. Greater than 45 analytes can be separated and quantitated in physiological fluids, most having a limit of quantitation (LOQ) well below 1.0 umol/L, with typical linear ranges of 1-1500 umol/L. Compared to the traditional platforms used for amino acid analysis, this method has the advantage of providing absolute amino acid identification through its combination of elution time and mass data, thus enhancing accuracy in reporting. Problems encountered due to interfering substances derived from diet or medications that are inherent to older amino acid analysis methods are not an issue with this test. In addition, the enhanced sensitivity of this method enables accurate quantitation of amino acids at very low levels, enhancing the utilization of such testing for nutritional status.

1437/W

Age-specific reference ranges and preanalytical stability of amino acids in plasma and urine. *T. Lynn, D. Salazar, J.A. Neidich, S. Goldman, C.M. Strom.* Biochemical Genetics Laboratory, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Quantitative amino acid analysis is important for the diagnosis of a large number of inherited defects of amino acid metabolism and is also used for ongoing therapeutic and dietary monitoring of patients once a diagnosis has been established. Normal amino acid levels can vary significantly depending of the age of the patient. Thus, accurate reference ranges are critical in establishing a diagnosis or assessing compliance with diet or medications. There is limited current published information regarding normal ranges for amino acids in physiological fluids, which may partly be due to the difficulty in obtaining samples from healthy neonates. We analyzed 438 plasma and 248 urine samples from apparently healthy individuals by LCMS to determine age-specific reference ranges for greater than 45 amino acids. Of the samples tested, 304 plasma samples and 137 urine samples were pediatric, thereby strengthening the reference ranges in this crucial population. In addition to the reference range determination we also studied the preanalytical stability of amino acids in both sample types. Data were collected regarding the stability of individual analytes at frozen, refrigerated, and room temperatures. The majority of analytes were found to be stable at refrigerated temperatures for up to a week, and many were also stable at room temperature for several days. Stability information will be extremely useful to clinicians attempting to interpret amino acid results for potentially compromised samples that may have been thawed in transit.

1438/W

Mutations in *MMADHC* in two patients with the *cblD* form of inborn error of cobalamin metabolism. *I.R. Miousse[1,2], D. Watkins[1], D. Coelho[3], T. Suormala[3], J.P. Lerner-Ellis[1,2,3], B. Fowler[3], D.S. Rosenblatt[1,2].* 1) Department of Human Genetics, McGill University, Montreal, Qc, Canada; 2) Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Montreal, Qc, Canada; 3) Metabolic Unit, University Children's Hospital, Basel, Switzerland.

Derivatives of cobalamin (vitamin B_{12}) are essential cofactors in two reactions in mammalian cells: methylmalonyl-CoA mutase and methionine synthase. Defects of cellular cobalamin metabolism that prevent the proper function of these two enzymes have been assigned to specific complementation groups (*cblA-cblG*). Eleven patients are known with the *cblD* inborn error of cobalamin metabolism, and the responsible gene, *MMADHC*, has recently been identified. Patients with *cblD* present with either isolated methylmalonic aciduria, isolated homocystinuria or combined methylmalonic aciduria and homocystinuria, and the location of the mutation correlates to the phenotype. We report two additional patients with the *cblD* disorder. Patient 1 presented during the first days of life with isolated methylmalonic acidemia. Cultured fibroblasts had decreased incorporation of label from propionate into macromolecules; the proportion of total cobalamin present as adenosylcobalamin was decreased. Complementation analysis classified this patient as *cblD variant 2* (isolated methylmalonic acidemia). Patient 1 was compound heterozygous for two putative truncating mutations in *MMADHC*: c.60insAT (p.L20fsX21) and c.455dupC (p.T152fsX162). Patient 2 presented at four months of age with elevated levels of both methylmalonic acid and homocysteine in blood and urine. Incorporation of label from both propionate and methyltetrahydrofolate in macromolecules was decreased. Synthesis of both adenosylcobalamin and methylcobalamin was decreased. Complementation analysis classified this patient as *"classical" cblD* patient (methylmalonic acidemia and hyperhomocysteinemia). Mutation analysis of *MMADHC* demonstrated that patient 2 was homozygous for the missense mutation c.683C>G (S228M). These findings reinforce phenotype-genotype correlations previously reported in *MMADHC*.

1439/W

GENOTYPING OF PATIENTS WITH PYRIDOXIN-DEPENDENT EPILEPSY (PDE) BY RT-PCR IN cDNA OF LEUKOCYTES IS DISTURBED BY AN ANTIQUITIN PSEUDOGENE. *E. Paschke[1], K. Paul[1], W. Erwa[3], B. Plecko[1,2].* 1) Department of Pediatrics, Medical University of Graz, Graz, Austria; 2) British Columbia Childrens Hospital , Vancouver, BC, Canada; 3) Institute of Clinical and Chemical Laboratory Diagnosis, Medical Universtiy of Graz, Graz, Austria.

Patients with Pyridoxine Dependent Epilepsy (PDE, MIM# 266100) present with pyridoxine-responsive seizures and elevated concentrations of pipecolic acid as well as alpha-aminoadipinic semialdeyde in urine, plasma and cerebrospinal fluid. PDE is caused by a deficiency of alpha-aminoadipinic semialdehyd-dehydrogenase (Antiquitin; ALDH7A1, MIM#107323;), located on chromosome 5q31. At the gene level, a total of 17 pathogenic mutations in 29 patients have recently been described by Mills P al. (Nature Med (2006) 12: 307-309) and Plecko B et al. (Human Mutation (2007) 28:19-26). Among these, we recently detected four heterozygous patients with a new common transversion of G>T at a highly conserved acceptor splice site in intron 17 (c.1482-1G>T) and a novel A>G transition affecting the acceptor splice site in intron 7 (c.612-2A>G). Further characterization of these mutations by RT- PCR revealed homozygous cDNA products containing 39 mismatches to the ALDH7A1 sequence. We subsequently sequenced preparations of 6 RT-PCR products spanning the entire cDNA sequence in leukocytes of normal individuals. We found that only the first amino-terminal fragment of six replicons covering the ALDH7A1 cDNA was 100% identical to the ALDH7A1 gene, while the others contained the exact ALDH7A1 pseudogene (NG_001082) sequence. When, in contrast, analogous replicons were prepared from fibroblasts at an elevated annealing temperature of 64 °C, all products were entirely free of pseudogene sequences. These findings are of special importance for the evaluation of presumptive disease-causing effects of novel splice site mutations in the ALDH7A1 gene considering m-RNA structure and protein expression.

1440/W

The effect of gestational age, transfusions, and dietary supplementation with medium chain triglyceride on ms/ms profiles of presumptive positive patients for medium chain acyl coa dehydrogenase deficiency. *C. Dvorak[1, 2], T. Narumanchi[1,2], A. Cunningham[1], K. Weissbecker[1], M. Jenkins[1], J. Smith[1], D. Werling-Baye[3], C. Myers[3], J. Thoene[1, 2], H. Andersson[1, 2].* 1) Human Genetics Program, Tulane Sch Medicine, New Orleans, LA; 2) Dept of Pediatrics, Tulane Sch Medicine, New Orleans, LA; 3) Louisiana DHH - Office Of Public Health - Genetic Diseases Program.

Newborn screening for medium chain acyl CoA dehydrogenase deficiency (MCADD) is based on elevations of medium chain fatty acids in the blood spot, but numerous factors in the newborn period may cause elevations of medium chain fatty acids which are unrelated to inherited disease. We present the final results of a study on the effect of gestational age, packed red blood cell transfusions, and dietary supplementation with Medium Chain Triglyceride (MCT) oil on the MS/MS profile of patients who were presumptive positives for Medium Chain Acyl CoA Dehydrogenase deficiency [MCADD]. Data was collected from September 2005 until Januray, 2007 for a total of 55 presumpitve positive MCADD patients (51 screened in Iowa, 4 screened by Pediatrix). Because Louisiana's NBS lab was damaged by Hurricane Katrina, newborn screening is being performed in Iowa, with final diagnosis being done following confirmatory testing by clinicians in LA. The single greatest predictor of a false positive for MCADD was gestational age. No patients under 32 weeks gestation proved to truly have MCADD. Also, these patients were more likely to reccieve PRBCs, and MCT-containing nutritional supplementation. Based on our findings, we recommend that for preemies < 32 weeks, blood for NBS be obtained prior to the start of TPN or transfusion, as this may reduce the number of presumptive positives. If this is not possible, an acylcarntine profile, along with free and total carnitine, and urine organics should be obtained, once the child is on PO feeds that do not contain MCT oil.

1441/W

Elevated Serum LDH-3 Level in sputum positive TB patients of Sahariya tribe of Central, India: A possible diagnostic marker for TB. *P.R. Sharma[1], S. Jain[2], P.K. Tiwari[1].* 1) Centre for Genomics SOS ZOOLOGY, Jiwaji University, Gwalior, Madhya Pradesh, India; 2) RNTCP unit, Sheopur District Hospital, Sheopur, M. P., India.

Indian populations are culturally stratified as tribals and non-tribals. Madhya Pradesh (Central India) is a home to large numbers of tribal groups. Sahariya tribe is one of the major primitive tribal groups confined mainly to North Madhya Pradesh. Unfortunately, the incidence of tuberculosis is very high in this tribal group. Lactate Dehydrogenase (LDH) is a tetrameric enzyme of pathophysiological importance in disease diagnosis. It has five isoforms each representative of individual tissue type. The present investigation was to find out if sputum test, which is generally performed to assess mycobacterial infection status, is in anyway correlated with one or more of the LDH isoforms level to employ it for diagnostic purpose. We conducted a case control study in 200 cases (Pulmonary tuberculosis patients) and 180 controls from the tribe to estimate the levels of various Lactate dehydrogenase isoforms in serum. The sputum (twice) and blood sample were collected after well informed & written consent of donors. The Ziehl-Neelsen's staining of sputum smear was carried out as per RNTCP (Revised National Tuberculosis Control Programme), WHO protocol. Each sputum slide was categorized into sputum one, two and three positive, according to the numbers of bacilli seen per oil immersion field. Total serum LDH level was estimated spectrophotometrically. The levels of individual isoforms were assessed on 6% native PAGE and quantitated using Uvitek gel-documentation system. The LDH content was found significantly ($p< 0.0001$) elevated in blood sera of sputum positive, especially in three positive individuals (444 ± 270 IU) as compared to sputum negative samples (242 ± 125 IU). Of the all LDH isoforms (LDH1, 2, 3, 4 and 5) LDH-3 was found to be highly elevated in sputum positive samples as compared to sputum negative samples. The statistical comparisons between sputum negative and sputum positive groups were made using Wilcoxon rank-sum test and Mann-Whitney's U test. $P < 0.01$ was considered statistically significant. Since, LDH-3 represents leakage from lung tissue in human body, its significant elevation in sera of sputum three positive TB patients suggests its possible use in diagnosing pulmonary tuberculosis along with standard sputum test. This combination of test is recommended because a pulmonary tuberculosis patient becomes sputum negative in one month after the start of TB chemotherapy, while serum LDH-3 levels are still found elevated (ongoing investgation) and may take more time to come down to its normal level in serum.

1442/W

BLOC-2 and BLOC-3 deficient melanocytes demonstrate distinct defects in TYRP1 trafficking. *A. Helip Wooley, H. Dorward, W. Westbroek, R. Hess, B. Pederson, M. Huizing, W.A. Gahl.* Medical Genetics Branch, NHGRI NIH, Bethesda, MD.

Hermansky-Pudlak syndrome is an autosomal recessive disorder characterized by oculocutaneous albinism and bleeding resulting from defects in any of eight distinct genes (HPS-1 through HPS-8). With the exception of HPS-2, the human HPS genes encode proteins of unknown function. Several of these proteins interact with each other in Biogenesis of Lysosome-related Organelles Complexes or BLOCs. Specifically, HPS1 and HPS4 form BLOC-3 and HPS3, HPS5 and HPS6 comprise BLOC-2. To characterize and distinguish these BLOCs at the cellular level, we examined cultured melanocytes from individuals with HPS-1 and -4 (BLOC-3) and HPS-3, -5 and -6 (BLOC-2). BLOC-3 deficient melanocytes contained fewer dark melanosomes than BLOC-2 deficient melanocytes. Localization of melanosomal proteins by confocal immunofluorescence microscopy revealed TYRP1 staining in BLOC-3 melanocytes concentrated in the perinuclear region, with a large degree of overlap with the TGN. In BLOC-2 melanocytes, TYRP1 staining extended into the dendrites but failed to appropriately collect in the tips. Antibody uptake experiments demonstrated increased trafficking of TYRP1 via the cell membrane in BLOC-2 but not in BLOC-3 deficient melanocytes. BLOC-2 appears to sort TYRP1 from an early endosomal compartment to developing melanosomes. In the absence of BLOC-2, TYRP1 is mis-sorted to the plasma membrane. BLOC-3 likely functions earlier in the pathway such that TYRP1 does not reach the BLOC-2 endosomal compartment in BLOC-3 deficient melanocytes. BLOC-2 and BLOC-3 deficient melanocytes demonstrate distinct defects in TYRP1 trafficking, reflecting their actions in disparate steps of the pathway.

1443/W

Weighted Gene Coexpression Network Analysis Identifies Biomarkers in Glycerol Kinase Deficient (GKD) Mice: Systems Biology Informs Pathogenesis. *N. MacLennan[1], J. Dong[2], J.E. Aten[2], S. Horvath[2,3], L. Rahib[4], K.M. Dipple[1,3,4], E.R.B. McCabe[1,3,4,5].* 1) Pediatrics, UCLA, Los Angeles, CA, USA; 2) Biostatistics, UCLA, Los Angeles, CA, USA; 3) Human Genetics, UCLA, Los Angeles, CA, USA; 4) Biomedical Engineering, UCLA, Los Angeles, CA, USA; 5) Bioengineering, UCLA, Los Angeles, CA, USA.

To investigate the pathogenesis of GKD, we identified biomarker genes in a glycerol kinase (Gyk) knockout (KO) mouse using a network analysis algorithm, Weighted Gene Co-Expression Network Analysis (WGCNA) that relates a measure of differential expression to intramodular connectivity. Highly connected, highly correlated intramodular hub genes are associated with disease pathogenesis. We used WGCNA to reduce dimensionality of microarray expression data from livers of day of life (Dol) 1 and Dol 3 KO and wild type (WT) mice and we identified genes involved in pathogenesis using intramodular connectivity. WGCNA revealed significant network overlap between Dol 1 and Dol 3 mice. Both Dol 1 and Dol 3 livers contained network modules enriched with genes involved in organic acid metabolism and cell cycle. Differences in network expression were revealed also. Unlike Dol 3, the Dol 1 gene module containing Gyk as a member was enriched with apoptotic genes. WGCNA identified networked genes that were not anticipated by apriori hypotheses. We examined the validity of these novel genes in tissue culture. Confirmation studies for Acot, Psat and Plk3 identified by WGCNA using nuclear receptor agonists and antagonists, and causality (NEO) analysis validated the results of WGCNA. Acot gene expression preceded Plk3 and Psat gene expression in the inferred dol 3 gene network from NEO analysis. We conclude that WGCNA reduces high dimensionality expression data to a low dimensionality output to identify networks and biomarkers in GKD. We speculate that GK may have an apoptotic moonlighting role that is lost in GKD. These investigations in Gyk KO mice demonstrate that systems biology approaches improve our understanding of disease pathogenesis and may provide insights into treatment through identification of previously unanticipated networks.

1444/W

A mild neurological phenotype of mucolipidosis type IV in a patient with an altered C-terminus of mucolipin-1. *E. Goldin[1], A.M. Cooney[2], C.R. Kaneski[2], Y. Blech-Hermony[1], S. Stahl[2], R. Schiffmann[2].* 1) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Developmental and Metabolic Neurology Branch, NINDS, NIH, Bethesda, MD.

Mucolipidosis IV (MLIV) is caused by mutations in mucolipin-1 (MCOLN1), a gene encoding a cation channel of the TRP family. Most Ashkenazi Jewish patients have one of two severe mutations that result in a null phenotype, presenting with eye abnormalities that lead to progressive blindness and severe psychomotor retardation. Patients also suffer achlorohydria, causing extremely elevated levels of blood gastrin. Lysosomal inclusions are found in most cells, and fibroblasts are autofluorescent. MRI shows partial agenesis in the corpus callosum and a degenerative process in the cerebellum. A variety of mutations have been described in other patients, causing intermediate forms of the disease that range from the most severe phenotype to mild developmental abnormalities and slowing of the retinal degeneration. The patient described here presented with only a mild loss of vision and a very mild motor deficit, and otherwise lives a normal life appropriate for his age. In contrast, his fibroblasts demonstrate autofluorescence similar to all other MLIV patients and his blood gastrin is elevated. DNA sequencing identified two frame-shift mutations in MCOLN1, but only one of them is expressed. This mutation alters the C-terminus of the protein. Electrophysiology of a construct of the mutant protein was tested in liposomes and minor abnormalities in the protein function were detected. The lack of brain abnormalities in this patient implies that a minor deficiency of mucolipi-1 does not necessarily impact brain development. This case contributes significantly to the literature because it broadens the spectrum of clinical heterogeneity encountered in ML-IV, and underscores the important role of the ophthalmologist in making this diagnosis.

1445/W

D409H homozygosity and the cardiovascular form of Gaucher disease: A case report. *K. Cusmano-Ozog, V.T. Sweet, G.M. Enns.* Medical Genetics, Stanford University, Stanford, CA.

Gaucher disease, a lysosomal storage disorder, is caused by a deficiency of glucocerebrosidase. There are several subtypes, one of which is the cardiovascular form. We present a 13 year-old female who recently relocated to the United States from Mexico with a seven year history of exertional chest pain and shortness of breath. Family history was significant for a brother who passed away at six months of age secondary to a bleeding abnormality and parental consanguinity. Physical exam was significant for thrombocytopenia and hepatosplenomegaly. An echocardiogram revealed thickened aortic and mitral valves. She was noted to be in congestive heart failure and a decision was made to repair her valves. During cardiac surgery, calcification of the ascending aorta and aortic arch was noted. St. Jude valves and a Dacron graft were placed. She was started on anti-coagulation therapy. Post-operatively, anisocoria was noted and a head CT revealed an intraventricular hemorrhage and hydrocephalus. A ventriculoperitoneal shunt was placed. The anisocoria resolved and a formal ophthalmologic evaluation was normal. She then developed a subdural hematoma that required evacuation. Electron microscopy of thymus tissue obtained during cardiac surgery revealed elongated and distended lysosomes. Beta-glucosidase activity was low at 4 nmoles/hr/mg protein (controls 8-14). *GBA* mutation analysis revealed that she was homozygous for the D409H allele. Following these results, she was started on enzyme replacement therapy (ERT) with imiglucerase. Individuals homozygous for the D409H allele share a common phenotype characterized by calcification of the aortic and mitral valves, splenomegaly, corneal opacities and supranuclear ophthalmoplegia. If patients survive cardiac surgery, improvement of the hematologic abnormalities and organomegaly with ERT has been described. Anti-coagulation treatment for mechanical valves may be challenging, especially with an underlying hematologic abnormality. A diagnosis of Gaucher disease should be considered in individuals with calcification of the aortic and/or mitral valves.

1446/W

Development of a disease severity scoring system for patients with Pompe disease. *E.H. Giannini[1], D.L. Marsden[2].* 1) Rheumatology, Cincinnati Children's Hospital, Cincinnati, OH; 2) Genzyme Corporation, Cambridge, MA.

Background: A Disease Severity Scoring Index (DS3) assesses the burden of disease severity in a patient and can compare patients with the same disease. It can distinguish between separate organ system scores and overall scores, allowing for comparison within organ systems. The clinical progress of a patient or the response to treatment can be followed. It is particularly useful in rare, heterogeneous diseases. A system is being developed for Pompe Disease, a rare autosomal recessive neuromuscular disorder due to a deficiency of lysosomal enzyme acid-α-glucosidase (GAA), which results in accumulation of glycogen in cardiac, skeletal and smooth muscle. Clinical phenotype ranges from severe, rapidly progressive disease in infants, to slower, more heterogeneous disease in children and adults. Enzyme replacement treatment (ERT) with recombinant GAA, Myozyme, is now available. **Methods:** A panel of Pompe experts was assembled to identify critical health domains. A Delphi group of physicians was consulted to capture standard medical practice(s) for severity measurement within each critical domain. Selected domains were: Cardiac, Respiratory, Proximal Muscle, Physician Reported Outcomes and Patient Reported Outcomes. Within each domain, 1-2 clinical assessments were identified. **Results:** To test this preliminary model, 9 cases from the Pompe Registry representing a severity spectrum were scored, and compared to results from a blinded small expert group assessment of the same cases using the Clinical Global Impression (CGI) Severity scale, yielding a 0.93 coefficient of correlation, indicating preliminary DS3 consistency with expert opinion, confirming DS3 validity, reliability and relevance. Validation will be completed by comparing DS3 results with the expert Delphi group opinion for multiple patient cases at multiple time points. **Conclusion:** Preliminary results indicate the Pompe DS3 model will help standardize disease terminology, highlight key clinical assessments to quantify disease severity. Ultimately this tool will evolve into a universal disease staging system where specific medical interventions may be recommended.

1447/W

High carrier frequency of an unusual deletion mutation of the *GALT* gene in the Ashkenazi population. *N. Goldstein, Y. Cohen, E. Sigalov, B. Vilensky, Y. Anikster.* Metabolic disease unit, Safra Children Hospital, Sheba Medical Center, Tel Hashomer.

Classical Galactosemia is a disorder of galactose metabolism presented in the first weeks of life and characterized by vomiting, diarrhea, lethargy, hypotonia, jaundice, hepatomegaly, septicemia and bleeding tendencies. Galactosemia is inherited in an autosomal recessive manner due to mutations in the *GALT* gene. Loss of GALT enzymatic activity prevents the conversion of galactose-1-phosphate (Gal-1-P) and UDP-glucose into glucose-1-phosphate and UDP- galactose causing the accumulation of Gal-1-P in various organs leading to the clinical signs and symptoms described above. Treatment for this disorder constitutes diet restrictions on galactose during infancy and all lactose-containing foods throughout life. However, long term complications such as cognitive and developmental delays and ovarian failure may not be prevented. Recently, an unusual deletion mutation of the *GALT* gene was characterized. The deletion includes most of the gene, retaining only a short internal segment. The ethnic origin of several unrelated patients having this mutation is Ashkenazi Jewish. Examination of this mutation in our Ashkenazi patients diagnosed with Galactosemia showed that all of the Ashkenazi alleles had the mutation. The aim of this study was to estimate the carrier frequency of this mutation in the Ashkenazi population in Israel. 760 DNA samples from anonymous subjects of Ashkenazi descents were available for molecular diagnosis at our lab. *GALT* gene deletion mutation was genotyped using a chip-based matrix-assisted laser desorption-time-of-flight (MALDI-TOF) mass spectrometer. Using MassARRAY® software (Sequenom) we designed a multiplex assay that includes detection of both normal and deleted alleles. Six out of 760 DNA samples screened had the deleted allele. Thus, the carrier frequency in this population is 1 in 127 (0.0079) and the predicted incidence of Galactosemia is 1 of 64,500 live births. Resolving the carrier frequency of this disease and an easy method of molecular diagnosis of Galactosemia in Ashkenazi Jews facilitates prenatal counseling for this population.

1448/W

Angiokeratoma: an important clue for the diagnosis of Fabry disease. *L.M.J. Albano[1], C. Rivitti[2], R. Giugliani[3], L.C.F. Sá[1], D.R. Bertola[1], R.S. Honjo[1], C.A. Kim[1].* 1) Pediatrics, Instituto da Criança, São Paulo, SP, Brazil; 2) Dermatology, HC-FMUSP, São Paulo, SP, Brazil; 3) Genetics, HCPA, Porto Alegre, RS, Brazil.

Fabry disease (FD) - an X-linked inborn error of glycosphingolipid catabolism - is the second most frequent glycosphingolipid lysosomal storage disease, after Gaucher disease. Unfortunately, the diagnosis is usually late, at third or fourth decades of life. As it is important to recognize this group of patients, especially now, with the proven efficacy of the enzymatic replacement therapy, we studied 16 men with angiokeratoma confirmed by biopsy. The relatives of these cases were also included, totalizing 29 individuals. After a clinical and laboratorial evaluation, we performed the enzymatic assay in 10 patients that showed strong evidence of FD. Among them, we detected three cases. Case 1: a 29 year-old man with penoscrotal and umbilical angiokeratoma since age 12. In childhood, due to severe attacks of pain and burning sensation on extremities, a rheumatic disorder was suspected. The values of α-Gal A enzyme were reduced in both the patient and his mother. They have never realized that they could be affected by FD or other kind of medical problem until our screening. The mother's investigation showed bilateral renal cysts, left ventricular hypertrophy and uterine myoma. Cornea verticilata was present in both mother and son. Case 2: a man already deceased due to renal disease with a clinical diagnosis of FD. Asking the family, we found out that her sister, a 54 year-old woman, had been investigated some years ago due to the positive familial history of FD The activity of the α-Gal A was normal in this woman, but molecular study showed a mutation (Y86H) in the α-Gal A gene. Case 3: a kidney transplanted 41 year-old man with clinical findings suggestive of FD. His enzymatic assay disclosed an α-Gal A activity in the inferior limit. This type of screening, looking for FD in patients with angiokeratoma, could be a good strategy for its diagnosis, since we detected 3 cases among 10, whose clinical and laboratory findings favored this hypothesis.

1449/W

Neonatal Screening for Pompe Disease: A Two-tier Screening Strategy. *J. Keutzer[1], Y.H. Chien[2], S.C. Chiang[2], X.K. Zhang[1], N.C. Lee[2], W.L. Hwu[2].* 1) Scientific Affairs, Genzyme Corp, Cambridge, MA; 2) Department of Pediatrics and Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan.

Pompe disease is caused by the deficiency of acid alpha-glucosidase (GAA). Recombinant human GAA has been used to treat infantile-onset Pompe disease (IOPD), resulting in prolonged survival, reversal of cardiomyopathy, and growth and in a subgroup of patients, the achievement of independent ambulation. Best motor outcomes are reached when recombinant human GAA treatment is initiated early. A neonatal screening pilot program for Pompe disease was started in October 2005 at National Taiwan University Hospital (NTUH). Blood spot GAA activities were measured on the dry blood cards collected from babies for routine neonatal screening three days after birth. The methods employed 4-MU-glucoside as the substrate, and acarbose as an inhibitor of maltase-glucoamylase. A two-tier screening strategy was used. In the first tier, assay GAA activity was measured. In the second tier, GAA activity and neutral maltase activity were measured. A second sample was requested from babies with low GAA activity and a high ratio of neutral maltase to GAA. Currently, more than 130,000 newborns have been screened and 4 cases of IOPD have been confirmed. No false negative results have been encountered. The results from this pilot program, which is ongoing, suggest that neonatal screening for Pompe disease is feasible.

1450/W

Mutation analysis of VLCAD gene in neonates -a sensitive and cost effective tiered approach. *M. Koul[1], J. Stoddard[1], J. Hempel[1], S. Edstrom[1], B. Cohen[2].* 1) Clinical Diagnostics,Transgenomic Labs, Transgenomic, Omaha, NE; 2) Cleveland Clinic, Cleveland.

Very-long-chain acyl-CoA dehydrogenase- VLCAD deficiency is an autosomal recessive disorder resulting from an inborn error of fatty acid oxidation. Fatty acid oxidation defects, including VLCAD deficiency, may account for as many as 5% of sudden infant death patients. VLCAD protein is loosely bound to inner mitochondrial membrane unlike the other acyl-CoA dehydrogenases-short, medium and the long. Over 150 mutations have been identified in the VLCAD gene. 40% of mutations seen in VLCAD gene are accounted by 779C>T, 830_832del and 848 T>C mutations, it is imperative to screen the entire gene following an initial screen of three common mutations routinely for asymptomatic neonates. This tier screening approach would avoid false-negative diagnoses of VLCAD deficiency in newborns. Genomic DNA isolated from samples have been analyzed with our tiered approach in which a total of 13 amplicons covering the entire VLCAD gene including coding regions and splice junctions are PCR-amplified with a high-fidelity DNA polymerase and analyzed by DNA double strand sequencing under fully optimized conditions for mutation screening. A initial single amplicon screen identifies the three most common mutations while the rest 12 amplicon screen identify any other mutation in the gene thus a cost-effective and sensitive screen. Advent of genotype-phenotype correlation in this disorder, the information derived from mutational analysis is essential in designing the appropriate follow-up and therapeutic regime for these patients. This screening process would also provide carrier frequencies of the most common VLCAD mutations in the population.

1451/W

MPS-Brazil Network: 3 years improving diagnosis and management of Mucopolysaccharidoses in Brazil. *I. Schwartz[1,2], A. Federhen[1], C. Rafaelli[1], U. Matte[1], J. Coelho[1], M. Burin[1], R. Giugliani[1], MPS-Brazil Network Group.* 1) Medical Genetics Serv, Hosp Clinicas de Porto Alegre, Porto Alegre RS, RS, Brazil; 2) Genetics Departament, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.

Purpose: To present the results of the first 3 years of operation of the MPS-BRAZIL NETWORK, a collaborative initiative involving centers from different Brazilian regions (Southeast-SE, South-S, Northeast-NE, North-N and West-Center-WC) to improve the diagnosis and management of MPS diseases in the country. Methods: The Medical Genetics Service of Hospital de Clínicas de Porto Alegre is the coordinating center, providing the information on the management of patients and making available the laboratorial tests necessary for their diagnosis. Results: 1) During this period, 493 Brazilian patients suspected of having MPS were investigated; the diagnosis of MPS was confirmed in 289/493 patients (58.6%); 2) MPS I was confirmed in 68/289 patients (mean age at diagnosis: 6yr6mo; origin: 37 SE, 18 S, 8 NE, 3 CO, 2 N); 3) MPS II was confirmed in 87/289 patients (mean age at diagnosis: 7yr11mo; origin: 39 SE, 24 NE, 19 S, 3 N, 2 WC); 4) MPS III was confirmed in 36/289 patients (mean age at diagnosis: 7yr2mo; origin: 23 SE, 6 NE, 5 S, 1 N, 1 CO); 5) MPS IV was confirmed in 22/289 patients (mean age at diagnosis: 11yr5mo; origin: 9 NE, 7 SE, 6 S); 6) MPS VI was confirmed in 71/289 patients (mean age at diagnosis: 6yr11o; origin: 29 SE, 28 NE, 5 N, 5 S, 4 WC); 7) MPS VII was confirmed in 5/289 patients (mean age at diagnosis: 4yr10mo; origin: 3 SE, 2 NE). Conclusions: MPS II, I and VI seem to be the most frequent types of MPS in Brazil, and MPS III seems to be underdiagnosed. There seems to be a difference in regional distribution of MPS, since MPS I is more common in the S and SE regions, while MPS VI seems to be less frequent in the S region. Mean age at diagnosis was found to be high in all the MPS types. Support: CNPq, Shire, Genzyme, Biomarin.

1452/W

An Improved Dried Blood Spot Screening Method for Gaucher Disease. *M. Titlow, H. Kallwass, J. Barranger, J. Keutzer.* Therapeutic Protein Research, Genzyme Corporation, Framingham, MA.

Gaucher disease is caused by a deficiency of the lysosomal enzyme glucocerebrosidase. Many patients are misdiagnosed or remain undiagnosed. A simple screening method could increase diagnosis rate and allow for early implementation of therapy when needed to prevent the serious complications of Gaucher disease. We developed a rapid and reliable screening assay for measuring glucocerebrosidase activity in dried blood spots (DBS) based on the method of Chamoles et al. [Clin. Chim. 317:191(2002)]. A fluorescent assay was developed using the substrate 4-methylumbelliferyl-alpha-D-glucopyranoside and conduritol B epoxide (CBE), an irreversible inhibitor of glucocerebrosidase. The difference in activity in the presence and absence of CBE is used to distinguish glucocerebrosidase activity from that of other β-glucosidase isoenzymes. We measured glucocerebrosidase activity in DBS samples from 43 untreated Gaucher disease patients and 153 normal adults. Activity in the Gaucher disease samples ranged from below the limit of detection to 4.4 pmol/(punch*h). Activity in the normal samples ranged from 5.6 to 34.7 pmol/(punch*h) with a mean of 10.9 pmol/(punch*h). The assay was sensitive enough to differentiate DBS from patients with Gaucher disease from normal controls. The DBS assay's speed, throughput, and low cost make it an ideal method to screen for Gaucher disease. The applicability of this method for diagnosing Gaucher disease remains to be determined.

1453/W

Does Geography influence the phenotype of Fabry disease in females? *M.A. BARBA[1], D. HUGHES[2], P. DEEGAN[3], A. LINHART[4] on behalf of the European FOS Research Group.* 1) UNIVERSITY HOSPITAL, ALBACETE, Spain; 2) Royal Free, University College Medical School, London, UK; 3) University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; 4) Charles University, Prague, Czech Republic.

Fabry disease (FD) is a lysosomal storage disorder with heterogeneus expression in females. Vascular involvement is prominent in FD. Environmental factors influence atherosclerotic vascular disease, so we hypothesize that similar factors may influence the disease expression in females with FD. To explore differences in the severity of manifestations of females in different European countries, we used the multi-centre Fabry Outcome Survey (FOS). A modified version (FOS-MSSI) of the Mainz Severity Score Index (MSSI) was calculated in females without treatment. That score was compared between patients living in Northern Europe (n=244), and those in Mediterranean Europe (n=125), according the SCORE system. It was performed an analysis of covariance, with age as covariate. **RESULTS**:

	All ages	< 20 years	20-40 years	40-60 years
Northern Europe	12.4 (9.3)	7.5 (6.8)	10.9 (7.5)	13.4 (8.9)
Mediterranean E	7.9 (7.6)	3.4 (4.0)	5.5 (6.0)	10.7 (7.6)
p value	<0.0001	0.0055	0.0010	0.0844

The most consistent difference was in the General and Neurological indexes, but not in the Cardiovascular nor Renal ones. Data on Pain attacks, Muscle Pain, Peripheral Oedema, Angiokeratomas and Diarrhoea, show a significant lower frequency in women from the Southern countries. **CONCLUSION**: The mean severity of females with FD (FOS-MSSI) is greater for females living in Northern Europe compared to the Mediterranean area, except for those aged older than 40. Further studies are required to confirm these data and analyse dietary and environmental factors in more detail.

1454/W

Gene expression analysis of quadriceps muscle from patients with infantile-onset Pompe disease. *R. Palmer[1], K. Ciociola[1], M. Zhang[2], S. Richards[1], R. Mattaliano[3], R. Pomponio[1]*. 1) Clinical Laboratory Science, Genzyme Corp, Framingham, MA; 2) Gene Analysis, Genzyme Corp, Framingham, MA; 3) Therapeutic Protein Development Genzyme Corp, Framingham, MA.

Pompe disease is an autosomal recessive disorder caused by a deficiency of acid alpha glucosidase (GAA), the enzyme required to hydrolyze lysosomal glycogen to glucose. Despite the common underlying deficiency, disease phenotype and response to enzyme replacement therapy (ERT) varies and may be influenced by other genetic and environmental factors. As a means to identify possible genetic modifiers of disease and response, we used Affymetrix HU133 Plus 2.0 Genechips to analyze RNA expression profiles of baseline quadriceps muscle biopsies from patients with infantile-onset Pompe enrolled in a clinical trial for treatment with MYOZYME® (alglucosidase alfa). Comparison of chip data between patients based on clinical outcome data yielded a list of differentially expressed genes, a subset of which we have confirmed by qRT-PCR. While many of the observed differences likely reflect stress-related changes due to the overall glycogen load observed in each sample, a smaller subset, including MYH1, MYH4, MYH8, ANKRD1, and ANKRD2, point to subtle differences in muscle fiber composition and response to stretch that may contribute to differences in severity and therapeutic response between patients. Additionally, concordant upregulation of several genes involved in insulin receptor signaling suggest possible alterations in glucose uptake and utilization that could accelerate the ongoing glycogen accumulation in some patients. Taken together, this data provides insight into the transcriptional differences present between patients which may contribute to differences observed in patient severity and outcome to therapeutic intervention.

1455/W

Genetic analysis of the GAA gene for 47 newborn screening samples. *P. Labrousse[1], L.M. Hire[1], Y.H. Chien[2], S.C. Chiang[2], W.L. Hwu[2], J. Keutzer[1], R. Pomponio[1], T. Scholl[1]*. 1) Genzyme Corp. Cambridge, MA; 2) National Taiwan University Hospital, Taipei, Taiwan.

Genzyme Genetics has launched a sequencing assay for genotyping the entire coding region and intron/exon boundaries of the acid α-glucosidase (GAA) gene. This clinically validated assay identifies sequence variants in DNA isolated from various specimen types. Extensive use of quantitative metrics enabled the development of this sensitive, specific, and robust assay. In addition, a workflow implementing assay-ready frozen reagent plates for PCR and sequencing enhanced quality assurance and turn-around time. Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of lysosomal GAA. One application of the assay described above was in support of a Taiwanese newborn screening (NBS) pilot program. To aid clinicians in better delineating the enzyme ranges between normal and those defining infantile or late-onset Pompe disease, we sequenced 47 samples from the NBS program that had enzyme activity just above the range indicating Pompe disease. The majority of the samples were carriers of either a known deleterious mutation or contained sequence variants of unknown significance (78%). It is known that some variants (e.g. p.G576S and p.E689K) have been shown to contribute to reduced enzyme activity in phenotypically normal individuals. Overall, 13 novel variants and 6 distinct haplotypes were identified in this population. Two haplotypes have not been previously reported. In this study we were able to characterize the molecular defects that most likely were the contributing factor to the observed low GAA activity in these infants. By combining genotyping with the practice of analyzing enzyme activity and other clinical parameters, clinicians will have comprehensive information when decisions for intervention and treatment are required in symptomatic individuals or those identified by NBS. Likewise, genotype determination may offer some predictive value as to the patient status as it relates to Pompe disease. Genotyping also provides individuals with a family history a means of assessing carrier status which aids in genetic counseling of these patients.

1456/W

GALNS Gene Analysis and Expression profiles in Morquio A Patients' Fibroblasts. *L. Carraresi[1], C. Filoni[1], A. Caciotti[1], R. Parini[2], M.A. Donati[1], S. Tomatsu[3], E. Zammarchi[4], R. Guerrini[1], A. Morrone[1]*. 1) Metabolic and Muscular Unit, Clinic of Ped. Neurol., AOU Meyer, Florence, Italy; 2) Metabolic Unit, S. Gerardo Hospital, Monza; Milan; Italy; 3) Ped. Res. Inst., St. Louis University, St. Louis, USA; 4) Dept of Ped., University of Florence, Florence, Italy.

Mucopolysaccharidosis IVA (MPS IVA, Morquio A) is an autosomal recessive storage disorder caused by deficiency of lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS), required for degradation of keratan sulphate and condroitin-6-sulfate. MPS IVA patients show a broad spectrum of clinical severity with classical forms characterised by severe bone dysplasia and normal intelligence. Here we report the clinical, biochemical and molecular analysis of two classical MPS IVA patients. Direct sequencing of the patients' GALNS gene identified in patient 1 the known splice-site mutation c.120+1G>A at homozygous level and in patient 2 the new mutations p.K129X (nonsense mutation) and c.899-1G>C (splice-site mutation). RT-PCR analysis on total RNA preparations from the patients' fibroblasts showed that the splicing mutation c.899-1G>C in patient 2 causes the skipping of exon 9, and results in a frameshift and a premature stop codon, while no transcript was detected in patient 1 with the known c.120+1G>A mutation. In order to shed light on the molecular basis of pathophysiology of the mutation causing disease in MPS IVA patients here described and study the mutant mRNA stability, we used the high efficient and reproducible quantitative Real-Time RT-PCR. The analysis carried out on patients' fibroblasts RNA confirmed the absence of GALNS mRNA in patient 1, harbouring c.120+1G>A splice site mutation, demonstrating, instead, in patient 2 the presence of both mutated mRNA transcripts. This indicates that c.385A>T (p.K129X) and c.899-1G>C mutations produces mRNAs able to escape NMD pathway, according to the fact that PTC are 50<nt upstream of the respective exon-exon junction. The absence in patient 1 and the presence in patient 2 of two mRNAs leading truncated inactive proteins enable us to make a genotype-phenotype correlation.

1457/W

Functional characterisation of 3 novel missense mutations in the ferroportin 1 gene (Hemochromatosis type 4). *E. Létocart[1], G. Le Gac[1], C. Ka[1], C. Férec[1], H. Fierens[2], W. Wuyts[2], S. Majore[3]*. 1) Inserm U 613, BREST, France; 2) Univ Hospital, Antwerp, Belgium; 3) Camillo-Forlanini Hospital, Rome, Italy.

Background: Hemochromatosis (HC) refers to 5 inherited disorders of iron metabolism. HC type 4 can be distinguished from the other forms as it is transmitted through a dominant mode and that it can predominantly affect Kupffer cells rather than hepatocytes. Ferroportin 1 (FPN1) is the only known membrane protein that can export iron from enterocytes, macrophages and hepatocytes. Upon an iron overload condition, FPN 1 is down-regulated by hepcidin. The FPN1 mutants fall into two functional categories: loss-of-function mutants, which are not able to export iron into the blood circulation, and gain-of-function mutants, which resist to hepcidin. Goal of the study: To demonstrate an association of 3 novel missense mutations with the disease through the development of functional experiments. Patients and methods: The 3 studied mutations were identified in single pedigrees. The patients originated from Belgium, Italy and Ivory Coast. Our functional tests allowed cellular localisation studies (using cell fragmentation and Western-Blottings), iron export measurements (by directly assessing radioactive iron release or indirectly by quantifying the intracellular ferritin levels) and resistance to hepcidin evaluations (using a synthetic 25 aa peptide and subsequently quantifying the intracellular ferritin levels). Results: We have achieved the functional experiments proving that the three tested protein mutants are efficiently addressed onto the cell surface and are able to export iron. We are currently testing resistance to hepcidin to evidence a gain-of-function. Conclusion: All the reported SLC40A1 mutations are rare or private and most of them are missense. This situation excludes the classical genotype/phenotype strategy and clearly requests the development of functional approaches. Moreover, the description of three novel amino-acid changes could give new opportunities to better understand the structure/function relationship between hepcidin, which is a key regulator of the iron homeostasis, and its cell target, namely the ferroportin 1 protein.éé.

1458/W

Gaucher disease therapeutic biomarker: Dried blood spot assays for chitotriosidase genotype and enzyme activity. *M.E. Grace, M. Balwani, R.J. Desnick.* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Gaucher disease (GD), due to the deficient activity of acid β-glucosidase, is the most prevalent lysosomal storage disease. The enzymatic defect results in the progressive accumulation of glycosylceramide (GL-1), primarily in the monocyte/macrophage system throughout the body. Due to the macrophage involvement, GD patients have high levels of plasma chitotriosidase, an enzyme secreted by activated macrophages. CHITO activities in untreated GD patients typically are ~600-fold greater than those in normal controls, and generally, are correlated with disease severity. Plasma CHITO activity has proven useful for monitoring disease severity and the effectiveness of various therapies for GD. The CHITO genotype is key to interpreting plasma CHITO levels in patients, as there are common low activity (G102S) (Grace et al. Hum Mut, Epub 2007) and null (dup24) alleles in ~50% and ~40% of patients, respectively. To facilitate CHITO genotyping and activity assays, a dried blood spot (DBS) assay was developed. The DBS provided a source of DNA for genotyping. The enzyme assay was performed by incubating a 2 mm DBS (equivalent to ~1.4 μL blood) with substrate for 2 hr at 37°C with shaking. There was excellent correlation between the direct plasma and DBS assays over a range of 50 to ~1000 nmol/h/mL. In a test of 30 blinded GD samples, two samples with no activity in the DBS assay were homozygous for the dup24 null allele. The sample with the lowest plasma activity (22 nmol/h/mL) had the lowest mean DBS activity (52 nmol/h/mL). Analogously, the sample with the highest plasma activity (917 nmol/h/mL) had the highest mean DBS activity (860 nmol/h/mL). Replicate assays over a two month time period demonstrated the stability of CHITO activity on the filter paper. These studies demonstrated the feasibility of offering CHITO enzymatic and DNA analyses by DBS for initial evaluation and periodic monitoring.

1459/W

Newborn Screening for Pompe Disease using tandem mass spectrometry. *K. Tuschl[1], A. Muhl[1], J. Keutzer[2], K. Zhang[3], J. Orsini[3], V. DeJesus[4], O.A. Bodamer[1]*. 1) General Pediatrics, University Children's Hospital , Vienna, Austria; 2) Genzyme Inc, Boston; 3) New York State Laboratory, Albany; 4) CDC, Atlanta.

Background: With the advent of novel treatment modalities in lysosomal storage diseases (LSD) such as bone marrow transplantation and/ or enyzme replacement therapies, newborn screening for LSD has become a focus point. From a technological perspective high-throughput newborn screening for LSD may be feasible using different analytical approaches. Among these, screening by tandem-mass spectrometry using unique, specific substrates and internal standards seems to be the most promising method as enzyme activities can be readily measured in dry blood spots from neonatal filter cards. In particular newborns with Pompe Disease are amenable to early enzyme replacement therapy and would potentially benefit the most from newborn screening. Methods: Routine neonatal screening filter cards were punched into 96 well plates and extracted with methanol. Unique Pompe specific substrate and internal standard were added to the solution and the plates incubated overnight at 37OC. Following liquid/liquid and solid phase extraction steps 10 μl of solution were injected into the MS/MS. The formation of product and internal standard were monitored using MRM. Results: GAA activity in 1560 dry blood spot samples: 15.50 + 7.86 umol/l/h; median 14.06; GAA activity in 3 adult Pompe Disease: mean 0.37 umol/l/h (0.16-0.93); median 0.19. Conclusion: Although newborn screening for Pompe Disease using MS/MS may be technically feasible, additional pilot studies have to demonstrate its validity, sensitivity, specificity and the potential to multiplex with additional LSD. In addition, strategies for confirmatory testing, treatment, follow-up care and scientific evaluation have to be defined and agreed upon at an international level.

1460/W

Late-onset Krabbe disease due to loss of expression of the maternal allele and a novel paternal missense mutation in the galactocerebrosidase gene. *I. Warshawsky[1], B. Tsao[2], J.F. O'Brien[3], M.R. Natowicz[1,4].* 1) Dept. of Clinical Pathology, Cleveland Clinic, Cleveland, OH; 2) Dept. of Neurology, Loma Linda University, Loma Linda, CA; 3) Dept. of Laboratory Medicine, Mayo Clinic, Rochester, MN; 4) Genomic Medicine Institue, Cleveland Clinic, Cleveland, OH.

Krabbe disease is a rare autosomal recessive neurodegenerative disorder with diverse clinical presentations and is characterized by CNS myelin loss and peripheral nerve involvement. Mutations of the lysosomal enzyme galactocerebrosidase (GALC) gene and the resulting enzyme deficiency cause the various forms of Krabbe disease. We describe a 37 year old wheelchair-bound man with a 17 year history of progressive leg stiffness/weakness, bladder urgency, decreased erectile function, and difficulty with fine motor function. GALC enzyme activity was variably low in two clinical laboratories. GALC DNA sequence analysis showed: (1) heterozygosity for a paternally-derived novel missense mutation (p.I368T, c.1103T>C); (2) heterozygosity for a paternally-derived synonymous SNP (p.S434S, c.1302C>T); (3) heterozygosity for a maternally-derived non-coding region variant (g.-335G>A); (4) heterozygosity for a maternally-derived synonymous SNP (p.D94D, c.282C>T); (5) homozygosity for a common low enzyme activity polymorphism (p.I546T, c.1637T>C). The proband's 31 year old sister with similarly low enzyme activity and an identical genotype denied symptoms but has pes cavus, mild gait abnormality, and lower extremity hyperreflexia. RNA analyses showed loss of expression of the maternal allele in the proband and his more mildly affected sister; allelic loss was seen in the mother's RNA. In conclusion, studies of this kindred reveal: (1) marked intrafamilial clinical heterogeneity in siblings with late-onset Krabbe disease and identical GALC mutations; (2) the complex combination of mutant alleles in the context of homozygosity for a low enzyme activity polymorphism and variably low GALC activity; (3) the unusual occurrence of loss of expression of one of the two mutant alleles in causing a lysosomal storage disease; and (4) the challenges this case poses for clinical biochemical genetic and molecular diagnostic laboratories.

1461/W

Inherited Metabolic Disorders Clinic Referral Patterns. *J.M. DeLuca, A.R. Siegel, E. Blakely, T. Marchetti, G.L. Arnold.* Pediatrics, University of Rochester, Rochester, NY.

The determinants of access to Inherited Metabolic Disease (IMD) clinics have not been adequately characterized. This pilot study was developed in order to evaluate referral patterns and improve access to services. We retrospectively reviewed the charts of 120 new patients referred to the IMD clinic at the Golisano Children's Hospital between January 2005 and December 2006.

Referral sources included: primary providers (43%), NY state newborn screening (NBS) program (47%), and others (10%). Reasons for referral included: suspected metabolic abnormalities (20%), abnormal biochemical lab results (10%), failure to thrive (6%), developmental delay (6%), hypoglycemia (3%), and other indications (such as evaluation for affected relatives, multiple congenital anomalies, and dysmorphic features). Fourteen of 57 NBS referrals were positive. Of the 63 non-NBS referrals, 12 (19%) received formal diagnoses including Niemann-Pick Type A, Angelman syndrome, peroxisomal disease, neurofibromatosis type 1 and others. In addition, 31 patients (50%) require ongoing management for recurrent hypoglycemic episodes or persistent biochemical abnormalities. Excluding newborn screens, the median interval from onset of symptoms to clinic visit was 1 year; the median age at the time of visit was 2 years. There was no correlation between patient age and length of interval between symptom onset and referral. Thirty-five of these patients were evaluated by other specialties prior to the initial clinic visit including: developmental pediatrics, cardiology, neurology, and other services.

The substantial percentage of patients receiving diagnoses and ongoing clinical management suggests that patients might benefit from earlier referral to IMD clinics. These data will form the basis for an educational outreach initiative for health care providers to encourage earlier referral for patients suspected of having metabolic disorders.

1462/W

***KCNJ11* 23E, not 23K, correlates with an increased risk of obesity and metabolic syndrome in Japanese.** *H. Morisaki[1], E. Mizuta[1], I. Yamanaka[1], Y. Miyamoto[2], Y. Kokubo[3], Y. Yoshimasa[2], T. Morisaki[1].* 1) Dept Bioscience, NCVC Research Inst, Suita, Osaka, Japan; 2) Dept Internal Med, NCVC Hosp, Suita, Osaka, Japan; 3) Div Cardiovascular Preventive Med, NCVC, Suita, Osaka, Japan.

KCNJ11 encodes an ATP-sensitive inward rectifier potassium channel 11 (Kir6.2), a major component of K_{ATP} channel which plays a critical role in regulation of glucose-induced insulin secretion in pancreatic beta cells. The E23K polymorphism (rs5219) in the *KCNJ11* was repeatedly shown to be associated with type 2 diabetes in Caucasians, suggesting individuals with 23K allele are prone to type 2 diabetes (T2D), while several studies in other populations including Japanese failed to reproduce the result. We examined the association of *KCNJ11* E23K with several clinical parameters, including body mass index (BMI), blood pressure, serum lipid levels, HbA1c, fasting blood glucose (FBS) and serum insulin levels in a large population-based Japanese cohort (the Suita Study, n=3637). We also evaluated the association of E23K with T2D and metabolic syndrome in Japanese. The 23E allele, but not 23K, showed a significant association with greater BMI as well as greater weight gain after age 20. We also confirmed this tendency in another group of Japanese healthy subjects. Also, 23E allele showed a significant association with metabolic syndrome, while no association was observed between E23K and T2D in our study. Interestingly, when stratified with FBS levels, 23K allele showed a significant association with decreased basal insulin secretion only with higher FBS groups, while fasting insulin levels or HOMA-beta showed no significant differences between genotypes as a whole. These results indicate that 23K allele is responsible for decreased insulin secretion in higher FBS circumstances that lead to susceptibility to T2D, while 23E alleles can maintain sufficient insulin secretion resulting in obesity and metabolic syndrome possibly through the anabolic effect of insulin. Our observations not only provide the possible explanation of discordances in previous reports, but also provide the caution that *KCNJ11* 23E allele has an increased risk of metabolic syndrome in Japanese.

1463/W

Correlation between dried blood spot thin layer chromatography and plasma high performance liquid chromatography of leucine/isoleucine levels among Filipino patients with Maple Syrup Urine Disease. *C.D. Padilla[1], J.Y. Lee[2], M.A. Demata[1], K.T. Dela Cruz[1], M.D. Chiong[1], C.C. Cavan[1].* 1) Institute of Human Genetics, National Institutes of Health Philippines, Manila, Philippines; 2) Metabolic Service, Genetic Health Services, Victoria, Australia.

Management of patients with maple syrup urine disease (MSUD) includes low protein diet supplemented with special formulas and constant monitoring of branched chain amino acids (BCAA). The gold standard for monitoring BCAA is plasma amino acid analysis using High Performance Liquid Chromatography (HPLC). In a developing country like the Philippines, however, the cost of this test is prohibitive to majority of the patients. In our center, dried blood spot leucine/isoleucine(leu/ile) level by thin layer chromatography (TLC) is often used to diagnose and monitor these patients. This study was done to determine the correlation of leu/ile levels using the two analyses (TLC and HPLC). Paired samples (dried blood spot and plasma) of twelve MSUD patients were collected. There were 8 males and 4 female with ages that range from 6 weeks to 4.9 years. Majority had the classical type of MSUD and the protein diet was restricted between 0.6 gram/kg/day to 1 gram/kg/day of natural protein. Results showed a significant linear correlation (Spearman correlation=0.800) between the two methods (p- value <0.05). A dried blood spot leu/ile level by TLC is an alternative method that can be used in the diagnosis and monitoring of MSUD patients especially in a developing country.

1464/W

Flow cytometric study of leukocytes and cell markers from Fabry disease patients. *P. Rozenfeld[1,3], E. Agriello[2], P. Martinez[2,3], N. De Francesco[1], I. Kisiniovsky[3], C. Fossati[1].* 1) LISIN (Immunolgia), Univ Nacional de La Plata, Buenos Aires, Argentina; 2) 2Servicio de Hematología, Hospital Penna, Bahía Blanca, Argentina; 3) 3AADELFA (Asociación Argentina de Fabry y otras enfermedades lisosomales).

There is much evidence linking glycolipids with the immune system. However, studies of immune cells and molecules in glycolipidosis are scarce, specially in Fabry disease. Aims: To analyze whether the disorder of glycolipid catabolism in Fabry patients is associated with changes in leukocyte subpopulations and their cell markers. Methods/Patients: nine Fabry patients were included in the study, 4 pediatric (median age=10 years) and 5 adults (median age=43 years). Four of the patients were on ERT with agalsidase alfa. Whole peripheral blood samples from Fabry patients and 3 normal controls were used for flow cytometric analysis, using the following monoclonal antibodies: CD4-FITC, CD8-PE, CD3-PerCP, CD56+16-PE, CD19-PerCP, Lin1-FITC, CD14-FITC, CD31-PE, CD1d-PE, TCR Vα24-FITC, CD77-FITC (intracellular staining). Results: The percentages of T and B lymphocytes were within the normal range. A significantly reduced percentage of NK (p=0.0005) and dendritic cells (p= 0.038) was observed, as compared to controls. ERT treated patients showed a higher level of Vα24+ cells compared to non treated Fabry patients (p=0.049) and controls (p=0.045). CD31 expression was lower in granulocytes, monocytes and lymphocytes, being statistically significant in the latter population (p= 0.0058). However, no difference was observed in CD38 expression in any subpopulation. Cell surface expression of CD1d showed lower levels in Fabry patients when compared to the control group (p<0.001). On the contrary, HLA-DR expression was elevated (p= 0.01). Intracellular content of Gb3/CD77 was also analyzed and increased amounts of Gb3 was detected in monocytes (p=0.001), lymphocytes (p= 0.02) and granulocytes (p=0.033). Conclusions: These results suggest that glycolipid disorders may cause changes in leukocyte populations and in expression of cell markers.

1465/W

Information Service on Inborn Errors of Metabolism (SIEM): 5-year report from a pioneer Brazilian call-free service. *C.F.M. Souza[1], S. Herber[1], C.D. Lima[1], L. Giugliani[1], L.F. Refosco[2], M.T. Sanseverino[1], C.B.O. Netto[1], C.L. Rafaelli[1], R. Giugliani[1,3].* 1) Medical Genetics Service, Hospital de Clinicas, Porto Alegre, RS, Brazil; 2) Diet & Nutrition Service, Hospital de Clinicas, Porto Alegre, RS, Brazil; 3) Department of Genetics, UFRGS, Porto Alegre, RS, Brazil.

The SIEM is a pioneer toll-free service in Brazil and South America, having a team specialized in IEM available to help health professionals to diagnose, manage and treat suspected and affected patients. Since IEM are poorly recognized by physicians in Brazil, as in most developing countries, improve diagnosis and management is crucial to provide a better prognosis for patients and/or better counseling to families. Between October 2001 and March 2007, 1043 cases were registered at SIEM. From these, 77% were from South and Southeast Brazil and 52% of the contacts were made by pediatricians or neonatologists. The majority of the professionals (85%) called the SIEM for diagnostic support and early management orientation, 8% to obtain information concerning IEM and 7% for suppport in the follow-up as the diagnosis was already established. From the 1043 entries, 629 (60%) had their investigation for IEM concluded, and on 97 (15.4%) of these cases a diagnosis of IEM was confirmed. From these, 22.7% presented organic acidemias, 20.6% amino acid disorders, 15.5% lysosomal diseasesm, 10.3% energy defects, 10.3%carbohydrate metabolism defects, 6.3% peroxisomal diseases and 14.3% other disturbances. We are convinced that SIEM is an extremely important source of information about IEM, specially in a country were such group of disorders is often unrecognized, helping health professionals to obtain a faster and more efficient diagnosis and treatment, reducing morbidity on these patients and families (PROREXT/UFRGS/Fundacao Medica/Support).

1466/W

Methylation of the promoter of the gene responsible for the *cblC* type of combined methylmalonic aciduria and homocystinuria (*MMACHC*) decreases its gene expression and is associated with a malignant phenotype in a human cancer cell line. *A.D. Loewy, K.M. Niles, D. Watkins, J.P. Lerner-Ellis, J.M. Trasler, D.S. Rosenblatt.* Department of Human Genetics, McGill University, Montreal QC.

A highly metastatic variant (MeWo-LC1) of the poorly malignant MeWo human melanoma cell line cannot grow on tissue culture medium in which homocysteine replaces methionine. MeWo-LC1 is a cellular phenocopy of the *cblC* disorder, characterized by decreased synthesis of adenosylcobalamin and methylcobalamin, decreased incorporation of label from [^{14}C]propionate and 5-[^{14}C]methyltetrahydrofolate into macromolecules, and failure to correct this after cell fusion with *cblC* fibroblasts. Upon identification of *MMACHC*, the gene responsible for the *cblC* disorder, sequencing gDNA from MeWo-LC1 failed to reveal mutations in the coding sequence. However, no *MMACHC* mRNA transcript was detected by PCR amplification after reverse transcribing cellular RNA with polyd(T) primers. Other genes involved in cobalamin metabolism (*MMAA*, *MMAB*, *MTR*, *MTRR*) were all expressed in MeWo-LC1, as were the control genes *GAPDH* and *ACTB*. All of the genes including *MMACHC* were expressed in MeWo, a human lung carcinoma cell line A549, and a normal fibroblast cell line. Transfection of wild-type *MMACHC* under control of a constitutive promoter corrected 5-[^{14}C]methyltetrahydrofolate and [^{14}C]propionate incorporation, as well as the synthesis of methylcobalamin and adenosylcobalamin in MeWo-LC1. The transfection also corrected the methionine dependent phenotype in these cells. Bisulfite sequencing showed that the CpG island 5' of *MMACHC* had low methylation in a control fibroblast cell line (0-14%, n = 15), MeWo had intermediate methylation (5-76%, n = 18) and MeWo-LC1 had high methylation (90-100%, n = 21). Similar methylation results were found by quantitative analysis of DNA methylation using real-time PCR (qAMP). Gene silencing by promoter hypermethylation is a phenomenon known in tumor-suppressor genes, and also appears to be associated with the malignant phenotype in MeWo-LC1 cells.

1467/W

Mitochondrial DNA instability and recessive POLG1 mutations in patients with isolated adult-onset sensory ataxic neuropathy. *S. Bannwarth[1, 2], K. Fragaki[1, 2], J. Pouget[3], D. Figarella-Branger[4], V. Paquis-Flucklinger[1, 2].* 1) Department of Medical Genetics, CHU Nice,; 2) UMR CNRS 6543, Medicine School, University of Nice-Sophia Antipolis; 3) Department of Neurology, CHU Timone, Marseille; 4) Department of Anatomopathology, CHU Timone, Marseille.

Nuclear gene defects affecting mtDNA stability include the mtDNA polymerase (POLG), the adenine nucleotide transporter (ANT1) and the Twinkle helicase. There is a considerable variability in the phenotype associated with POLG1 mutations which are responsible for autosomal dominant and recessive progressive external ophtalmoplegia (PEO), Alpers syndrome, a sensory ataxic neuropathy, dysarthria and ophtalmoparesis (SANDO) and a mitochondrial recessive ataxic syndrome. Nevertheless, patients with POLG1 mutations and sensory ataxic neuropathy always presented with associated muscular and/or central neurological system features. The aim of our study was to test whether POLG1 mutations can be responsible for isolated sensory ataxic neuropathy. We screened 15 patients by direct sequencing. Seven patients were men and the median age of the population was 57 years. The presenting and only feature was ataxia caused by axonal sensory neuropathy. A 50 year-old woman was found to be a compound heterozygous carrying the c.1391T>C mutation (M464T) in combination with the c.2302A>G substitution (K768E). No POLG1 mutation was found in other patients. Nevertheless, a muscle biopsy was performed in two cases. Ragged-red and COX negative fibers were found in one patient, with multiple mtDNA deletions by both long range PCR and Southern blot analysis. The patient was a 67 year-old man who developped ataxic symptoms at the age of 47. ENMG revealed normal motor potentials and absent sensory potentials in the four limbs. No mutations were detected in ANT1 or Twinkle. In conclusion, mitochondrial disease has to be considered as a cause of isolated adult-onset sensory ataxic neuropathy.

1468/W

Evolutionary, structure-function and physiological considerations of a second mitochondrial ornithine transporter, ORNT2. *J. Camacho, D. Nguyen, N. Rioseco-Camacho.* Dept Pediatrics, Univ California, Irvine, CA.

Human ORNT2 is a functional retroposon and member of the mitochondrial carrier subfamily (MCF) of proteins that includes ORNT1, SLC25A20 (carnitine), SLC25A29 (carnitine and ornithine) and SLC25A45 (unknown function). Although a functional ORNT2 protein is present in mammalian species, ORNT2 is not functional in rodentia. Clinically, ORNT2 is important because it may act as a modifier gene in patients with ORNT1 deficiency (Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH) syndrome) and is very likely responsible for the residual mitochondrial ornithine transport seen in these patients' cell lines. Previous studies of HHH patients revealed three ORNT2 polymorphisms that increased (V181G), decreased (G159C) or had no effect on function (V226I). V181G is noteworthy because it occurs in the putative solute recognition site located in the 4th transmembrane domain (TMD) of ORNT1 & ORNT2, a region containing most of the documented ORNT1 mutations, and is prominent in controls of American Indian descent. Our current goal is to determine if ORNT2 polymorphisms differ in their geographic distribution and to characterize the level of residual ornithine transport in control fibroblast cell lines carrying different ORNT2 polymorphisms. Results demonstrate that both G159C and V226I polymorphisms are prevalent in controls of Old World descent; conversely, the V181G change is more prevalent in controls of New World descent. To achieve our second objective, we knocked down (KD) the ORNT1 gene using targeted siRNAs in cultured human and mouse cell lines. Our preliminary siRNA work demonstrates that the ORNT1 mRNA can be significantly KD 24 hours after electroporation. Functional studies are under way. In conclusion, our preliminary observations suggest that changes in ORNT2 amino acids may have been under selective pressure. A caveat of our work is that seemingly neutral amino acid changes may actually have an important integrative physiological function given their unequal frequency distribution. Our work will further elucidate the potential contribution of MCF protein polymorphisms to the variable phenotype of metabolic disorders such as HHH syndrome.

1469/W

In vivo radical species quantification in C. elegans mitochondrial mutants. *R. Lightfoot[1], T. Lamitina[2], E.P. Polyak[1], H. Ischiropoulos[1], M.J. Falk[1].* 1) Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Physiology, The University of Pennsylvania, Philadelphia, PA.

The mitochondrial respiratory chain is associated with oxidant production and altered longevity in C. elegans. These associations have been made largely using in vitro markers of oxidant damage. Dysfunction in either complex I or II is associated with shortened lifespan and increased oxidant damage, while impaired coenzyme Q biosynthesis or complex III function lead to increased longevity and resistance to oxidative stress. To better assess the involvement of individual mitochondrial complexes in oxidant species generation, we developed an in vivo method to quantify C. elegans mitochondrial superoxide production using microscopic fluorescence intensity quantification in the mitochondrial-rich pharyngeal bulbs of worms fed Mitosox, a mitochondrial superoxide indicator dye. Synchronous young adult populations of C. elegans were fed standard E. coli on nematode growth media plates spread with Mitosox. C. elegans strains studied included wildtype (N2) animals, as well as single gene missense mitochondrial mutants in complex I (gas-1), complex II (mev-1), complex III (isp-1), and the long-lived insulin receptor mutant (daf-2). No significant variation in fluorescence intensity was detected among these strains following overnight 5 uM Mitosox exposure. However, a significant fluorescence increase was observed in the mitochondrial mutants compared to N2 following overnight exposure to both 5 uM Mitosox and 100 uM methyl viologen, an agent which induces superoxide production. This suggests mitochondrial mutants have either an increased sensitivity to oxidant stress or a decreased capacity to scavenge oxidant species. Among the long-lived mutants compared to N2, fluorescence intensity was significantly lower in the insulin receptor mutant (daf-2) and greater in the complex III mutant (isp-2). This suggests longevity is not always characterized by resistance to oxidative stress, particularly in mitochondrial mutants. Further studies with additional markers, strains, and stressors are currently underway.

1470/W

Renal carnitine reabsorption in primary and secondary carnitine deficiency. *M.G. Lloyd[1], R. Guymon[2], P. Jungerberg[2], N. Longo[1,2,3], M. Pasquali[1,2,3].* 1) ARUP Inst for Clin and Exp Pathology, Salt Lake City, UT; 2) ARUP Laboratories; 3) Univ Utah, Dept. of Pathology.

Primary carnitine deficiency, a recessive disorder caused by defective OCTN2 carnitine transporters and affecting long-chain fatty acid oxidation, can present with hypoketotic hypoglycemia and/or cardiomyopathy. Affected patients have very low plasma carnitine levels due to increased renal losses. Asymptomatic mothers with primary carnitine deficiency are identified because of low carnitine levels in their infants by newborn screening. Here we evaluate renal carnitine and amino acid reabsorption in adults with primary carnitine deficiency. We analyzed plasma and urine samples from 3 mothers and one child with primary carnitine deficiency, patients with secondary carnitine deficiency due to other metabolic disorders and normal controls. Free and total carnitine concentrations were measured by tandem mass spectrometry. Amino acids were measured by ion-exchange chromatography. Reabsorption of free and total carnitine and individual amino acids for each patient was calculated as percent of filtered load estimated from creatinine clearance. Reabsorption of urinary free and total carnitine was 99.8±0.4% and 98.6±1.4%, respectively, in our controls (7 months-43 years of age). By contrast, three asymptomatic mothers with primary carnitine deficiency had free and total carnitine reabsorption reduced to 69.7±2% and 71±2.5% ($p<0.01$ vs. controls), respectively. Urinary free and total carnitine reabsorption was <20% in a symptomatic 13 year old patient with primary carnitine deficiency. Carnitine reabsorption was comparable to that of normal controls in patients with secondary carnitine deficiency. The average reabsorption of all amino acids was 93.7±7.4%, with no significant differences between normal controls and patients with primary or secondary carnitine deficiency. The quantitative study of urine carnitine reabsorption can distinguish primary from secondary carnitine deficiency. Asymptomatic adult mothers with primary carnitine deficiency have higher residual carnitine reabsorption than that classically described in symptomatic children with primary carnitine deficiency.

1471/W

Lethal neonatal presentation of Medium-Chain Acyl-CoA Dehydrogenase Deficiency. *J.M. Brumblay[1], T.M. Cowan[2], A. Manoukian[3,4], C. Matsumoto[5], L.H. Seaver[1,6].* 1) Kapi'olani Medical Specialists, Honolulu, HI; 2) Stanford University Dept. of Pathology, Palo Alto, CA; 3) John A. Burns School of Medicine Dept. of Pathology, Honolulu, HI; 4) Clinical Laboratories of Hawaii, Maui, HI; 5) Hawaii Dept. of Health, Newborn Metabolic Screening Program, Honolulu, HI; 6) John A. Burns School of Medicine Dept. of Pediatrics, Honolulu, HI.

Medium-Chain Acyl-Coa Dehydrogenase Deficiency (MCADD) is the most common inborn error of fatty acid oxidation. Clinical presentation varies greatly and is unpredictable. Infants born in the state of Hawaii are tested for MCADD on the newborn screening panel through measurement of acylcarnitines by tandem mass spectrometry (MS/MS). We report an infant who died at home on day 3 of life prior to the newborn screning result.

The infant was the first child of unrelated parents. The mother was of Korean ancestry and the father was of Swedish/Irish/French ancestry. The full term infant male was born via emergent cesarean section due to failure to progress and fetal heart rate decelerations. He briefly required oxygen after delivery. He was breastfed and discharged from the hospital at 35 hours of age. He was subsequently found unresponsive in his crib at 61 hours of age. The newborn screening result was reported 3 days later and reveal elevation of (C6) hexanoyl, (C8) octanoyl, (C10) decanoyl carnitines and elevated C8/C10, suggestive of MCADD. Postmortem acylcarnitine profile confirmed the newborn screen result. Autopsy revealed a non-dysmorphic, slightly jaundiced, well developed infant with extensive microvesicular fatty metamorphosis of the liver by microscropy. Further laboratory testing revealed that the father had a normal acylcarnitine profile and the common K329E mutation in ACADM, the MCADD gene. His mother had normal plasma acylcarnitine and urine acylglycine profiles, and was found to have a rare mutation, 449_452delCTGA. This mutation has previously been reported in persons of Asian descent, and has also been associated with symptoms in the first week of life. This case illustrates the extreme variability in presentation of MCADD and the importance of rapid newborn screen results.

1472/W

Screening of Total N-Glycans by Mass Spectrometry of Filter Paper Specimens. *D.C. Dannaway[1], B. Xia[2,3], J.J. Mulvihill[1], R.D. Cummings[2,3].* 1) Department of Pediatrics, Oklahoma University Health Sciences Center, Oklahoma City, OK; 2) Department of Biochemistry and Molecular Biology, Oklahoma University Health Sciences Center, Oklahoma City, OK; 3) Oklahoma Center for Medical Glycobiology, Oklahoma University Health Sciences Center, Oklahoma City, OK.

Many human congenital disorders of glycosylation (CDGs) are caused by defects in assembling or processing of *N*-glycans on glycoproteins, which are typically diagnosed by isoelectric focusing and immunoblotting to identify altered glycoforms of serum transferrin. Since CDGs affect all tissue-derived glycoproteins, we sought to develop a sensitive and non-invasive mass spectrometry (MS) approach to analyze *N*-glycan structures and composition from total blood glycoproteins and to facilitate analysis of neonatal heel-stick blood samples on filter paper. *N*-glycans in total blood glycoproteins from eight normal adult volunteers were released by direct treatment with *N*-glycanase and processed by their binding to Carbograph cartridges and subsequently profiled by matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI-TOF-MS). The expected *N*-glycan structures in serum were found in all donors with a high sensitivity and a high degree of reproducibility. We also prepared total *N*-glycans for MALDI-TOF-MS profiling using whole blood spotted on filter paper and found reproducible sensitivity with as few as 75 microliters of blood. This sensitive procedure or its modifications for analyzing total *N*-glycan structures using serum or filter paper samples and MALDI-TOF-MS may be useful in future studies of screening newborn blood samples for CDGs.

1473/W

Sequencing from dried blood spots in neonates with positive NBS results for MCADD who die before confirmatory testing. *S.E. McCandless[1], R. Chandresekar[2], S. Linnard[2], W. Becker[2], L. Rice[1].* 1) Department of Genetics, Case Western Reserve Univ, University Hospitals Case Medical Center, Cleveland, OH; 2) Ohio Department of Health, Newborn Screening Program, Reynoldsburg, OH.

Medium chain acyl-CoA dehydrogenase deficiency (MCADD) is one of the most common disorders identified by newborn screening (NBS) programs. NBS for MCADD uses measurement of octanoylcarnitine (C8) from dried blood spots. Occasionally, newborns with elevated C8 on NBS die before confirmatory testing can be obtained. Neonates with MCADD can have metabolic decompensation in the neonatal period, raising the question of whether MCADD contributes to some of these deaths. Six such infants were identified by the Ohio NBS Lab in the first 3 years of MS/MS NBS. **Methods:** DNA was extracted from dried blood spots and screened for the common A985G mutation in exon 11 of the MCADD gene, ACADM, using a specific restriction digest method, followed by sequencing of the 12 exons, intron-exon junctions, and several hundred base pairs of the 5' untranslated region. **Results:** The cut-off value for C8 used was 0.7 μg/L. The mean C8 for the six infants was 1.0, much lower than the mean value for confirmed cases. Four of the 6 neonates weighed <700 g, another was 800 g, the last 3200 g. One neonate had multiple abnormalities on NBS. No sequence variants were found in 4 of the 6 neonates. Two subjects had a total of 3 previously known SNPs identified in exons 7 (1) and 11 (2). A heterozygous single nucleotide change deep in the intron between exons 1 and 2 was identified in two patients that is unlikely to be disease causing. **Conclusions:** Sequencing of ACADM in six neonates with elevated C8 on NBS did not identify any significant mutations in the coding region of the gene, suggesting that MCADD was not a contributing factor in these deaths. It is possible that elevated C8 is a non-specific marker for infant distress, particularly in very low birthweight infants. These results suggest that sequencing of ACADM from dried blood spots is a useful follow-up tool to provide the most accurate genetic counseling in the situation of an infant with elevated C8 on NBS who dies before confirmatory testing is obtained.

1474/W

Gamma-hydroxybutyric aciduria and severe lactic acidosis in a young Chihuahua dog. *D.P. O'Brien[1], E. Kelmer[1], G.D. Shelton[2], B.A. Barshop[3], G.S. Johnson[4], S. Kahn[4], E.A. Struys[5], C. Jacobs[6].* 1) Veterinary Medicine & Surgery, University of Missouri, Columbia, MO; 2) Department of Pathology, University of California San Diego, La Jolla, CA; 3) Department of Pediatics, University of California San Diego, La Jolla, CA; 4) Department of Pathology, University of Missouri, Columbia, MO; 5) Department of Pediatrics, University of Pittsburg, Pittsburg, PA; 6) VU University Medical Center, Amsterdam, The Netherlands.

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare, autosomal recessive disorder of children characterized by neurological impairment and gamma-hydroxybutyric acid (GHB) in the urine. We report a 5 month old Chihuahua that presented with a history of waxing and waning ataxia and altered mental status. The dog was severely acidotic (pH 6.938) with elevated serum lactate (18.27 mmol/L). Urine organic acid analysis showed dramatic elevations in lactic acid (>30,000), pyruvic acid (>2,000), and GHB (812 mmol/mol creatinine). SSADH activity in leukocytes was 30% of parallel controls (n=2 dogs). Activity of hydroxyacid-oxoacid transhydrogenase (HOT), an enzyme metabolizing GHB, was comparable to control (n=4 dogs) in autopsied liver from the proband. The *SSADH* coding region was sequenced and no pathological mutations were identified. Severe lactic acidosis seen in this dog is atypical in children with SSADH deficiency. Similarly, although the SSADH activity was decreased, it was not to the extent typically seen in children with SSADH deficiency. We speculate that the decrease could reflect a mutation in the non-coding region affecting gene expression, or a secondary effect on the enzyme perhaps linked to overwhelming lactic acidosis and/or oxidant stress.

1475/T

A 5-year old Argininemia patient diagnosed by Newborn Screen achieved normal growth and development. *S. Yano[1], K. Moseley[1], R. Schein[1], Y. Watanabe[2].* 1) Pediatrics/Genetics Div, 1G24, Women's & Children's Hp, USC, Los Angeles, CA; 2) Department of Pediatrics and Child Health, Kurume University, Kurume, Japan.

Introduction: Argininemia is a rare disorder due to Arginase 1 deficiency. Approximately 20 patients have been reported and only a few reports describing long-term clinical observations are available. Patients are usually asymptomatic in early infancy and are diagnosed late after the onset neurological symptoms. The clinical presentation includes spastic paraplegia, mental retardation, and seizures. With the initiation of newborn screen by MS-MS, arginine levels are measured and noted to be elevated, allowing for diagnosis shortly after birth. It is not well known if early intervention can prevent the neurological insults. Case Report: The patient is an almost 5-y-old Hispanic male born at term by NSVD (BW 7lb 13oz). Newborn screening showed a blood arginine of 327mM (ref: 0-140) at 30 hours of age, a repeat serum arginine at 2 months of age was 768mM (ref: 12-133). Arginase I was undetected on enzyme assay. Initiation of diet management with protein restriction as well as treatment with sodium benzoate, carnitine, and vitamin supplementation started at 3 months of age. He had mild speech delay that resolved by age 2 1/2. The patient's serum arginine level has remained between the range of 2-4 times the normal levels. He has mild liver dysfunction with elevated transaminases and prolonged PT and PTT. Currently the patient has normal physical findings without evidence of spasticity. He underwent a complete developmental assessment at age 4 10/12, which showed normal development. Discussion: Argininemia is a rare urea cycle defect. Long term prognosis of patients with argininemia has not been well known, particularly for patients who are diagnosed prior to occurrence of neurological symptoms. The presented case with history of initiation of treatment from 3 months of age, showed normal growth and development over the past 5 years. This case illustrates that newborn detection of argininemia is possible and early treatment may enable patients to avoid or at least postpone the onset of neurological involvement.

1476/T

Evaluation of glycerol homeostasis and metabolism in glycerol kinase (Gyk) knockout (KO) heterozygous mouse using intraperitoneal glycerol tolerance test (IPGlyTT). *M. Kosuga[1], N.K. Maclennan[1], Y.H. Zhang[1], B.L. Huang[1], E.R.B. McCabe[1,2].* 1) Pediatrics, UCLA, Los Angeles, CA, USA; 2) Human Genetics, UCLA, and Bioengineering, Henry Samueli School of Eng and App Sci, UCLA, and Mattel Children's Hospital at UCLA, Los Angeles, CA, USA.

Glycerol kinase deficiency (GKD) is an X-linked inborn error of metabolism with phenotypes, ranging from asymptomatic hyperglycerolemia to a severe metabolic disorder with vomiting, acidosis and central nervous systems crises. Because glycerol kinase (GK) activity and the GK genotype do not predict phenotype in GKD patients, it is essential to examine glycerol homeostasis and metabolism in a murine model to understand pathogenesis and to evaluate treatment efficacy. We designed an intraperitoneal glycerol tolerance test (IPGlyTT) and studied glycerol tolerance in vivo using Gyk KO heterozygous and wild type (WT) female mice. Mice were weighed to determine the dose of glycerol (2mg/g body weight from a 20% solution) and fasted for 8 hours. The glycerol solution was injected ip into mice and blood samples were collected before injection and then at 30, 60, 90, 120, 180 minutes after injection. Serum glycerol concentrations and hepatic GK activities in Gyk KO heterozygous mice and WT mice were measured using radioassay. Hepatic GK activities in Gyk KO heterozygous female mice were from 30% to 50% of WT female mice. In the IPGlyTT, Gyk KO heterozygous mice had normal serum glycerol concentrations before glycerol injection. Serum glycerol concentrations reached maximum at 30 minutes after injection in both KO heterozygous and WT mice. Serum glycerol concentration in WT mice and GKD KO heterozygous mice remained similarly elevated through 90 min and then began to diverge. The WT mice returned to normal at 120 min, whereas the GKD KO heterozygous mice continued to have elevated glycerol levels through 180 min. Glycerol tolerance was impaired in GKD KO heterozygous mice compared to WT mice. IPGlyTT is useful in assessing glycerol homeostasis and metabolism GKD KO heterozygous mice. We will utilize this test for evaluating the efficacy of cell transplantation and lentiviral gene therapy for GKD KO mice.

1477/T

Evidence that oxidative stress is increased in plasma of patients with peroxisome biogenesis disorders. *M. Deon[1,3], A. Sitta[1,3], A.G. Barschak[1,3], T. Terroso[1,2], M. Pigatto[1,2], A. Barden[1,2], A.B. Oliviera[1], G.O. Schmitt[1], D.M. Coelho[1], L.B. Jardim[1], R. Giugliani[1,3], M. Wajner[1,3], C.R. Vargas[1,2,3].* 1) Medical Genetics Service, HCPA, Porto Alegre, RS, Brazil; 2) Department of Clinical Analysis, Pharmacy Faculty, PPGCF, UFRGS, Porto Alegre, RS, Brazil; 3) Department of Biochemistry, ICBS, UFRGS, Porto Alegre, RS, Brazil.

The peroxisome is a single membrane organelle present in nearly all eukaryotic cells with different metabolic functions. The importance of the peroxisome became more evident by the existence of various severe genetic disorders associated by the failure of the functions of peroxisomes. Defects in peroxisomal functions are associated with major, and often fatal, changes at the neurological level during human development. The peroxisomal disorders are subdivided into two major categories: those in which the organelle is not formed normally (the peroxisomal biogenesis disorders - PBDs) and those that are associated with defects of a single peroxisomal proteins [the single peroxisomal enzyme (transporter) deficiencies - PEDs]. Neurological symptoms and brain abnormalities are characteristic of patients with PBDs. However, very little is known about the pathomechanisms involved in the tissue damage of these disorders. Considering that peroxisome is involved in oxidative reaction and that in a previous study we showed evidence that oxidative stress is probably involved in pathophysiology of other peroxisomal disease - X-linked adrenoleukodystrophy, in the present study we evaluated two oxidative stress parameters, namely as membrane protein thiol content and thiobarbituric acid-reactive species (TBA-RS) in plasma of patients with PBD. It was observed a significant decrease of membrane protein thiol content, indicating a possible protein oxidation, and a significant increase of plasma TBA-RS measurement, indicating a stimulation of lipid peroxidation. It is therefore proposed that oxidative stress may be involved in the pathophysiology of the disorders of peroxisome biogenesis. Financial support: CNPq, CAPES, PROPESQ/UFRGS, PROREXT/UFRGS, FIPE/HCPA, FAPERGS.

1478/T

Generation of the anti-mouse prosaposin specific antibody: Regional accumulation of prosaposin in the hippocampus of saposin D knockout mouse. *J. Matsuda, A. Yoneshige, K. Suzuki.* Institute of Glycotechnology, Future Science and Technology Joint Research Center, Tokai University, Kanagawa, Japan.

Sphingolipid activator proteins (saposins A, B, C, D) are small homologous glycoproteins which are indispensable for *in vivo* hydrolysis of some sphingolipids by lysosomal hydrolases. We generated specific saposin A and D knockout mice that clarified their *in vivo* functions, saposin A as an essential activator of lysosomal galactosylceramidase and saposin D as a lysosomal acid ceramidase activator. In this study, in order to further investigate the biological role of the precursor protein (prosaposin) of the four saposins in the nervous system, we generated an anti-mouse prosaposin-specific antibody by immunizing rabbit with three oligopeptides, selected from the prosaposin sequences that do not encode any saposins and investigated its regional expression in the brain of murine models of lysosomal storage disorders including saposin D knockout mouse (Sap-D-/-). Immunoblotting study of brain homogenates demonstrated a major band at around 65kDa corresponding to the predicted size of prosaposin. Most of the 65 kDa band shifted to 58 kDa by glycoproteinase F treatment. The intensity of the 65kDa band was most dramatically increased in Sap-D-/-. Immunohistochemical study of the brain showed that the expression of prosaposin was observed predominantly in the hippocampus, olfactory bulb and cerebellum. In Sap-D-/-, its immuno-reactivity was dramatically increased with some regional increases, most notably in the hippocampal CA3 pyramidal neurons. By confocal microscopic analysis, hippocampal pyramidal neurons in the CA3 area contained prosaposin immuno-reactive inclusions, co-localizing with an endoplasmic reticulum (ER) marker protein. Immunoelectron microscopic analyses also revealed the retention of prosaposin immuno-reactive products in the ER. These findings indicate that the prosaposin dose exist uncleaved in the specific type of cells suggesting a regional role in the nervous system, especially in the hippocampal CA3 pyramidal neurons. This prosaposin specific antibody may be useful to further investigate regional functions of prosaposin itself in the nervous system.*in vivo*.

1479/T

Glycogen Storage Disease Type 1b and Myasthenia Gravis: casual or causal association? *D. Melis[1], F. Balivo[1], R. Della Casa[1], A. Romano[1], M. Sibilio[1], F. Fontana[1], B. Capaldo[2], A. Imbroinise[2], G. Parenti[1], G. Andria[1].* 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Dept Internal Medicine, Federico II Univ, Naples, Italy.

We describe a patient affected by Glycogen Storage Disease type 1b (GSD1b) and myasthenia gravis (MG). The diagnosis of GSD1b was suspected when she was 8 months old on the basis of the phenotype including growth retardation, hepatomegaly, fasting hypoglycemia, hyperlactic acidemia; the diagnosis was confirmed by enzyme studies and by mutation analysis of the glucose-6-phosphate transporter gene, showing the C911T and 1211-1212 delCT mutations. The patient developed a multi-systemic disease, typical of GSD1b; in particular, she showed neutropenia (treated with Granulocyte-Colony Stimulating Factor -G-CSF), hyperuricemia (treated with allopurinol), nephropathy (treated with ACE-inhibitors) and inflammatory bowel disease (treated with metronidazole). When she was 26 years old, a diagnosis of MG was performed, on the basis of the clinical picture, characterized by dropped lid, diplopia, dysarthria, dysphagia and easy tiredness. The biochemical, imaging and electrophysiological studies showed: no antibodies blocking a receptor of acetylcholine, small thymic gland at chest TC, signs of exhaustion of neuromuscular transmission at electromyography. Different therapeutic approaches were used including pyridostigmine, intravenous immunoglobulins, steroids, plasmapheresis and azhathioprine; however symptomatology exclusively improved with plasmapheresis. The association of GSD1b and MG has never been reported in the literature. We hypothesize that metabolic derangement, and/or G-CSF treatment are risk factor for MG in GSD1b patients. To support the last hypothesis we underline that sporadic occurrence of autoantibodies to GM-CSF has been reported in patients affected by MG, in particular with 'seronegative' MG.

1480/T

Molybdenum Cofactor Deficiency: Extension of Phenotype and Neuroradiology. *S. Prasad, S. Sharma, A. Petros, K. Nischal, J. O'Connell, P. Daubeney, A.K. Saggar.* Paediatric Intensive Care, Cromwell Hospital, London, SW5 0TU, United Kingdom.

Molybdenum Cofactor Deficiency (MCD) is a rare, autosomal recessive, inborn error of metabolism, characterised by seizures and lens dislocation. Cardiomyopathy has not previously been recognised as an association. We report an 18 month old Arab girl, born of consanguineous parents. Following an unremarkable pregnancy, hypotonia and delayed milestones were observed at 5 months. She was admitted to hospital at the age of 13 months, with a severe respiratory illness requiring ventilation. Her symptoms progressed to include seizures, dystonia and bulbar dysfunction. An MRI demonstrated necrosis in the thalami and lentiform nucleus, and cerebral volume loss; these findings were reported as compatible with hypoxic ischaemic injury. The patient was transferred to the UK for further evaluation. Examination revealed gross developmental delay, microcephaly (0.4th percentile), dystonia and posturing. Ophthalmic examination confirmed bilateral dislocated lenses. She required ventilatory support, and due to persistent tachycardia and intermittent hypertension, an echocardiogram was performed showing significant cardiomyopathy. The combination of lens dislocation and refractory seizures is consistent with sulphite oxidase deficiency. High sulphite, high xanthine and low uric acid levels in the urine suggested MCD. This was confirmed by the finding of a homozygous deletion at the MOCS2 gene. The patient was given a low cystine, low methionine diet which made no difference to her clinical symptoms. The literature suggests that MRI changes in this disorder are similar to hypoxic brain injury; on further review of the MRI films, the appearances were felt to be consistent with metabolic disease. A subsequent MRI confirmed these appearances. This case highlights the unusual clinical features of hypertension and cardiomyopathy in MCD, lack of response to dietary restriction, and MRI findings similar to those found in hypoxic injury. MCD should be considered in cases of refractory seizures and lens dislocation; review of MRI scans by a specialist neuroradiologist is advised.

1481/T

Patterned cerebellar Purkinje cell degeneration in mouse models of saposin D deficiency and Niemann-Pick type C disease is associated with selective expression of sphingosine kinase. *A. Yoneshige, J. Matsuda, A. Sasaki, K. Suzuki.* Institute of Glycotechnology, Future Science and Technology Joint Research Center, Tokai University, Hiratsuka, Kanagawa, Japan.

Selective death of cerebellar Purkinje cells (PCs) is a prominent feature of the neuropathology in several lysosomal storage disorders (LSD). It is well known that both Niemann-Pick type A/B and type C (NPC) show PC death. However there has been no accepted explanation of this selective loss of PCs in these metabolic disorders. We generated saposin D knockout mouse (Sap-D-/-) which is the deficiency of the essential *in vivo* activator of lysosomal acid ceramidase, and found selective PC death with accumulation of α-hydroxyl fatty acid-ceramide. In 2004, Terada *et al.* demonstrated a compartmentalized expression of sphingosine kinase 1 (SPHK1), which phosphorylates sphingosine (Sph) to form a bioactive lipid mediator, sphingosine 1-phosphate (S1P), in PCs of wild type mice. In this study, we investigated the relationship between SPHK1 expression and the pattern of PC death in two murine models of sphingolipidosis, Sap-D-/- and mouse model of human NPC disease (NPC1-/-). Immunoblotting study using anti-mouse-SPHK1 antibody revealed that SPHK1 was localized in the cytosolic fraction and its protein level was higher in the cerebellum than that in the cerebrum. By immunohistocemical study, both Sap-D-/- and NPC1-/- showed selective and progressive loss of PCs. The pattern of PC death was symmetrical in stripes in coronal sections corresponding to the expression of SPHK1. Especially in Sap-D-/-, most of the SPHK1-negative PCs were completely lost. In contrast, SPHK1-positive PCs could survive even in the terminal stage. The study with primary cultured PCs also confirmed that PCs from Sap-D-/- die earlier than those from the wild type. In the primary cultured PCs from Sap-D-/- mice, SPHK1 was expressed dominantly in the dendritic spines of PCs. These findings indicate that the intracellular levels of sphingomyelin, ceramide, Sph and S1P have important roles in the survival and maintenance of PCs. Regulating the expression of SPHK1 could be a possible way to protect neurons from cell death in some LSDs.

1482/T

Obesity-associated SNP distribution in the Mexican Mestizo population. *D. Velazquez[1], I. Silva-Zolezzi[1], K. Carrillo[1], R. Reynoso[2], M.F. Herrera[2], E. Garcia-G[2], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine, Mexico; 2) National Institute of Medical Sciences "Salvador Zubiran", Mexico.

Obesity is considered a global pandemia. Two thirds of the Mexican population has an abnormal body mass index and close to 24% can be defined as clinically obese. Published studies have suggested that hispanic-americans, and possibly Mexicans have a higher risk for the disease. Most of the Mexican Mestizo population results from admixture of any of 65 ethnic groups, with Spaniards, an in a lesser extent Africans. A number of genetic polymorphisms have been associated to obesity in different populations. To characterize obesity-associated SNPs in the Mexican population we genotyped 5 SNPs in obesity genes: *ADRB2* 1666C>G (rs1042741), *ADRB3* 387C>T (rs4994), *AGRP* 499A>G (rs5030980), *POMC* 934G>A (rs1042571), and *PPARG2* 34C>G (rs18012182) in 1,033 Mestizos from 5 different states of Mexico: Guanajuato, Sonora, Veracruz, Yucatan and Zacatecas. These SNPs have shown association to obesity in at least 2 different populations. Our results showed the following average frequencies: *ADRB2* 0.20 (CI95 0.14-0.27); *ADRB3* 0.34 (0.11-0.25); *AGRP* 0.02 (0.01-0.05); *POMC* 0.12 (0.07-0.18); and for *PPARG2* 0.12 (0.06-0.17). Comparative analysis of the Mexican allele frequencies with other world populations showed significant differences as in the case of *POMC* 934G>A (rs1042571) with an average minor allele frequency (MAF) of 0.15 for Mestizos vs. 0.30 for CEU (HapMap) and for *ADRB2* 1666C>G (rs1042741) MAF of 0.22 for Mestizos and 0.40 fro CEU. We are conducting a multicentric study including morbid obese patients from different parts of Mexico. Preliminary results show MAF of 0.33 (CI95 0.16-0.50) for *ADRB2*; 0.10 (0.01-0.2) for *ADRB3*; 0.06 (-0.02-0.15) for *AGRP*; 0.20 (0.06-0.34) for *POMC*; and 0.06 (0.01-0.22) for *PPARG2*. Our preliminary results show differences for some MAFs in obese patients compared to population controls. We propose that our case-control study in the Mexican admixed population can lead to the identification of novel genes associated to obesity.

1483/T

Multidisciplinary evaluation in 12 Mucopolysaccharidose type II or Hunter Syndrome patients prior Enzyme Replacement Therapy. *T. Vertemati[1,2], C. Micheletti[1,2], C. Mendes[1,2], E. Fraccaro[1,2], E. Menegatti[1,2], J. Correa[1,2], M. Rant[1,2], T. Pereira[1,2], A. Martins[1,2].* 1) CREIM, UNIFESP, São Paulo, São Paulo, Brazil; 2) Ambulatório de Doenças Metabólicas do Centro de Referência em Erros Inatos do Metabolismo (CREIM), Escola Paulista de Medicina da Universidade Federal de São Paulo (UNIFESP/EPM), São Paulo, São Paulo, Brazil.

BACKGROUND: Mucopolysaccharidose Type II (MPS II) is a Lysossomal Storage Disease (LSD) that results from human lysossomal function defects by inherited enzyme iduronate-2-sulfatase (I2S) deficiency, which is an X-linked disease. OBJECTIVE: The purpose was to evaluate the MPS II patients by our multidisciplinary group and perform clinical and laboratorial evaluations, at baseline and proceed with the control after beginning enzyme replacement therapy (ERT) at 26th and 52th weeks. METHODS AND PROCEDURES: In 2007, 12 MPS II patients, 11 male and 1 female, were evaluate by CREIM's multidisciplinary group. RESULTS: The MPSII patients had ages ranging from 3,7 to 14,3 years old, mean age of 9,3 years old. The age at first signs and symptoms ranging from 3 to 27 months old, mean age of 15,1months; after the first evaluation. At physical examination, all of them presented facial dysmorphisms characteristic of the MPSII, hepatomegaly, short stature, skeletal deformities and joints stiffness. We found macrocephaly in 6 (50%), neurodegeneration leading to profound mental retardation in 11(91,66%), seizures in 4 (33,33%), hyperreflexia or hyporreflexia in 6 (50%), hypotonia or spastic hypertonia in 10 (83,33%), Babinski and Clonus in 3 (25%), agitation and attention deficiency in 6 (50%), hemiparesis or spastic tetraparesis in 6 (50%), past hydrocephalie in 1 (8,33%), abnormal cardiac exam in 9 (75%). DISCUSSION AND CONCLUSION: The possibility of specific treatment of this disease with Idursulfase impose the necessity of evolutive attendance on several clinical and laboratorial aspects of the MPS II patients for better knowledge of the benefits regarding the treatment, irreversible damages prevention and promotion of better quality of life.

1484/T

Adult onset congenital erythropoietic porphyria, an extra challenge in the diagnosis of porphyria. *S. Gustafson[1], A. Lichtin[2], K. Astrin[3], C. Eng[1].* 1) Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH; 2) Department of Hematology, Cleveland Clinic Foundation, Cleveland, OH; 3) Mt Sinai School of Medicine Porphyria DNA Testing Laboratory, New York, NY.

The porphyrias are a group of metabolic diseases of heme biosynthesis for which clinical and laboratory findings show significant heterogeneity. Congenital erythropoietic porphyria (CEP) is a rare autosomal recessive porphyria, caused by germline mutations in the uroporphyrinogen III synthase gene (UROS), typically presenting in childhood with severe photosensitivity, urine discoloration, and erythrodontia. Rare cases of adult-onset CEP have been reported, with homozygous germline UROS mutations causing reduction in enzyme production. In some cases of late-onset CEP associated with hematologic abnormalities suggestive of myelodysplastic syndrome, somatic loss-of-heterozygosity or acquisition of a second loss-of-function mutation within bone marrow cells has been suggested as a cause. A 47 year-old woman who first presented in her late 20's with mild cutaneous photosensitivity resembling porphyria cutanea tarda, a family history suggesting AD inheritance, and urine, blood and stool porphyrin levels consistent with CEP, was referred to genetics for clarification of the diagnosis and discussion of treatment options. Mutation analysis of UROS identified germline compound heterozygosity for a common CEP missense mutation (C73R), and a novel missense change (R138H). This is the first report of R138H, which, based on the presentation of our patient, is suspected to be a mild CEP mutation. Literature review revealed at least 17 cases of adult-onset CEP, with male predominance; 9 (52.9%) were associated with thrombocytopenia or myelodysplasia. Age at diagnosis ranged from 23 years of age to 72 years, and presentation varied from mild to severe in skin findings, urine discoloration, and other organ involvement. Our patient, with a mild phenotype, adds to the limited literature reporting adult-onset CEP and helps to expand the clinical spectrum of CEP. Further studies are needed to define whether UROS testing should be included as a diagnostic adjunct in atypical CEP and CEP-like presentations.

1485/T

Demographics in FOS - the Fabry Outcome Survey. *A. Mehta[1], M. Beck[2], J. Clarke[3], A. Linhart[4], G. Sunder-Plassmann[5].* 1) Dept Haematology, Royal Free Hosp, London, UK; 2) Children's Hosp, Univ Mainz, Germany; 3) The Hosp for Sick Children, Toronto, Ontario, Canada; 4) Charles Univ, Prague, Czech Republic; 5) Div Nephrology & Dialysis, Dept of Medicine III, Medical Univ Vienna, Austria.

Background: Fabry disease (FD) is a progressive multisystemic X-linked lysosomal storage disease caused by deficiency of the enzyme α-galactosidase A. FOS - the Fabry Outcome Survey - is an international, multicentre database established to monitor patients with FD and their response to enzyme replacement therapy (ERT) with agalsidase alfa. Aims: To examine changes in the characteristics of a large group of patients at enrolment in FOS over a 2-year period. Methods: Data from patients enrolled in FOS were analysed in terms of demography and clinical manifestations of FD. Results and discussion: As of February 2007, FOS contains data from 1329 patients with FD (41% men; 42% women; 8% boys; 9% girls). More females than males have been enrolled since 2005 (82 females v 32 males in 2006/7). A total of 220 children are enrolled in FOS, 54% of whom are girls. These data may reflect increased understanding that women and children with FD can be symptomatic. FOS contains treatment data for 882 patients (51% men, 36% women, 13% children); 66% of the patient population. A larger proportion of women is receiving ERT than reported in 2005 (57% v 47%, respectively). Since 2001, the age at start of ERT has remained relatively stable overall; however, in children, the mean age at start of ERT has decreased (mean age in boys, 12.2 years in 2006/7 v 13.8 years in 2001/2; mean age in girls, 12.8 years in 2006/7 v 15.5 years in 2001/2). Furthermore, the mean severity of signs and symptoms of FD at the start of treatment, as measured by the FOS adaptation of the Mainz Severity Score Index, has decreased in both male and females since 2001. This may indicate that physicians are becoming more aware of the early manifestations of FD and the importance of prompt therapeutic intervention with ERT. Conclusion: These data suggest that heightened awareness in the medical community may have decreased the delay between the onset of symptoms and diagnosis of FD, resulting in the earlier initiation of ERT.

1486/T

Clinical manifestations and consequences of the P479L mutation of carnitine palmitoyl transferase type 1 deficiency in the Alaskan native population. *M.L. Raff[1,2], C. Trahms[2], S.H. Hahn[1,2], P. Schubert[1], M.A. Parisi[1,2], M. Hannibal[1,2], I.A. Glass[1,2], C. Leblond[3], M.J. Bennett[4].* 1) Div Genetics, Children's Hospital, Seattle, WA; 2) Dept Pediatrics, Univ of Washington, Seattle, WA; 3) State of Alaska Dept of Health, Anchorage, AK; 4) Depts of Pathology and Lab Med, Univ of Penn, Philadelphia, PA.

Carnitine palmitoyl transferase type 1 catalyzes the transport of long-chain fatty acids into the mitochondria where they undergo oxidation of energy production. The P479L mutation in the CPT1A gene exists in high frequency in the native populations of Alaska. The pathogenicity of this mutation has previously been questioned. Physical examination of 50 children with confirmed P479L CPT1A deficiency identified by state newborn screening, testing of siblings of affected children, or investigation of suspicious cases, noted clinical findings in 16 individuals. Clinical findings were found among infants and toddlers who had not followed a regimen of strict avoidance of fasting. Complications included hypoglycemia, elevated liver transaminases, seizures, hypotonia, motor delays, and death. Two cases diagnosed by DNA testing were undetected by newborn screening using tandem mass spectrometry, indicating that an increased level of suspicion of the conditon is necessary to avoid missing a diagnosis of cpt-1 deficiency. Recognition of this disorder in populations at risk followed by swift initiation of measures to avoid fasting, particulary during times of illness, can reduce morbidity and mortality. P479L has been shown elsewhere to cause insensitivity to malonyl-CoA inhibition of fatty acid oxidation, suggesting that there is a low-level constitutive enzyme activity with this mutation. Others have demonstrated that failure to inhibit cpt-1 function in rats is associated with increased feeding behavior. P479L may promote increased caloric intake in times of plenty, perhaps improving survival in times of famine, but conversely may contribute to higher rates of obesity and insulin resistance. The existance of other mild deficiency alleles is under investigation.

1487/T

Clinical Outcomes in Menkes Disease Patients with a Potentially Treatment-Responsive ATP7A Mutation, G727R. *J.R. Tang, A. Donsante, S.G. Kaler.* Unit Pediatric Genetics, LCG, NICHD/NIH, Bethesda, MD.

Menkes disease is a fatal neurodegenerative disorder caused by diverse mutations in an X-linked copper transport gene, ATP7A. Emerging evidence from a long-term clinical trial indicates that favorable response to early copper treatment in this disorder requires a mutation that allows partial copper transport. We identified and characterized such a mutation, G727R, in two infants treated beginning at 25 and 228 days of life, respectively. G727R occurs in exon 10 of ATP7A and affects the second transmembrane segment of the copper-transporting ATPase encoded. Western analysis showed equivalently reduced quantities of the full length protein in both G727R patients' fibroblasts compared to wild type, indicating post-translational degradation or possibly aberrant splicing, mechanisms we are formally investigating. Importantly, the mutant allele complemented the *S. cerevisiae* copper transport mutant, Δccc2, a finding consistent with partial functional activity. Patient A was diagnosed at 22 days of age, based on a positive family history, and entered the clinical trial of daily copper injections at 25 days of age. After two years, his neurodevelopment was normal in fine motor, personal-social, and language spheres, and delayed in gross motor (13 months). Brain MRI at 15 months of age revealed slightly delayed myelination. Serial electroencephalographs showed no abnormalities. Patient B had no family history of the disorder and was diagnosed at 6 months of age based on clinical phenotype, biochemical findings (low serum copper), and molecular testing that revealed G727R. Based on the relatively favorable response to copper treatment in patient A, we enrolled patient B, beginning at 228 days (≈7.6 mos) of age. After 6 months, however, there were no major improvements in his clinical status. Neurodevelopmental levels ranged from 1 to 2 months. The EEG was markedly abnormal and his seizures persisted. The outcomes in these two patients, each with the same missense mutation associated with residual copper transport function, confirm the importance of early medical intervention and highlight the potential benefit of newborn screening for Menkes disease.

1488/T

Clinical characteristics of patients with mucopolysaccharidosis type II: the Hunter Outcome Survey (HOS). *J.E. Wraith[1], B.K. Burton[2], J. Muenzer[3], M. Beck[4], R. Giugliani[5], J. Clarke[6], R. Martin[7] on behalf of the HOS investigators.* 1) Royal Manchester Children's Hospital, Manchester, UK; 2) Children's Memorial Hospital, Chicago, IL, US; 3) University of North Carolina, Chapel Hill, NC, US; 4) Children's Hospital, University of Mainz, Mainz, Germany; 5) Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; 6) Hospital for Sick Children, Toronto, Ontario, Canada; 7) Saint Louis University, Saint Louis, MO, US.

Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a rare, X-linked metabolic disorder caused by deficiency of the enzyme iduronate-2-sulfatase and characterized by the progressive accumulation of glycosaminoglycans throughout the body. To assess the natural history of MPS II and the long-term safety and efficacy of enzyme replacement therapy (ERT), a global survey, the Hunter Outcome Survey (HOS), was initiated in 2006. As of May 2007, HOS contained data on 263 patients with MPS II, 63 of whom were receiving ERT. Mean ages (± SD) at enrollment, onset of symptoms and diagnosis were 13.5 (8.4), 1.8 (1.6) and 4.3 (4.2) years, respectively. Patients had many signs and symptoms; facial dysmorphia was reported in 95%, enlarged liver/spleen in 89%, neurological manifestations and joint stiffness in 84%, chest and lung symptoms in 83%, cardiovascular manifestations in 82%, hernia in 78%, enlarged tonsils/adenoids in 68%, nasal obstruction in 34%, seizure disorders in 18% and hydrocephalus in 17% of patients for whom data were available. Many of these manifestations were present before diagnosis. Among patients with cardiovascular manifestations, 57% reported valve disease, 53% murmur and 8% cardiomyopathy. The prevalences of tachycardia (7%), bradycardia (2%) and arrhythmia (4%) were low. On average, patients developed cardiovascular manifestations 3.0 (0.6) years after diagnosis. By 6 years of age, approximately 50% of patients had developed cardiovascular abnormalities, increasing to 90% by age 15 years. HOS is proving to be a valuable resource to help better understand the clinical features of MPS II. Future analysis of HOS data should be able to determine the long-term impact of ERT and allow the development of evidence-based clinical management guidelines.

1489/T

A Novel Karyotype Involving a Pericentric X Chromosome Inversion and Mosaicism for Two Cell Lines with Different 5p Deletions, Presenting as Neonatal Hyperammonemia. *J. Gillis, A. George, M. Shago, D. Antinucci, A. Feigenbaum, M. Rohrbach.* Division of Clinical and Metabolic Genetics, University of Toronto, Toronto, ON, Canada.

We present a case of a term female infant presenting on day 3 of life, with poor feeding, hypotonia, and lethargy. She subsequently developed seizures and vomiting and was found to have hyperammonemia and respiratory alkalosis, suggestive of a urea cycle defect. Low levels of plasma citrulline and arginine, as well as elevated levels of urine orotic acid were in keeping with a diagnosis of ornithine transcarbamylase (OTC) deficiency. OTC deficiency, an X-linked trait, is the most common inherited urea cycle disorder, with the most severe form usually restricted to males. Chromosome analysis revealed the karyotype; 46,X,inv(X)(p11.4q26.1),del(5)(p15.2)[16]/46,X,inv(X)(p11.4q26.1),del(5)(p14)[15] consistent with a mosaic female having 2 abnormal cell lines, each possessing one X chromosome with an apparently balanced pericentric inversion with estimated breakpoints at regions Xp11.4 and Xq26.1. Late replication studies demonstrated that the inverted X chromosome was active in the majority of cells. In addition, mosaicism was apparent for 2 different deletions on chromosome 5 resulting in monosomy 5p15.2 to 5pter in 52% and monosmomy 5p14 to 5pter in 48% of cells. FISH analysis of region 5p15.2 confirmed a deletion in all cells in keeping with Cri du Chat syndrome, which is known to be associated with severe psychomotor/mental retardation. Parental karyotypes were normal. Molecular analysis of the OTC gene did not identify a mutation, and assay for OTC enzyme activity in liver tissue is planned. In summary, we describe previously unreported de novo chromosomal rearrangements involving three chromosome abnormalities resulting in at least two clinically unrelated diseases, manifesting as a severe and complex phenotype in a female infant. We recommend that chromosome analysis be considered even in cases of apparent simple metabolic disease, in order to further elucidate the underlying molecular and cytogenetic mechanisms, as well as to provide appropriate genetic counseling and future prenatal testing.

1490/T

Risk assessment of acute vascular events in Congenital Disorder of Glycosylation type Ia. *J.B. ARNOUX[1], V. VALAYANNOPOULOS[1], N. BODDAERT[2], F. BRUNELLE[2], N. SETA[4], M.D. DAUTZENBERG[3], P. DE LONLAY[1].* 1) Pediatric Metabolism Unit, Hôpital Necker - Enfants Malades, Paris, France; 2) Pediatric Radiology, Hôpital Necker - Enfants Malades, Paris, France; 3) Hematology Laboratory, Hôpital Necker - Enfants Malades, Paris, France; 4) Department of Biochemistry, Hôpital Bichat, Paris, France.

Background: The congenital disorder of glycosylation type Ia (CDG-Ia) presents a broad clinical spectrum. Some patients suffer from acute vascular events (AVE; thrombosis and bleeding) and stroke-like events. No correlations have been made between the marked haemostasis abnormalities of CDG-Ia and the occurrence of acute vascular events.

Methods: We report on 6 patients with CDG-Ia presenting vascular events, then we analyzed the clinical and haemostasis data of 39 CDG-Ia patients described in the literature, 17 with vascular events (E) and 21 unscathed of any event (EF), to determine risk factors for acute vascular events in CDG-Ia.

Results: Acute vascular events occurred in patients younger than 15 years, especially when there was fever and prolonged immobilization. Haemostasis and liver cytolysis were statistically abnormal in patients younger than 5 years whatever the occurrence of vascular events, and they normalized with time. Higher factors VIII and IX activities were statistically observed in the E cluster (p=0.03) compared to the EF cluster. The activity/antigenicity ratio for Protein C (p=0.02) was also higher in the E group.

Conclusion: CDG-Ia patients younger than 15 years old are at risk of acute vascular events. The paradoxical results - abnormal VIII and IX factors in EF patients and normal results in E patients, while XI, AT, PC and ASAT, ALAT are abnormal in both groups - could suggest a disequilibrium between prothrombotic and antithrombotic factors in the E group. Vascular events may also occur in patients where glycoproteins are proportionally more hypoglycosylated, particularly Protein C.

1491/T

Natural course of MELAS -Japanese cohort compaired with literature study-. *S. Yatsuga, Y. Akita, J. Nishioka, K. Katayama, T. Matsuishi, Y. Koga, MELAS Study Group.* Pediatrics and Child Health, Kurume University School of Medicine, Kurume, Fukuoka, Japan.

<Objective> MELAS, a maternally inherited multi-system disorders, is the most common mitochondrial disorders. However the natural course and epidemiology has not been discovered. The specific aim of the present study is to estimate the incidence, natural course, and severity of the disease seen in MELAS based on the nationwide survey in Japan, and compared them with the literature. <Methods> In Japanese cohort study, we analyzed the questionnaires. In literature study, we searched the date base from all publications identified via Medline search using "MELAS" as a keyword. We categorized the MELAS patients into two subtypes. The patients who firstly recognized any signs of psychomotor developmental delay before the age of 18 year-old defined as juvenile type of MELAS. The patients who firstly recognized any abnormality after the age of 18 year-old defined as adult type MELAS. <Result> In Japan, MELAS showed 32% of total mitochondrial disorder. In Japanese cohort study, the age of onset, and the death was 9, and 32.2, and 15 and 40 years old in juvenile and adult type. In the literature, the age of onset was 8.3, and 31, the age of death was 18.9 and 41.5 in juvenile and adult type. Among symptoms recognized at the diagnosis of MELAS, the short stature and developmental delay was significantly highly recognized in juvenile than those seen in the adult type. On the other hand, deafness and diabetes mellitus were more significantly recognized in adult than those seen in the juvenile type. Using Japanese mitochondrial disorders rating scale, some juvenile type showed rapidly increase their exacerbation within 5 to 9 years after the onset of disease. However adult type showed slowly increase their exacerbation during all the course of the disease. Using Kaplan-Meier survival analysis, juvenile type has 3.2 times more chance of death than those seen in adult type. <Conclusion> In Japanese cohort study, MELAS was divided into two subtypes, juvenile and adult type, and was the progressive and currently untreatable inherited disorder.

1492/T

Differential distribution of type 2 diabetes-related polymorphisms in Mexican Mestizo and indigenous populations. *L. del Bosque-Plata, J. Fernandez-Lopez, E. Hernandez-Lemus, M. Arrieta-Gonzalez, K. Carrillo-Sanchez, A. Inchaustegui, C. Rangel, I. Silva-Zolezzi, J. Estrada-Gil, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Most of the Mexican population results from admixture of any of 65 ethnic groups, with Spanish, and in a lesser extent Africans. To explore whether this population origin have influenced T2D-associated allele frequencies in our population, we analyzed SNPs in 13 genes previously associated with T2D in at least two studies with a $p < 0.01$. We analyzed 1,104 samples from the Mexican Genomic Diversity Project, from six states of Mexico: Yucatan, Guanajuato, Guerrero, Sonora, Veracruz, and Zacatecas. In addition, we analyzed samples from five Amerindian groups: 60 Mazatecan (MT), 34 Nahua (ST) (San Luis Potosí), 60 Purepecha (Pu), 25 Nahua (XV) (Veracruz), and 62 Otomi (OT). We compared allele frequencies with those of the international HapMap populations. The following SNPs of T2D-associated genes were genotyped using TaqMan *KCNJ11* (rs5219), *PPARG* (rs1801282), *HNF4A* (rs2144908), *SLC2A1* (rs841853), *CAPN10* (rs3792267), *TCF7L2* (rs7903146), *ADIPOQ* (rs2666729), *PTPN1* (rs914458), *GCK* (rs3757840), *LMNA* (rs4641), *SLC30A8* (rs13266634), *HHEX* (rs1111875), *EXT2* (rs3740878). Results were tested for HWE, allele and genotype frequency differences were calculated by Fisher exact test and Fst. This comparative analysis show SNPs with small variation within mestizos between regions as in the case of *CAPN10, PTPN1* , and others with differences of more than 10% as in the case of *PPARG* , *SLC2A1* , *HNF4A* , *ADIPOQ* and *TCLF7L2* ; in some cases a marked frequency variation between the Amerindian groups: The allele frequencies of the SNPs *HNF4A* rs2144908 (XV 08-Pu 28;Mestizo:Gue 39-Son 55); HapMap:CEU 81;HCB 51,JPT 57YRI 93) and *TCF7L2* rs7895340 are lower in all the Amerindian groups(MT.05-ST.11) from those in mestizo groups (Yuc .15-Son .26) and from those reported in the Hap Map populations (CEU 25,HCB 02,JPT 02,YRI 29). Our results show a wide range of MAFs in T2D-associated SNPs, making evident that our complex population history may have implications.

1493/T

Diagnosis of Pompe disease in different age groups using a dried blood spot assay. *D. Bali, M. Changela, JL. Goldstein, SP. Young, PS. Kishnani, H. Zhang, J. Dai, DS. Millington.* Dept Pediatric Med Genetics, Duke Univ Medical Ctr, Durham, NC.

Pompe Disease (acid maltase deficiency; Glycogen Storage disease type II) is caused by a deficiency of the lysosomal enzyme, acid alpha-glucosidase (GAA). GAA deficiency results in glycogen accumulation in multiple tissues, particularly skeletal, cardiac and smooth muscles. Measurement of GAA activity in dried blood spots (DBS) (Zhang et al, Genet. Med. 2006, 8: 302-306) is a rapid and reliable method for diagnosing Pompe disease, being less invasive than assays in cultured skin fibroblasts or muscle biopsies. We report our diagnostic experience of this assay, in both younger (≤3 yrs) and older (≥ 3 yrs) patient populations, including correlation with urinary tetrasaccharide biomarker, GAA activity in fibroblasts and muscle and DNA mutation analysis. In the older patient population we have reviewed the reported clinical indications for testing, including symptoms of limb girdle muscular dystrophy and other myopathies that resemble late-onset Pompe disease. 18% of younger patients tested (14 of 79) had DBS GAA deficiency, and follow-up testing was performed in 9 patients for whom samples were received. 12 of 14 younger patients were referred because of cardiomyopathy. A similar incidence of GAA deficiency (20%; 45 of 220 samples) was observed for older patients and follow-up testing was performed for 19. Muscle weakness (71%) was the most common reason for testing in the older patient population. One third of these cases were defined as proximal muscle weakness and 2/3rd were undefined. Family history (21%) was the second most common reason. 16% of the older patient group were reported to have respiratory involvement. The majority of patients (>50%) were referred from genetics or neurology clinics. Approximately 20% of tested patient population had GAA activity below the control range, but above the range for known affected patients. Additional testing was recommended for these patients. These results will aid our understanding of patients who will benefit from DBS GAA testing. Correlation with other diagnostic tests will improve recommendations for confirmatory testing.

1494/T

Overexpression of mitochondrial Leucyl-tRNA synthetase restores the mitochondrial dysfunctions caused by the MELAS-associated tRNALeu(UUR) A3243G mutation. *M. Guan[1,2], R. Li[1].* 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229.

The A3243G mutation in the tRNALeu(UUR) gene causes mitochondrial encephalomyopathy, lactic acidosis, stroke-like symptoms (MELAS) and other disorders including diabetes and deafness. Cytoplasmic hybrids (cybrids) cell studies demonstrated that this mutation results in decreased level and aminoacylation capacity of the tRNALeu(UUR), thereby leading to a decrease in the steady-state levels of affected tRNA. A failure in the tRNALeu(UUR) metabolism is responsible for the reduced rate of mitochondrial protein synthesis and the respiration defects. However, attempts to correct the mitochondrial dysfunctions caused by this mtDNA mutation have so far been unsuccessful. We hypothesized that overexpression of mitochondrial Leucyl-tRNA synthetase (LeuRS) in the cybrids carrying the A3243G mutation can correct the defects in mitochondrial tRNA metabolism, consequently increasing the level of mitochondrial translation. For this purpose, human LeuRS cDNA was cloned into a pcDNA3 vector and transfected into 43B cell line carrying nearly homoplasmic A3243G mutation and a cell line derived from same subject but lacking this mutation. Resultant stable transfectants expressing the LeuRS cDNA exhibited an increase in the level of aminoacylated and steady-state tRNALeu(UUR) as well as other mitochondrial tRNAs, compared with parental cybrids carrying the A3243G mutation. These are likely responsible for an increasing of in the rates of mitochondrial protein synthesis and respiration in these resultant stable transfectants expressing the LeuRS cDNA, relative to the parental cybrids carrying the A3243G mutation. This suggests that the overexpression of mitochondrial Leucyl-tRNA synthetase restore the mitochondrial dysfunctions caused by the MELAS-associated tRNALeu(UUR) A3243G mutation.

1495/T

Clinical Characteristics of MPS I Patients in the MPS I Registry. *O. Bodamer, for the MPS I Registry European Board of Advisors.* General Pediatrics, University Children's Hospital, Vienna, Austria.

Background and Methods: Mucopolysaccharidosis I (MPS I) is a progressive and often life-threatening autosomal recessive disease caused by deficiency of the lysosomal enzyme α-L-iduronidase and resultant multisystemic accumulation of glycosaminoglycans. Data collected by the MPS I Registry (established by BioMarin/Genzyme LLC) provide insights on the natural history and broad phenotypic spectrum of MPS I. Demographics and frequency and age of onset of signs and symptoms were evaluated for all patients and different phenotypic presentations in the Registry.

Results: As of January 2007, the MPS I Registry contained data from 585 patients (292 males, 293 females) in 29 countries. Median current age was 9.4 y. Most patients (59%) were classified as Hurler (H), followed by Hurler-Scheie (H-S) (26%) and Scheie (S) (12%). Median age at diagnosis was 0.8 y for H patients, 3.7 y for H-S patients and 9.0 y for S patients. The five most common signs and symptoms varied according to phenotype. In H patients they were: coarse facial features (94%), corneal clouding (88%), hepatomegaly (83%), kyphosis (81%) and hernia (75%); in H-S patients: corneal clouding (84%), coarse facial features (83%), hepatomegaly (81%), hernia (73%) and joint contractures (72%); and in S patients: valve abnormalities (87%), corneal clouding (86%), joint contractures (81%), hernia (65%) and carpal tunnel (62%). These signs and symptoms were first reported at median ages of ≤1.1 y for H patients, 3.3-4.2 y for H-S patients and 2.5-12.5 y for S patients. In all phenotypes, median age at onset of corneal clouding was similar to age at diagnosis and median age at onset of hernia was ≤3.3 y.

Conclusions: Corneal clouding and early hernia are prominent in all MPS I patients. Coarse facial features and hepatomegaly are more prominent on the severe end of the MPS I spectrum (H and H-S) whereas joint contractures and carpal tunnel are more prominent on the attenuated end (S).

1496/T

Aortic root dilatation is highly prevalent in male patients affected with Fabry disease and correlates with the presence of a megadolicho-ectatic basilar artery. *D.P. Germain[1], B. Diebold[2], S. Peyrard[3], A.I. Martin-Mista[1], K. Benistan[1].* 1) Centre de reference de la maladie de Fabry et des maladies hereditaires du tissu conjonctif. Deparment of Genetics, Hopital Raymond Poincare, Garches, France; 2) Department of Cardiology, HEGP, Paris, France; 3) URC, HEGP, Paris, France.

Background : Fabry disease (FD, OMIM 301500) is an X-linked metabolic storage disorder due to the deficiency of lysosomal alpha galactosidase A, and the subsequent accumulation of glycosphingolipids throughout the body. While FD pathophysiology basically results from multifocal small-vessel involvement, little is known about the involvement of large vessels with the exception of the classically described ectatic vertebral and basilar arteries. Methods: Using echocardiography, we prospectively investigated aortic root diameter in 71 consecutive hemizygous males (mean age : 40 years, range 16-66 years) and 67 heterozygous women with a (mean age : 41 years, range 15-67 years)affected with classic FD. Cranial MRI was also simultaneously performed in all patients (n=138). Results: The mean aortic diameter was 33.2 mm (SD = 5.8) in males and 32.9 mm (SD = 5.7) in females. Of 71 male patients, 17 (24 percent) had an aortic root dilation (diameter > 40 mm). In contrast, only one heterozygote had aortic root diameter > 40 mm. Out of the 17 hemizygotes with aortic dilatation, 9 had a megadolicho-ectatic basilar artery on cranial MRI. Out of the remaining 54 hemizygotes, 7 additional cases of ectatic basilar artery were found. Discussion : This is the first study demonstrating that, in addition to its microvascular involvement, FD is associated to an increased risk of developing aortic root dilatation in male patients. Aortic root dilation was detected in 24 percent (n=17) of our 71 hemizygous male patients. Aortic root dilatation was statistically associated with the presence of a dolicho-ectatic basilar artery (corrected Chi square, P=0.004). We recommend to search for aortic root dilatation and dilative arteriopathy of the vertebrobasilar circulation in male patients affected with FD.

1497/T

International Registry and Growth Charts for Morquio A: Insights in the natural course of the disease. *A.M. Montano[1], S. Tomatsu[1], G.S. Gottesman[1], M. Smith[2], T. Orii[3].* 1) Dept Pediatrics, St Louis Univ, St Louis, MO, USA; 2) Int Morquio Org, Phoenix, AZ, USA; 3) Dept Pediatrics, Gifu Univ, Gifu, Japan.

Mucopolysaccharidosis IVA (MPS IVA; Morquio A disease) is a lysosomal storage disorder caused by deficiency of N-acetylgalactosamine-6-sulfate sulfatase. A progressive skeletal dysplasia is commonly observed among the MPS IVA patients. The assessment of physical activity and growth of MPS IVA patients is essential for monitoring disease activity, progression and response to treatment. To understand the natural course of this disease and to provoke awareness, we conducted a study in which MPS IVA patients were asked to fill out a questionnaire with inquiries regarding family history, diagnosis, signs and symptoms, height, weight, surgical history, physical activity, and general complaints. In this study, Morquio A growth charts are based on the cross sectional and longitudinal data provided by the questionnaire. 2,695 measurements of height and weight were obtained from 326 patients (172 males, 154 females) from 42 countries enrolled in the Morquio A Registry program. The mean age of patients was 14.9 years for males and 19.1 years for females. Initial symptoms and diagnosis were reported at a mean age of 2.1 and 4.7 years, respectively. 50% of patients underwent surgical operations to improve their quality of life. The most frequent surgical sites include neck (51%), ear (33%), leg (26%) and hip (25%). The birth length for affected males and females was 52.2 ± 4.7 cm and 52.2 ± 4.5 cm, respectively. The mean of birth weight for affected boys was 3.53 ± 0.66 kg and for affected girls was 3.44 ± 0.61 kg, which is similar to values of normal control charts. On the other hand, the final adult height for affected males and females was 122.5 ± 22.5 cm and 116.5 ± 20.5 cm, respectively. The mean weight for men over 18 years old was 43.02 ± 18.02 kg and for women 36.7 ± 14.5 kg. Resulting charts of height and weight centiles for Morquio A patients were compared with those of the Center of Disease Control and Prevention. The results of this study provide a reference for assessment in the medical routine follow-up and in the evaluation of the efficacy of novel therapies.

1498/T

Pathogenesis of the Mucopolysaccharidoses: Differential Effects of Glycosaminoglycan Storage on Cartilage Versus Synovial Tissue. *C. Simonaro[1], X. He[1], E. Eliyahu[1], N. Shtraizent[1], M. Haskins[2], E. Schuchman[1,3].* 1) Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY 10029; 2) University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104; 3) Department of Gene & Cell Medicine, Mount Sinai School of Medicine, New York, NY 10029.

We have previously shown that glycosaminoglycan (GAG) storage in the mucoploysaccharidoses (MPS) leads to inflammation and apoptosis within cartilage, most likely through activation of the lipopolysaccharide (LPS) signaling pathway. We have now extended these findings to synovial tissue, and further explored the mechanism underlying GAG-mediated disease. Gene and protein expression analysis of synovial fibroblasts from rats with MPS type VI revealed that numerous inflammatory molecules were elevated, including several molecules important for LPS signaling (e.g., toll-like receptor 4 and lipoprotein binding protein). Elevation of tumor necrosis factor-alpha, in particular, led to up-regulation of an essential osteoclast survival factor, ligand of receptor activator of NF-kB (RANKL), resulting in the appearance of multinucleated osteoclast-like cells in the bone marrow and osteopenia. Treatment of normal synovial fibroblasts with GAGs also led to production of the “pro-survival” lipid, sphingosine-1-phosphate, resulting in enhanced cell proliferation consistent with the hyperplastic synovial tissue observed in MPS patients. In contrast, GAG treatment of normal chondrocytes led to production of the “pro-apoptotic” lipid, ceramide, confirming the enhanced cell death we had previously observed in MPS cartilage. These findings have important implications for the pathogenesis and treatment of MPS, and have further defined the mechanism of GAG-stimulated disease.

1499/T

Multiple OXPHOS deficiency and mitochondrial DNA depletion in the liver of a patient with CblA methylmalonic aciduria sensitive to vitamin B12. *A. Brassier[1,2], V. Valayannopoulos[1], S. Romano[1], M. Sarzi[2], D. Chretien[2,3], P. Hue[2], J. Kaplan[2,3], D. Rabier[4], A. Rötig[2,3], A. Munnich[2,3], Y. de Keyzer[2], P. de Lonlay[1,2].* 1) Metabolic Unit, Necker-Enfants Malades Hosp, Université Paris V, Paris, France Paris, France; 2) INSERM-U781; 3) Genetic Department; 4) Biochemistry Laboratory.

Background: Defects of adenosylcobalamin A, which is the coenzyme of the methylmalonyl-CoA mutase (MUT) is responsible for methylmalonic aciduria (MMA) responsive to vitamin B12. A few reports have supported the hypothesis that secondary respiratory chain deficiency could be the cause of complications observed in MMA patients. Case report: A patient with cblA MMA responsive to vitamin B12 (homozygous c.387, nonsense mutation in exon 2 of the MMAA gene) and considered to have a well-controlled metabolic disease with a very low urinary excretion of methylmalonic acid, presented with an extremely sudden and severe visual impairment due to optic atrophy without retinal degeneration. Six months later, he presented with a severe metabolic distress, with lactic acidosis and multiorgan failure leading to death. Results: A multiple OXPHOS deficiency was found in the patient’s liver with reduced absolute activity values of mtDNA-encoded complexes (I, III, IV and V) and abnormal activity ratios. A profound mtDNA depletion was also identified in the liver, with a residual mtDNA content of 16%. Conclusion: We describe for the first time multiple OXPHOS deficiency and mitochondrial DNA depletion in the liver of an MMA-CblA, B12 sensitive patient. Deficient methylmalonyl-CoA mutase results in an accumulation of methylmalonyl-CoA, but also in a reduction of succinyl-CoA, which affects the activity of the succinyl-CoA synthase (SCS), known to be responsible for mtDNA depletion, and influences the overall flux of the tricarboxylic acid (TCA) cycle. This hypothesis confers a major role to the TCA cycle in the physiopathology of long-term complications in MMA.

1500/T

Interleukin-6 (IL-6) promoter and C-reactive protein (CRP) gene polymorphisms and levels compared to Mainz Severity Score Index (MSSI) in Fabry disease±. *G. Chicco[1], G. Altarescu[2], C. Whybra[3], S. Delgado-Sanchez[3], N. Sharon[4], M. Beck[3], D. Elstein[1].* 1) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Genetics Unit, Shaare Zedek Medical Center,Jerusalem, Israel; 3) Universitäts-Kinderklinik, Mainz, Germany; 4) Department of Statistics, School of Public Health, Hebrew University, Jerusalem, Israel.

Objectives: Fabry disease is a multi-system disorder with phenotypic hetereogeneity partially explained by genotype. Elevated IL-6 and CRP plasma levels are associated with increased risk and worse outcome of ischemic events, serious prognostic signs in Fabry disease. Methods: 56 patients (34 hemizygous males, 22 females) were studied. A promoter polymorphism (-174 G→C) of IL-6 gene associated with serum IL-6 levels was compared to the Mainz Severity Score Index (MSSI) in patients. C-reactive protein (CRP) serum levels and polymorphism (1059 G→C) were evaluated as inflammation markers to ascertain a possible inflammatory etiology. Non-parametric ANOVA, Fisher’s exact, Bonferroni, and Hardy-Weinberg (HW) statistics were used. Results: Mean age of adults = 42 (range: 26-58) years; 29 patients received enzyme therapy (ERT). Mean total MSSI = 26.7 (moderate disease) but females were lower (total: 23.4±12.6 vs 32.2±13.6). Controls but not patients were in HW equilibrium. Significant correlations existed between all MSSI scores and IL-6 genotypes in females but only 3 MSSI scores in males. IL-6 C/C genotype was significantly correlated with 3 MSSI sub-scores, generally two-fold higher. There were no significant correlations with CRP levels/polymorphisms and MSSI scores or with IL-6 polymorphisms. CRP levels decreased after ERT in patients with a G allele in IL-6 but increased in patients with C/C (p=0.003). Conclusions: Prevalence of C allele in IL-6 significantly influences MSSI i.e. clinical severity, especially in females. This is unrelated to IL-6 as a pro-inflammatory marker as demonstrated by lack of correlations with CRP levels/genotypes. IL-6 may be a prognostic marker in Fabry disease, especially the presence of a C allele and especially in females.

1501/T

Late-onset Tay-Sachs disease (LOTS): cognitive function. *D. Elstein[1], G. Pastores[2], G.M. Doniger[3], E. Simon[3], I. Korn-Lubetzki[4], E.H. Kolodny[2], A. Zimran[1].* 1) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Neurogenetics Unit, Department of Neurology, NYU School of Medicine, NYC, NY, USA; 3) Neurotrax Corp., Teaneck, NJ, USA; 4) Department of Neurology, Shaare Zedek Medical Center, Jerusalem Israel.

Objective: LOTS (chronic GM2-gangliosidosis) is a rare, erratically progressive neurodegenerative disorder, due to mutations of the α-subunit of Hexosaminidase A gene, with residual enzyme activity. Manifestations include progressive proximal muscle weakness, dysarthria, incoordination, tremor, and psychosis. Onset is in early adulthood. Substrate reduction and chaperone therapy are under investigation. Our purpose is to assess cognitive function using a computerized system that is user-friendly and non-threatening for follow-up of LOTS patients and after therapeutic interventions. Methods: Cognitive testing uses the computerized Mindstreams® system (NeuroTrax Corp., NJ) which is inexpensive, brief (<1 hour), and essentially self-administered. Outcome parameters are calculated using automatic algorithms. To permit averaging performance across outcome parameters (e.g., accuracy, reaction time), each parameter is standardized according to age and years of education and fit to an IQ-style scale (mean=100, SD=15). Standardized subsets of outcome parameters are averaged producing 7 ‘index scores’: memory, executive function, visual-spatial, attention, information processing, and motor skills, and a Global Cognitive Score (GCS). Results: Mean values of index scores for 16 evaluable patients (age: 23-65 years) from 2 centers ranged from 75.5-91.0; mean GCS was 79.2; one patient skewed results because of scores >104 in all domains. Despite small sample size, testing for the hypothesis that scores are equal to normal populations was rejected (p=.0003). Scores did not correlate with age or education but did correlate with a LOTS-specific severity score assessing neurological, non-cognitive, parameters. Conclusions: The computerized Neurotrax system allowed even moderately impaired patients to complete the battery without frustration. The most affected domains appear to be memory, executive function, and verbal function.

1502/T

FGF23 gene is associated with renal phosphate leak in calcium nephrolithiasis. *T. Esposito[1], G. Mossetti[2], D. Rendina[2], G. De Filippo[3], A. Ciccodicola[1], F. Gianfrancesco[1], P. Strazzullo[2].* 1) Institute of Genetics and Biophysics, Italian National Research Council, Naples, Italy; 2) Department of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy; 3) Pediatric Endocrinology, Gaetano Rummo Hospital, Benevento, Italy.

Nephrolithiasis is a common disorder, affecting about 10% of the western population. Approximately 20% of patients with calcium nephrolithiasis and normal parathyroid function show reduced serum levels of phosphate associated to reduced renal phosphate reabsorption (i.e. renal phosphate leak). In this setting, we previously demonstrated that circulating levels of fibroblast growth factor 23 (FGF23), a hormone-regulating phosphate homeostasis, were significantly higher compared to stone formers without renal phosphate leak and to healthy controls. We collected 106 stone formers, 17 of them with renal phosphate leak, and 87 healthy controls and we sequenced the entire regulative and coding regions of FGF23 gene. We detected in 10 out of 17 stone formers with renal phosphate leak a non-synonymous change T239M in FGF23 gene. The T239M allele and genotype frequencies in stone formers with renal phosphate leak were significantly higher compared to controls [CvsT allele frequencies (p=0.03) and genotype frequencies (p=0.007)], and to stone formers without renal phosphate leak [CvsT allele frequencies (p=0.024) and genotype frequencies (p=0.002)]. No significant differences were found for T239M allele and genotype frequencies between stone formers without renal phosphate leak and controls The missense variation Thr239Met could significantly influence the structure as well as the biological properties of the FGF23 protein. In conclusion, our results indicate, for the first time, that there is a genetic link between T239M missense variant of the FGF23 gene and renal phosphate leak in patients with calcium nephrolithiasis.

1503/T

Dilated aortic root: a previously unrecognized complication of mitochondrial diseases. *N. Fouladi[1], N. Brunetti-Pierri[1], J. Towbin[2], J.L. Jefferies[2], V.R. Sutton[1], J. Belmont[1], W. Craigen[1], L.-J. Wong[1], F. Scaglia[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatric Cardiology, Baylor College of Medicine, Houston, TX.

Mitochondrial cytopathies are a genetically, biochemically, and clinically heterogeneous group of disorders associated with abnormalities of oxidative phosphorylation. The heart is highly energy dependent and particularly vulnerable to energy production defects. Hypertrophic and dilated cardiomyopathy and left ventricular noncompaction are among the main cardiac manifestations in mitochondrial cytopathies. Dilated aortic root is typically found in connective tissue disorders, such as Marfan and Ehlers-Danlos syndrome and has not been previously reported in mitochondrial disorders. We found aortic root dilation in seven patients with mitochondrial cytopathies. The aortic root dilation was mild to moderate with a z-score ranging from +2.9 to +4.0. In at least two cases the aortic root dilation was progressive requiring treatment with β-blockers. We then investigated the aortic root diameter in a series of 45 patients followed in our Center with a diagnosis of mitochondrial disorder based on the modified Walker criteria. Interestingly, we found a statistically significant increase in aortic root diameter with the mean z-score being +0.96±1.14 (CI 95% +0.63 to +1.3), which is significantly increased compared to normal controls ($p<0.001$). The screening and follow-up of more patients with mitochondrial cytopathies are necessary to define the natural history of this newly recognized complication of mitochondrial diseases. The pathomechanism(s) leading to aortic root dilation in mitochondrial disorders is unknown. Mitochondrial dysfunction may lead to nitric oxide (NO) dysregulation and increased generation of reactive oxygen species triggering a signaling cascade of apoptosis. Therefore, we propose the increased endothelial and/or smooth muscle cell apoptosis induced by nonfunctioning mitochondria as a potential mechanism for the observed finding.

1504/T

Upregulation of transthyretin in the brain of a Phenylketonuria mouse model. *J.W. Park, E.S. Park, M.H. Lee, H.Y. Park, S.C. Jung.* Department of Biochemistry,School of Medicine, Ewha Womans University, Seoul, Korea.

Phenylketonuria (PKU) is an autosomal recessive disorder that arises from deficiency of phenylalanie hydroxylase (PAH), which catalyzes the conversion of phenylalanine to tyrosine. The resultant hyperphenylalanemia causes mental retardation, seizure, and behavior and movement abnormalities. The high levels of phenylalanine affect brain development in PKU, but the mechanism of neuropathogenesis has not been fully elucidated. Therefore, gene expression profiling was performed in the brain of a mouse model for PKU. Microarray expression analysis revealed overexpression of transthyretin (TTR), early growth response 2 (Egr2), sclerostin domain containing 1 (SOSTDC1), prolactin receptor (PRLR) and klotho (KL) by gene-dosage dependent manner in the brain of the PKU mouse. Among them, transthyretin (prealbumin) is a thyroid hormone-binding protein that transports thyroxine from the bloodstream to the brain. Upregulation of transthyretin and other genes was confirmed by real-time PCR. Western blot analysis also showed increased levels of transthyretin in the brain of the PKU mice, compared to the wild type. This study could be a clue to understand the mechanism of neuropathogenesis and to find a useful biomarker of PKU.

1505/T

Clinical and demographic characteristics of 122 patients with Type 3 Gaucher disease. *A. Tylki-Szymanska[1], A. Vellodi[2], A. El-Beshlawy[3], J.A. Cole[4], E. Kolodny[5].* 1) Child Mem Health Inst, Warsaw, Poland; 2) Great Ormond St. Hosp Child, London, UK; 3) Ped Hosp Cairo Univ, Cairo, Egypt; 4) Genzyme Corp, Cambridge, MA, USA; 5) NYU Sch Med, NY, USA.

Purpose: To describe the demographic and clinical characteristics of patients with type 3 Gaucher disease (GD3). **Methods:** Data from all patients diagnosed with GD3 enrolled in the Neurological Outcomes Sub-Registry of the ICGG Gaucher Registry as of March 2007. Demographics, clinical characteristics of Gaucher diagnosis, and neurological manifestations at first assessment were analyzed. Patients were on ERT for varying times as of the first assessment. **Results:** We identified 122 patients enrolled in the Sub-Registry. Neurological symptoms were first noted before 2y in 54%; 2-18y in 44%. Forty-three percent showed abnormal symptoms of ability to look to the extreme up or down, abnormal slow object tracking (40%), or convergent squint (35%). Wide based gait was noted in 22%, assistance with walking or non-ambulatory in 14%. Frequency (mean age of onset) for muscle weakness was 24% (2.7y); extrapyramidal features, 19% (4.9y); spasticity, 15% (6.9y); tremor when reaching, 21% (9.7y); tremor at rest, 14% (11.2y). Seizures were reported in 16 patients (14%); 2 patients reported myoclonic seizures. The clinical characteristics as of Gaucher disease at diagnosis were: anemia in (61%); thrombocytopenia ($<120 \times 10^3/mm^3$) in 52% of non-splenectomized patients; moderate splenomegaly (>5 to ≤15 MN) in 6% and severe splenomegaly (>15 MN) in 94% of patients; moderate hepatomegaly (>1.25 to ≤2.5 MN) in 65% and severe hepatomegaly (>2.5 MN) in 32% of patients; growth retardation (56%); and bone pain (11%). Forty-five percent reported Caucasian ethnicity and 36% reported Arab ethnicity. Most common genotypes reported were L444P/L444P (72%), L444P/D409H (9%), D409H/D409H (7%), and L444P/Rare allele (5%); full sequencing was not performed in all patients. **Conclusion:** GD3 affects patients of different ethnicities; most common genotype is L444P/L444P. Neurological symptoms typically begin before 2y (54%) and appear in nearly all patients before 18y (98%).

1506/T

Identification of transcobalamin as the cobalamin-binding protein in crude mitochondrial fractions in fibroblasts from patients with inborn errors of vitamin B_{12} metabolism. *L. Yamani[1,2], A. Hosack[2], B.M. Gilfix[2], D. Watkins[2], D.S. Rosenblatt[1,2].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada.

Vitamin B_{12} (Cobalamin, Cbl) binds two enzymes intracellularly. The cytosolic enzyme, methionine synthase (MS), utilizes methylcobalamin (MeCbl) as a cofactor in the conversion of homocysteine to methionine. The mitochondrial enzyme, methylmalonyl-CoA mutase (MCM), utilizes adenosylcobalamin (AdoCbl) as a cofactor in the conversion of L-methylmalonyl-CoA to succinyl-CoA. A defect in MeCbl metabolism or MS (*cblD variant 1, cblG,* and *cblE*) results in homocystinuria. A deficiency in AdoCbl synthesis or MCM (*cblA, cblB, cblD variant 2,* and *mut*) results in methylmalonic aciduria. A defect in steps common to the two pathways causes both conditions (*cblC, cblD,* and *cblF*). We have recently shown that at least one Cbl-binding protein of 28 kDa besides MCM exists in crude mitochondrial fractions. Human fibroblasts were incubated for 96 hours in 25 pg/mL [^{57}Co]CNCbl bound to human transcobalamin (TC). Crude mitochondrial fractions were isolated and analyzed by gel filtration chromatography. The presence of a Cbl-binding protein with an estimated molecular weight of 28 kDa was confirmed. The amount of labelled Cbl bound to this 28-kDa protein was increased in cells from *mut, cblB,* and *cblD var.2* patients, as opposed to wild-type cells. In an attempt to identify this protein, crude mitochondrial fractions from a *cblB* cell line were incubated with anti-TC antibody-coated beads. It was found that the protein was precipitated by the anti-TC antibody. Analysis of labelled Cbl from a *cblF* cell line, where all Cbl is trapped in lysosomes, showed that 22-28 % of the Cbl counts were in the crude mitochondrial fractions, suggesting our mitochondrial fractions contain lysosomal material. This also suggests that the previously identified Cbl-binding protein is in fact lysosomal TC that is present in the "crude mitochondrial fractions". The reason for differences in Cbl-bound TC levels among complementation groups remains to be determined.

1507/T

The Angiotensin System Mediates Renal Fibrosis in Glycogen Storage Disease Type Ia Nephropathy. *W.H. Yiu[1], C.J. Pan[1], R.A. Ruef[1], M.F. Starost[2], B.C. Mansfield[3], J.Y. Chou[1].* 1) Section on Cellular Differentiation, Program on Developmental Endocrinology and Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892; 2) Division of Veterinary Resources, National Institutes of Health, Bethesda, MD 20892; 3) Correlogic Systems, Inc., Rockville, MD 20850.

Glycogen storage disease type Ia (GSD-Ia) patients are deficient in glucose-6-phosphatase-α and manifest disturbed glucose homeostasis. While intensive dietary therapies can maintain euglycemia in GSD-Ia, renal disease of unknown etiology remains a long-term complication. In this study we examined whether the angiotensin system mediates renal fibrosis in GSD-Ia mice. The expression of angiotensinogen, the precursor of the multifunctional cytokine angiotensin II, angiotensin type 1 receptor, transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) are elevated in the kidneys of GSD-Ia mice compared to the controls. While the increase in renal angiotensinogen expression was evident in 2-week-old GSD-Ia mice, the increase in renal expression of TGF-β1 and CTGF was not observed until the GSD-Ia mice were at least 3 weeks old. This is consistent with the finding that Angiotensin II up-regulates the expression of TGF-β and CTGF. In addition, renal expression of genes for the extracellular matrix (ECM) proteins, fibronectin and collagens I, III, and IV were all elevated in GSD-Ia mice, compared to the controls. This was associated with renal fibrosis in the GSD-Ia mice which was characterized by a marked increase in the synthesis and deposition of ECM proteins in the renal cortex and renal histological abnormalities including tubular basement membrane thickening, tubular dilation, and multifocal interstitial fibrosis. Our results suggest that activation of the angiotensin system plays an important role in the pathophysiology of renal disease in GSD-Ia.

1508/T

Paternal transmission and resistance against selection of mutant alleles associated with late-onset ornithine transcarbamylase deficiency in male patients. *M. Yoshino[1], E. Harada[1], A. Yanagawa[2], Y. Watanabe[1], S. Numata[1], C. Fujii[1].* 1) Pediatrics, Kurume University School of Medicine, Kurume, Fukuoka, Japan; 2) Institute of Biostatistics, Kurume University, Kurume, Fukuoka, Japan.

In 13 families with late-onset ornithine transcarbamylase (OTC) deficiency in male patients, 3 mutant alleles - R40H, R277W and Y55D - were identified. In a total of 22 informative parent-offspring pairs, father-to-daughter transmission and mother-to-offspring transmission occurred in 5 (23%) and 17 (77%), respectively, indicating that paternal transmission would substantially contribute to the pool of these mutant alleles. Relative reproductive fitness of males and females carrying these mutant alleles were calculated to be 0.49 and 0.89, respectively. Comparison of life-span of these mutant alleles estimated on the basis of these fitness values with those associated with 'classical' phenotype (neonatal onset), in which reproductive fitness of male patients was nil, revealed that the former was selected against much slowly. This would allow these late-onset phenotype mutant alleles to be retained more frequently than those associated with classical phenotype in a population. Although heterozygous female carrying these late-onset phenotype mutant alleles were generally asymptomatic, one female carrying R40H allele died after a hyperammonemic episode at the age 18 years. Such heterozygous female should be alerted to possible hyperammonemic crisis.

1509/T

Bioenergetical analysis of mouse neuronal mitochondrial DNA during aging. *Y. Bai, Q. Zhao, Y. Li, T. Song.* Cellular & Structural Biol, Univ Texas Health & Sci Ctr, San Antonio, TX.

There is a significant amount of evidence suggesting that aging affect, particularly in neuronal cells, mitochondrial structure and function. The mitochondrial theory of aging thus proposes that there is a vicious cycle in which somatic mtDNA mutations cause defective electron transfer, increasing the generation of damaging reactive oxygen species (ROS) that, in turn, induce further mtDNA mutations. Compromised mitochondrial function, including a decrease in energy supply and tissue degeneration, leads to various aging-related phenotypes. However, there have been no comprehensive studies of overall mutation loads in the tissues during the aging process or of the bioenergetics consequences resulting from these mutations. To address these issues, we developed approaches to transfer mtDNA from mouse brain into established cell lines, and improved methods to isolate mutations in the cultured cell lines. In particular, we established 60 cell lines each in groups carrying near homoplasmic mitochondrial DNA from synaptosomes of old (25 months) and young (5 months) mice. Baseline respiration, maximal respiratory capacity and uncoupled respiratory activity were measured in those cells. Interestingly, there is no significant difference between young and old groups in the base line respiration and maximal respiration capacity. However, we found that the uncoupled respiratory activities in the aged group were significantly increased by 33%. Furthermore, the ratio of maximal respiratory capacity to uncoupled respiratory activities in the old group were lower by 27%, indicating a aging-dependent decrease in the control of respiration by the mitochondrial membrane potential. We also measured the growth capacity in galactose medium where cells were predominantly relied on mitochondrial oxidative phosphorylation for ATP production. The cell numbers after 4 days culturing in galactose medium were 18% lower in the old group compared with the young group. Theses findings pointed to an alteration of mitochondrial function associated with changes in mtDNA during aging.

1510/T

Clinical and Molecular Features of Mitochondrial DNA depletion due to Mutations in Deoxyguanosine Kinase. *D. Dimmock[1], C. Dionisi-Vici[9], Q. Zhang[1], J. Shieh[2], C. Truong[1], E. Schmitt[1], M. Sifry-Platt[3], R. Carrozzo[9], S. Lucioli[9], C. Ficicioglu[4], G. Enns[5], E. Arch[6], N. Longo[7], M. Lipson[3], H. Vallance[8], F. Scaglia[1], L.-J. Wong[1].* 1) Dept M & H Gen, Baylor C M, Houston, TX; 2) David Gladstone Inst at UCSF, San Francisco;; 3) Kaiser Permanente, Sacramento, Ca;; 4) Children's Hospital Philadelphia, Philidelphia, PA;; 5) Stanford University SoM, Stanford, CA;; 6) Massachusetts General, Boston, MA;; 7) University of Utah, Salt Lake City, Utah;; 8) University of BC, Vancouver, B.C., Canada; 9) Children's Hospital "Bambino Gesu", Rome, Italy;.

Background

Deoxyguanosine kinase (*DGK*) is a nuclear gene that along with *thymidine kinase-2* salvages deoxyribonucleotides (dNTPs) for mtDNA synthesis. Deficiency of either of these causes a mitochondrial depeletion syndrome.

Methods

We have undertaken a retrospective analysis of two center's 9 Kindreds representing 16 mutations, 13 of which are unpublished. These are compared with previously published cases to establish genotype/phenotype.

Results

DGK mutations are associated with both isolated hepatic and hepato-cerebral forms. In all patients in our series hepatocerebral disease was associated with an abnormal newborn screen, early onset of nystagmus and early death. Conversely, the absence of a neurological phenotype is predictive of long term survival independent of liver transplantation. The N46S mutation is associated with isolated hepatic disease in all ethnicities.

Conclusions

Mitochondrial depletion caused by mutations in *DGK* should be considered in children with hepatic dysfunction or cholestasis even without neurological findings Full gene sequencing is warranted if *DGK* deficiency is suspected.

1511/T

Metabolic pathway profiling in C. elegans mitochondrial respiratory chain mutants. *M.J. Falk[1], Z. Zheng[1], J.R. Rosenjack[2], E. Daikhin[1], I. Nissim[1], O. Horyn[1], M.M. Sedensky[2], M. Yudkoff[1], P.G. Morgan[2].* 1) Divisions of Human Genetics, Biomedical Informatics, & Metabolism, The Children's Hospital of Philadelphia & Univ of Pennsylvania, Philadelphia, PA; 2) Departments of Anesthesiology, Genetics, & Pharmacology, Univ Hospitals of Cleveland & CASE, Cleveland, OH.

C. elegans affords a model of mitochondrial dysfunction that allows insight into cellular adaptations that occur consequent to genetic alterations associated with human disease. We characterized genome-wide expression profiles of hypomorphic C. elegans mutants in various nuclear-encoded subunits of respiratory chain complexes I, II, and III. Our goal was to detect concordant changes of clusters of genes that comprise a defined metabolic pathway utilizing gene set enrichment analysis. Results indicate that respiratory chain mutants significantly upregulate a variety of basic cell metabolism pathways involved in carbohydrate, amino acid, and fatty acid metabolism, as well as cellular defense pathways such as the P450 system and the γ-glutamyl pathway of glutathione synthesis. Initial results were confirmed in an independent data set. In addition, metabolomic profiling at the protein level in C. elegans mitochondrial mutants confirms and extends expression analysis findings. Detection of consistent changes in nuclear gene expression patterns in a translational genetic model of mitochondrial dysfunction provides insight into disease mechanisms. Furthermore, our results provide novel evidence for an increase in transamination reactions occurring in primary mitochondrial mutants caused by their failure to oxidize ketoacids. This approach may permit exploration of the complex pathogenesis underlying primary mitochondrial disease. To this end, further metabolomic profiling of these mutants is underway using stable isotopic/mass spectrometric studies of precursor-product relationships as well as measurements of flux through specific biochemical pathways. These studies in a simple genetic model of mitochondrial disease will form the basis for developing screening tools for mitochondrial dysfunction in humans based upon pathway expression patterns and metabolomic profiling.

1512/T

A novel mutation of the NDUFS7 gene leads to activation of a cryptic exon and impaired assembly of mitochondrial complex I in a patient with Leigh syndrome. *S. Lebon[2], L. Minai[1], D. Chretien[1], J. Corcos[2], V. Serre[1], N. Kadhom[2], J. Steffann[2], JY. Pauchard[3], A. Munnich[2], JP. Bonenfont[2], A. Rotig[1].* 1) INSERM U781, hopital Necker, Paris, France; 2) Service de Génétique, Hôpital Necker Enfants Malades, Paris, France; 3) Service de Pédiatrie, Hôpital de Pontarlier, Pontarlier, France.

Complex I deficiency is a frequent cause of mitochondrial disease as it accounts for one third of these disorders. By genotyping several putative disease loci using microsatellite markers we were able to describe a new NDUFS7 mutation in a consanguineous family with Leigh syndrome and isolated complex I deficiency. This mutation lies in the first intron of the NDUFS7 gene (c.17-1167 C to G) and creates a strong donor splice site resulting in the generation of a cryptic exon. This mutation is predicted to result in a shortened mutant protein of 41 instead of 213 amino acids containing only the first five amino acids of the normal protein. Analysis of the assembly state of the respiratory chain complexes under native condition revealed a marked decrease of fully-assembled complex I while the quantity of the other complexes was not altered. These results report the first intronic NDUFS7 gene mutation and demonstrate the crucial role of NDUFS7 in the biogenesis of complex I.

1513/T

Relations between ethymalonic acid and isoleucine / methionine metabolism in ethylmalonic acidemia are unclear. *M. BARTH[1], V. VALAYANNOPOULOS[1], L. HUBERT[2], L. MINAI[2], S. ROMANO[1], D. CHRETIEN[2], A. ROTIG[2], D. RABIER[3], A. MUNNICH[2], Y. DE KEYSER[2], P. DE LONLAY[1,2].* 1) Unité de métabolisme, Hôpital Necker Enfants Malades , France; 2) INSERM U-781, Hôpital Necker Enfants Malades, France; 3) Service de Biochimie, Hôpital Necker Enfants Malades, France.

Background: Ethylmalonic encephalopathy is a rare autosomal recessive metabolic disorder caused by mutation in ETHE1 gene and presenting in infancy with psychomotor retardation, chronic diarrhea, orthostatic achrocyanosis and relapsing petechia. High levels of lactic acid, ethymalonic acid and methylsuccinic acid are detected in body fluids. A decreased cytochrome c oxydase activity has been reported in skeletal muscle. The source of ethylmalonic acid is hitherto unclear. Relations to isoleucin and methionine metabolism have been suggested.

Patient and Methods: We report a 15 months old male born to consanguineous parents, presenting with a typical ethylmalonic encephalopathy phenotype.Oral isoleucin (150mg/kg)and methionine (100 mg/kg) loading tests as well as an isoleucine restricted diet (200mg/d) were performed to analyze the consequences on ethylmalonic excretion.

Results: Cytochrome c oxidase (COX) activity was decreased in lymphocytes. COX deficiency was not associated with an abnormal assemblage of mitochondrial respiratory chain complexes as shown by blue native PAGE. Molecular studies showed a homozygous mutation in the ETHE1 gene, leadingto the deletion of exon 4 in the mRNA. Ethylmalonic acid was increased only after isoleucine loading (99 to 242 μmol/mmol creatinine) while the methionine load did not change ethylmalonic acid excretion (105 to 122 μmol/mmol creatinine). After isoleucine restricted diet, we neither observed a clinical improvement nor biochemical modifications in ethylmalonic acid excretion and COX activity in lymphoblasts.

Conclusion: Our results suggest that ethylmalonic acid formation is linked to isoleucine metabolism but not to methionine metabolism. The inhibition of COX activity in ethylmalonic acidemia does not involve alterations in assembly of OXPHOS complexes.

1514/T

CEBPδ is a candidate regulator of brain disease in prosaposin deficiency mice. *L. Jia[1], Y. Sun[1], M.T. Williams[2], M. Zamzow[1], H. Ran[1], B. Quinn[1], B.J. Aronow[3], C.V. Vorhees[2], D.P. Witte[4], G.A. Grabowski[1].* 1) Div Human Genetics,; 2) Div Neurology,; 3) Div Biomedical Informatics,; 4) Div Pediatric Pathology, Cincinnati Children's Hosp, Cincinnati, OH.

The physiological importance of prosaposin has been demonstrated by the genetic deficiencies of individual saposins or prosaposin that leads to various glycosphingolipid (GSL) storage diseases. Our hypomorphic prosaposin deficient mouse model, PS-NA, exhibits 45% of WT levels of saposins in the brain and showed neurological pathology that included GSL storage in neurons and loss of Purkinje cells. Deterioration of neuronal function was observed by 6 wks using narrow bridge test. To explore the molecular mechanism(s) responsible for disease progression, temporal transcriptome microarray analyses of mouse brain tissues were conducted using mRNA from three prosaposin deficiency models: PS-NA, prosaposin null (PS-/-) and 4L/PS-NA (a V394L/V394L glucocerebrosidase mutation and PS-NA). Central nervous system gene expression alterations were detectable at birth and were of a greater magnitude in cerebellum than cerebrum. Differentially regulated genes encompassed a broad spectrum of cellular functions. Down-regulated genes did not change with age, but up-regulated genes (75%) did tend to increase in number and magnitude suggesting that cellular coping and disease propagation mechanisms were operative. A common transcription factor, CEBPδ, was up-regulated in all three models at all time points. Network analysis revealed that CEBPδ has functional relationships with genes in transcription, proinflammation, death, binding, myelin and transport and represented the regionally specific gene expression abnormalities preceded the histological and behavioral changes. Our results indicate that temporal gene expression profile changes have provided novel insight into the molecular mechanism responsible for GSL storage disease progression. CEBPδ is a candidate regulator of brain disease in prosaposin deficiency and may represent a novel therapeutic target to modulate disease progression. It remains to be determined if CEBPδ signaling is playing an accelerating or progression suppressive role.

1515/T

Deficiency of mitochondrial respiratory activity in 6 patients with organic acidemias. *P. de Lonlay[1,2], V. Valayannopoulos[1], M. Sarzi[2], D. Chrétien[2], JB. Arnoux[1,2], S. Romano[1], D. Rabier[3], A. Munnich[2], A. Rötig[2], Y. de Keyzer[2].* 1) Pediatric Dept, Hosp Necker-Enfants Malades, Paris Cedex, France; 2) INSERM U781; 3) Biochimie.

Organic acidemias (OA) result from a defect of methylmalonic-CoA mutase (MMA) and propionyl-CoA carboxylase (PA). A few reports have supported the hypothesis that secondary respiratory chain deficiency could be the cause of complications observed in the long term follow-up of OA. Here, we report on respiratory chain deficiency and mitochondrial DNA depletion in several tissues of 6 patients with OA.

Case reports : Six patients, two with PA and four with MMA, were followed at Necker-Enfants malades Hospital. Both patients with PA developed severe cardiomyopathy. One improved quickly after a liver transplantation. Patients with MMA eventually developed neurological disease (3/4) and renal failure (2/4).

Results: A OXPHOS deficiency was found in the liver (multiple deficiency in 1 MMA, CII deficiency in 1 PA; CIII deficiency in 1 PA), skeletal muscle (CIII and CIV deficiency in 1 PA), heart (CII and CIII deficiencies in 2 PA), and kidney (generalized in 2 MMA). A mtDNA depletion was identified in the liver and skeletal muscle of these patients, with a residual mtDNA content of 9.2% and 24.8% in the liver of PA patients, and 11.5% in skeletal muscle (MMA).

Discussion: In OA, not only conversion of propionyl-CoA into methylmalonyl-CoA and succinyl-CoA but a reduction of the production of succinyl-CoA also occurs. This therefore affects the activity of the succinyl-CoA synthase (SCS) and the overall flux of the tricarboxylic acid (TCA) cycle. Because SCS deficiency is associated with mtDNA depletion, and accumulation of propionyl-CoA leads to hyperproduction of methylcitrate, an inhibition of the TCA cycle is suggested in the pathophysiopathology of long-term complications in OA.

Conclusion: We describe OXPHOS deficiency and mitochondrial DNA depletion in several tissues of 6 patients with OA, likely to be due to a reduced flux through the TCA.

1516/T

NTS promoter variants are associated with body mass index. *N. Kavaslar[1], N. Ahituv[2], T. Naing[1], S. Hebert[1], H. Doelle[1], R. Dent[1], A. Stewart[1], R. Roberts[1], L. Pennacchio[2], R. McPherson[1].* 1) University of Ottawa Heart Institute, Ottawa, ON, Canada; 2) Lawrence Berkeley National Laboratory, Berkeley, CA.

Neurotensin (NTS) is a brain-gut peptide with a role in appetite regulation. NTS levels are known to be decreased in obese subjects and to increase after bariatric surgery. The NTS gene has four exons and spans 8.7kb on chr12q21. To determine whether variants in the NTS gene are associated with obesity, we resequenced the exons and intron-exon boundaries in 378 lean (av BMI 19.4kg/m2) and 379 obese (av BMI 49.0kg/m2) subjects matched for age and sex. We identified a total of 5 nonsynonymous novel coding variants and 10 noncoding SNPs. Of these 15 variants, only two showed a difference in frequency between obese and lean cohorts: rs1800832 is located 3 bp upstream of the translation start site and 84% of the subjects (both cohorts combined) who are homozygous for the rare G allele of this SNP are lean ($p<0.001$), whereas the variant -23G/A is novel and unique to the obese population (1.6%, $p=0.014$). A third 5' variant, rs2234762, showed a small difference in minor allele frequency between the two cohorts (0.236 in obese and 0.246 in lean). In silico analysis suggested a functional role for the promoter variants rs1800832 and rs2234762, since they result in loss of a PPAR/RXR site and gain of an HBP1 site, respectively. We analysed these two common SNPs with 2000bp and 500bp constructs using the dual luciferase assay in two cell lines. The promoter activity of constructs containing the rare allele was decreased by 10-14% (ns). Using data from the Affymetrix 500K genotyping assay, we analysed six SNPs in the 20kb vicinity of the NTS gene in a cohort of 1622 subjects enrolled in the Ottawa Heart Study. Three SNPs upstream and one SNP downstream of the NTS gene showed an association with BMI in men (n=1032; rs4143239, $p=0.019$; rs11117064, $p=0.021$; rs11117060, $p=0.030$; rs12314274, $p=0.023$; adjusted for age). Our results indicate that variants in regulatory regions of the NTS gene have a modest role in body weight regulation, in accord with the hypothesis that multiple rare alleles in the coding and noncoding regions of candidate genes contribute to the complex phenotype of obesity.

1517/T

Serum levels of the KL-6 epitope of MUC1 correlate with pulmonary fibrosis in Hermansky-Pudlak syndrome. *T.C. Markello[1,2], M. Anahtar[2], I. Bernardini[2], B.B. Gochuico[2], K. O'Brien[2], G.A. Golas[2], W.A. Gahl[2].* 1) 1 Department of Genetics & Metabolism, Children's National Medical Center, Washington, DC; 2) 2Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda MD.

Elevated serum levels of the KL-6 epitope of MUC1 have previously been correlated with the presence of interstitial lung diseases, including Idiopathic pulmonary fibrosis (IPF). Little is known about serum levels of KL-6 prior to the onset of clinical symptoms, since patients with IPF typically present after significant fibrosis has occurred, prompting medical attention for the resulting respiratory compromise. Type 1 Hermansky-Pudlak Syndrome (HPS) has a frequency of pulmonary fibrosis that approaches 100% in the 4th through 6th decades of life. Because patients with HPS are first ascertained by their oculocutaneous albinism and their bleeding diathesis, it is possible to analyze serum samples in HPS patients prior to the onset of clinically significant pulmonary fibrosis. We tested archival serum samples from patients with Hermansky Pudlak syndrome who were seen at the NIH Clinical Center between 1998 and 2007. These samples include HPS types 1 and 3, i.e., subtypes with and without pulmonary fibrosis. Compared to normal controls, both HPS type 1 and type 3 patients have a 2.2 fold elevation in KL-6/MUC1 levels prior to onset of clinical pulmonary fibrosis (N=14, range 1.12 to 3.14 fold vs normal controls). HPS type 1 patients have a 12 fold elevation in serum KL-6/MUC1 levels after the onset of clinical pulmonary fibrosis (n=13, range 4.7 to 42.0 fold vs normal controls). These results suggest a potential relationship between a genetic disorder of intracellular vesicle trafficking (i.e., HPS) and a protein that requires intracellular trafficking for proper glycosylation and apical targeting. Further characterization of KL-6/MUC1 and other pneumoproteins in HPS may be useful for early diagnosis and prognosis of pulmonary fibrosis. Serum levels of pneumoproteins, including KL-6/MUC1, may also serve as outcome parameters for future therapeutic interventions in HPS patients with pulmonary fibrosis.

1518/F

Sarcosinemia: Analysis of the *SARDH* Gene in Three Patients. *Y. Anikster[1], N. Goldstein[1], H. Reznik-Wolf[2], E. Pras[2].* 1) Metabolic Disease Unit, Safra Children's Hosp, Tel-Hashomer, Israel; 2) Danek Gartner Institute of Human Genetics, Sheba Medical Center, Tel Hashomer.

Sarcosinemia is characterized by increased levels of sarcosine in plasma and urine and is inherited in as an autosomal recessive trait. Previous reports have associated sarcosinemia with a variety of symptoms such as mental retardation,cranisinosyosis,hepatomegaly and cardiomyopathy. However the vast majority of the individuals with sarcosinemia are symptom free and therefore it is assumed that Sarcosinemia by itself is a benign condition and that the associated symptoms are due to a referral bias. Sarcosinemia was suspected to be caused by a defect in Sarcosine dehydrogenase (SARDH) activity, a liver mitochondrial matrix flavoenzyme that catalyzes the oxidative demethylation of sarcosine, however in humans this hypothesis was never proven. We have analyzed the SARDH gene in 3 sarcosinemia female patients all born to consanguineous parents. The first, a 15 years of age, was diagnosed as suffering from cardimyopathy since an early age with no signs of CHF or developmental delay. The second, 9 months old, presented with developmental delay, hypotonia, deafness and a cherry red spot.The third patient, a 2 years old girl was evaluated due to global developmental delay. Sequence of the SARDH gene in the first patient, revealed a homozygous G to T substitution at nucleotide 211 (from the ATG initiation codon) substituting Valine for Phenylalanine at codon 71. This amino acid was found conserved throughout evolution. Analysis of the patient's family showed that both parents and one brother were heterozygous for the mutation, one sister (asymptomatic) was homozygous for it, while 2 additional brothers and one sister did not carry this mutation. The results were compatible with the family members' sarcosine level test. No mutations were found in the second and third patients and segregation studies performed with closely linked markers ruled out the SARDH gene in one of the families, implying genetic heterogeneity. The mutation described above is the first identified in the SARDH gene and strongly suggests that this gene is implicated in the pathogenesis of this disorder.

1519/F

A novel deletion mutation in *ARG1* gene found in a neonate. *O. Staretz-Chacham[1], N. Goldstein[2], B. Ben-Zeev[2], R. Loewenthal[2], H. Mandel[2], E. Sigalov[2], B. Vilensky[2], Y. Cohen[2], S.D. Cederbaum[2], Y. Anikster[2].* 1) NIH, Bethesda, MD; 2) Safra Children Hospital, Sheba Medical center, Tel Hashomer, Israel.

Argininemia is a rare autosomal recessive disorder caused by deficiency of Arginase I gene (*ARG1*), the final enzyme in the Urea Cycle that catalyzes the breakdown of arginine to ornithine and urea. Arginase I is a cytosolic enzyme expressed predominantly in the liver but also in erythrocytes. Argininemia typically presents as a progressive neurometabolic disorder with spastic paraplegia, developmental retardation, hyperactivity,irritability and episodic vomiting, hyperammonemia and seizures.Basic treatment is dietary protein restriction, and supportive therapy is to prevent and control the hyperammonemia. A 2 weeks old female infant of first cousin Ashkenazi Jewish parents was diagnosed with Argininemia in our clinic. For confirmation of clinical diagnosis, the patient's genomic DNA was PCR amplified for the 8 exons of the *ARG1* gene and analyzed by nucleotide sequencing. PCR amplification yielded PCR products for all exons except exon 2. Since the amplification primers were intronic, results suggested an exon 2 deletion. In order to define the deletion borders, sequence analysis was performed with primers at introns 1 and 2 leading to the identification of a 1337 bp deletion which included exon 2. This results in a reading frame shift after amino acid residue 19 followed by a premature stop codon after 4 amino acids. Examination of the sequence flanking the deletion revealed 5 base repeats at the 5' and 3' breakpoints. The presence of these repeats at the breakpoints might have facilitated the generation of the deletion through a slippage mispairing mechanism. In order to determine the carrier rate of this novel mutation in addition to a second known mutation (D128G) found in several Jewish families of Ashkenazi descent. *ARG1* mutations were genotyped using a chip-based matrix-assisted laser desorption-time-of-flight (MALDI-TOF) mass spectrometer for 760 DNA samples from anonymous Ashkenazi subjects. No carriers were found. These results suggest low carrier frequency for these mutations among Ashkenazi Jews and/or a *de novo* mutation in this neonate.

1520/F

A novel intronic point mutation of CPS1 gene in a Korean family with CPS1 deficiency. *G.H. Kim[1], J.M. Ko[2], J.J. Lee[1], H.W. Yoo[1,2].* 1) Med Genetics Clinic & Laboratory, Asan Medical Center; 2) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Carbamoyl phosphate synthetase I (CPS1) deficiency (OMIM#237300) is a rare autosomal recessive inborn error of the urea cycle causing hyperammonemia. The mutations of CPS1 gene located on 2q35 are responsible for CPS1 deficiency. To date, about 50 mutations have been reported. We encountered a 7-day-old Korean female infant with hyperammonemia and the diagnosis was confirmed by CPS1 gene mutation analysis as well as biochemical findings including low plasma citrulline level and absence of orotic aciduria. The patient was born to healthy parents of non-consanguineous marriage. Her previous 3 elder siblings died during neonatal period because of hyperammonemic encephalopathy. The mutation analysis was performed with reverse transcriptation (RT)-PCR and sequence analysis using cDNA isolated from liver tissue. Two different transcripts were found; one showed normal sequences and the other 60 bp insertion of intronic sequences. Subsequently, the sequence variations were confirmed in genomic DNA isolated from peripheral leukocytes. Mutations of the CPS1 gene were identified in the patient and her parents. The patient carries both a deletion mutation c.1529del, resulting in a frame-shift p.Gly510AlafsX4, and a novel intronic mutation, c.3666+64T>G, a base change at 64 bp down stream from the end of exon 30, making a base c.3666+61 more efficient 5' new splice donor site for intron 30. Automated splice site analysis using this base change revealed that it generated 1.4 fold more efficient splice site than the original one. Therefore, c.3666+64T>G mutation results in the retention of 60 intronic bases into mRNA during RNA processing, leading to a truncated mutation p.Val1223delins(Val-Ile-Ile-Tyr-Lys_X). The mutation c.1529del was inherited from her father, and the novel c.3666+64T>G from his mother. We report a novel intronic mutation of the CPS1 gene in a Korean family with CPS1 deficiency.

1521/F

Chemical Chaperone Effect on GLA Gene Mutations in Korean Patients with Fabry disease. *J.Y. Park[1], G.H. Kim[1,2], H.W. Yoo[1,2,3].* 1) Genome Research Center for Birth Defects and Genetic Disorders, Asan Institute for Life Sciences; 2) Medical Genetics Clinic and Laboratory, Asan Medical Center; 3) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Fabry disease, an X-linked inborn error of glycosphingolipid catabolism, results from the deficient activity of GLA (α-galactosidase A). We have identified 15 different mutations in the GLA gene in 13 classic and 2 atypical male Fabry patients from 15 unrelated Korean families. Out of 15 identified mutations, 2 were novel mutations, the p.Asp231Gly missense mutation and the p.Leu268delfsX1 deletion mutation. The study was undertaken to evaluate effect of chemical chaperone 1-deoxygalactonojirimycin (DGJ) on GLA missense mutant constructs in vitro. Nine missense mutations including one novel mutation were cloned into a mammalian expression vector. GLA activity and GLA expression were analyzed using fluorescence spectrophotometry and Western blot after transient expression in COS-7 cells. COS-7 cells were cultured for 2 days in DMEM medium with and without of 20 uM DGJ. The addition of DGJ to culture media enhanced GLA activity up to 2.5 fold in p.Met42Val, p.Ile91Thr and p.Phe113Leu. The p.Ile91Thr and p.Phe113Leu were clinically associated with atypical form. While mature form (46 kDa) protein of α-Gal A increased markedly in the p.Phe113Leu, it also increased less abundantly in the p.Met42Val and p.Ile91Thr. DGJ treatment in other mutations including p.Glu66Gln, pArg112Cys, p.Cys142Trp, p.Asp231Gly, p.Asp266Asn, and p.Ser297Phe did not show any significant effect both on GLA activity and protein expression. Interestingly, no GLA activity was observed in a novel mutation, p.Asp231Gly, but the protein was normally expressed as wild type GLA. Especially, the p.Glu66Gln showed approximately 40 % GLA activity in the absence of DGJ, and the protein was expressed normally, indicating that it is a mild mutation or functional SNP. In conclusion, the results suggest that chemical chaperone DGJ enhances GLA activity and mature protein expression in milder mutations associated with atypical form of Fabry disease.

1522/F

Accumulation of alpha-synuclein and ubiquitin in Gaucher disease mouse models. *YH. Xu[1], Y. Sun[1], R. Reboulet[1], H. Ran[1], B. Quinn[1], S. Clark[2], B. Wustman[2], GA. Grabowski[1].* 1) Div Human Gen, Children's Hosp Medical Ctr, Cincinnati, OH 45229; 2) Amicus Therapeutics, Cranbury, NJ 08512.

Gaucher disease (GD), the most prevalent lysosomal storage disease, is caused by insufficient activity of acid beta-glucosidase (GCase). Some non-neuronopathic (type I) GD patients have a disease course complicated by Parkinsonism that has an unusually early onset and refractoriness to conventional anti-Parkinson therapy. To understand the pathogenic correlations between GD and Parkinsonism, alpha-synuclein and ubiquitin levels in brain were evaluated by immunohistochemistry of serial brain sections from GCase point-mutated Gaucher mice [D409H (9H) and V394L (4L)] and prosaposin hypomorph together with GCase mutations (4L/PS-NA and 9H/PS-NA). 4L/PS-NA and 9H/PS-NA mice had excess GC accumulation in the brain and the levels were increased with age. In 10-wk old mice, significant alpha-synuclein aggregates were observed in hippocampus, basal ganglia (caudate putamen, substantia nigra, subthalamic nucleus), brain stem, and some cortical/cerebellar regions. Ubiquitin aggregates were also found in these regions and some co-localized with alpha-synuclein. However, alpha-synuclein aggregates were only observed at hippocampal and cerebella regions in >42-wks old 9H and 4L mice that are less severe models. Mouse models for other lysosomal storage diseases were screened in parallel and only sporadic signals were observed, e.g., alpha-synuclein signals in cortex and brain stem of prosaposin deficient (PS-NA) mice and low level in cerebellum (granular cell layer) of lysosomal acid lipase (LAL) knock-out mice; ubiquitin in the midbrain of MPS1 and NPC1 deficiency mice, or in the cortex and mid brain of PS-NA mice. These findings suggested that the defect of GCase activity is a risk factor for alpha-synucleinpathies. Understanding the pathogenic relationship between GCase deficiency and the development of parkinsonian manifestations will provide insights into the genetics, pathogenesis, and treatment of Parkinson disease.

1523/F

HIGH INCIDENCE OF DHPR DEFICIENCY IN SOUTH ITALY : REPORT OF THREE PATIENTS WITH THE SAME MUTATION (L14P). *D. Concolino, L. Muzzi, M. Rapsomaniki, M.G. Pascale, M.T. Moricca, F. Ceravolo, P. Strisciuglio.* Dept Pediatrics, Univ Catanzaro, Catanzaro, Italy.

Deficiency of dihydropteridine reductase (DHPR) causes a variant form of phenylketonuria associated with a devastating neurological disease. Hyperphenylalaninaemias (HPA) with BH4 deficiency are about 3% of all HPA. We describe three patients from Calabria, a southern region of Italy, affected by DHPR caused by same mutation. We used serum prolactin levels as a marker for optimal dosage of hydroxylated precursors in long-term treatment monitoring. All patients were children of unrelated parents DHPR diagnosis were made by BH4 oral loading test (20mg/Kg) and the measurement of DHPR activity in erythrocytes. None of patients showed neurological signs before the beginning of pharmacological treatment. In the case 1 the annual median dosage of L-Dopa was of 5,19mg/Kg/die. Other two patients showed increase of serum levels of prolactin that required adjustments of L-dopa independently of the body weight. Actually, case 2 (5 years and 3 months) needs L-Dopa at the dosage of 6.0mg/Kg/die and case 3 (4 years) needs L-Dopa at the dosage of 5.8 mg/Kg/die. The outcome of three patients is until now very favourable. Molecular analysis on QDPR gene on these three patients showed the mutation pL14P in homozygosity on exon 1. This mutation has been found in Mediterranean populations and a founder effect has been hypothesized. Thus our molecular data seem to confirm this hypothesis and could explain high incidence of DHPR deficiency in our region. In conclusion we found an high incidence of DHPR deficiency in our region and we show that the prolactin could be a good indicator of optimal dosage of neurotransmitter precursors.

1524/F

Molecular characterization of 31 patients with pyridoxine-dependent-epilepsy (PDE). *B. Plecko[1, 2], K. Paul[2], E. Struys[3], C. Jakobs[3], S. Stoeckler-Ipsiroglu[1], W. Erwa[4], M. Baethmann[5], A. Gatta[6], I. Gyoergy[7], G. Horvath[1], G. Kluger[8], B. Neubauer[9], A. Panzer[10], T. Scheffner[11], R. Van Coster[12], S. Vlaho[13], E. Paschke[2].* 1) Department of Pediatrics, UBC, Children's and Women's Health Center, UBC, Vancouver, BC, Canada; 2) Department of Pediatrics, Medical University Graz, Austria; 3) Department of Clinical Chemistry, University Medical Center, Amsterdam, The Netherlands; 4) Institute for Clinical and Chemical Laboratory Diagnosis, Medical University Graz, Austria; 5) Kinderklinik des Krankenhauses Dritter Orden, Munich, Germany; 6) Casa Sollievo della Sofferenza San Giovanni Rotondo, Italy; 7) Department of Pediatrics, University of Debrecen, Hungary; 8) Epilepsy Center Vogtareuth, Germany; 9) Department of Pediatric Neurology, Giessen, Germany; 10) DRK-Kliniken Westend Berlin, Germany; 11) Klinik für Kinder- und Jugendmedizin, Reutlingen, Germany; 12) Department of Pediatrics, University Hospital Gent, Belgium; 13) Department of Pediatrics, University of Frankfurt, Germany.

PDE (MIM #266100) is caused by mutations of the ALDH7 A1 gene, located on chromosome 5q31. We report on biochemical and molecular findings in 31 Caucasian PDE patients with neonatal seizure onset, 13 of whom have not been reported so far. In all patients with ALDH7 A1 mutations urinary α-AASA and plasma PA were elevated 1.6 to 62- fold and 1.5 to 38 - fold, respectively. α-AASA and PA concentrations were higher in the 3 patients with pre-treatment sampling. Within 60 of the 62 alleles a total of 16 different mutations were identified. Several mutations had increased prevalence, as p.Glu399Gln (exon 14; 34%), c.1482-1G>T acceptor splice site mutation (intron 17, 12%), Arg82X (exon 4; 10%) and a "silent mutation", p.V250V (exon 9; 9%). In 7 patients of 6 unrelated families 6 novel mutations were found: an insertion in exon 1, c.57insA (p.Arg20ThrfsX8), a donor splice site mutation, c.689+2T>C (intron 8), and 4 missense mutations, p.Arg82Gly (exon 4), p.Asn167Ser (exon 6), p.Asn420Lys (exon 15) and p.Gln425Pro (exon 15). All these mutations showed familiar cosegregation and were not present in 120 control alleles.

1525/F

AMPD2 deficient mice: A murine model for minimal change nephropathy. *T. Morisaki[1], K. Toyama[1], J. Cheng[1], H. Kawachi[2], F. Shimizu[2], M. Ikawa[3], M. Okabe[3], H. Morisaki[1].* 1) Department of Bioscience, National Cardiovascular Center Research Institute, Suita, Osaka, Japan; 2) Department of Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; 3) Genome Information Research Center, Osaka University, Suita, Osaka, Japan.

AMP deaminase (AMPD), an enzyme catalyzing AMP to IMP, plays an important role in purine metabolism, especially in maintaining adenylate energy charge. AMPD2 gene, a member of the AMPD gene family in vertebrates, is widely expressed in non-muscle tissues and cells including kidney, though its function has not been fully understood. In this study, we have established the AMPD2 knockout mouse at the first time to identify the gene function. In the AMPD2 knockout mice, the AMPD2 protein was not detectable and the AMPD activity was significant decreased in kidney. Also, we found the changes of nucleotide metabolism in kidney, such as increased AMP and decreased ATP and GTP. In addition, proteinuria was found in mice lacking AMPD2 in 3 week-old mice, followed by further increment of proteinuria at the peak levels in 6 week-old and then decreased but sustained proteinuria in AMPD2 knockout mice. Since the major protein composition in the proteinuria of AMPD2 KO mice was demonstrated to be albumin, it was suggested that AMPD2 could have a key role for glomerular filtration. Indeed, the ultra-structure study of glomerulus showed effacement of the podocyte foot processes, though microscopic analysis did not exhibit apparent morphological abnormality of glomerulus. These changes resemble to those found in minimal change nephropathy in human. Based on these results, we conclude that AMPD2 deficiency induces not only unbalance of nucleotide metabolism but proteinuria probably due to the dysfunction of podocytes.

1526/F

Status of HFE and other iron homeostasis gene in iron overload thalassemia patients and liver cirrhotic patients in India. *D. Tiwari, S. Agarwal.* Genetics, Sanjay Gandhi post graduate Institute of Medical S, Lucknow, U.P, India.

Hereditary hemochromatosis is an autosomal recessive disorder and most commonly inherited single gene disorder among Caucasians with a prevalence of 5 per 1000 and carrier frequency of 1 in 10. Two HFE point mutations are described and referred as C282Y and H63D. In the present study as per the classification of Beutler, we have analyzed DNA samples of North Indian subjects for HFE gene (C282Y & H63D), Ferroportin (A77D), Transferrin receptor 2 (Y250X), Hepcidine (C70R) and Hemojuvelin gene (G320V) by PCR-RFLP. Total numbers of samples screened were 1358 [436: cryptogenic cirrhosis, 410 thalassemia and 512: control]. Of these no allele of C282Y gene was found However, We found percent prevalence of H63D gene mutation in our cirrhosis group 11.5% (50 out of 436), in thalassemia group 13.4% (55 out of 410) and in control group 9.5 % (46 out of 512). Ferroportin SLC40A1 (A77D) mutation was for the first time reported by us in thalassemia patients. A significant association of H63D mutation with iron overload was observed [p< 0.01] The overall frequency of H63D in North Indian population is 11.2%, which is similar to the reported incidence in Northern Europe. The study emphasizes the value of routine screening of the HFE mutation in thalassemias and cirrhotic patients to modify treatment modalities occurring due to iron overload.

1527/F

Clinical and molecular characterization of Hermansky-Pudlak Syndrome type-6. *R. Hess[1], M. Huizing[1], A. Helip-Wooley[1], L. Vincent[1], R. Fischer[1], J. White[2], W.A. Gahl[1].* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Univ Minnesota, Minneapolis, MN.

Hermansky-Pudlak syndrome (HPS) is a rare disorder of vesicle formation characterized by oculocutaneous albinism, a bleeding diathesis and, in some patients, granulomatous colitis or pulmonary fibrosis. Eight autosomal human genes have been shown to cause various HPS phenotypes, and at least five additional genes correspond to murine models. We previously described clinical, molecular and cellular characteristics of HPS subtypes 1 through 5. Here we report our detailed clinical and genetic studies on patients with HPS-6. The human *HPS6* gene (murine *ruby-eye*) is located on 10q24.32 and consists of a single large exon coding for a protein of 775 aa. We screened 19 patients, without defects in other HPS causing genes, and identified 4 patients with 7 different novel *HPS6* mutations, including two frameshift (c.238dupG, c.1938delTG), three nonsense (Q305X, Q75X, Q412X), one large chromosomal deletion, and one missense mutation (T272I). Most nonsense and frameshift mutations generating premature termination codons cause nonsense mRNA mediated decay (NMD), while intronless genes, like *HPS6*, are usually not monitored by NMD. Expression analysis in two HPS-6 patients revealed no mRNA decay in fibroblasts; hence a truncated protein is most likely produced. Clinically, our HPS-6 patients exhibited a relatively mild HPS phenotype, including mild iris transillumination, variable hair and skin pigmentation, and absent platelet dense granules. Pulmonary fibrosis and granulomatous colitis were not observed in these patients, although they were all under 27, an age before which lung disease rarely develops in HPS. It is important to continue to follow adults with HPS-6 for the development of restrictive lung disease. The clinical features of HPS-6 resemble those of HPS-3 and HPS-5, presumably because HPS3, HPS5 and HPS6 interact with each other in BLOC-2 (biogenesis of lysosome-related organelles complex -2). These findings are important for the prognosis of newly diagnosed HPS-6 patients, and for studying the role of the HPS6 protein in the biogenesis of lysosome-related organelles.

1528/F

Leptin resistance and neuroendocrine defects in mouse models of Bardet-Biedl Syndrome. *S. Seo[1,2], K. Rahmouni[3], V.C. Sheffield[1,2].* 1) Dept. of Pediatrics, Univ. of Iowa, Iowa city, IA; 2) Howard Hughes Medical Institute, IA; 3) Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a pleiotrophic genetic disorder with cardinal features of obesity, polydactyly, and retinal degeneration. We have previously developed mouse BBS models for BBS2 (Bbs2-/-), BBS4 (Bbs4-/-), and BBS6 (Bbs6-/-). These mice recapitulate most of the phenotypes observed in humans. We have used these models to dissect the mechanisms involved in the metabolic disorders associated with BBS. We found that the development of obesity in BBS null mice is associated with hyperphagia, decreased activity and increased circulating level of the adipocyte-derived hormone leptin. Intraperitoneal administration of leptin failed to reduce appetite or body weight in Bbs2-/-, Bbs4-/-, and Bbs6-/- mice suggesting leptin resistance. Increased leptin levels in the cerebrospinal fluid and resistance to intracerebroventricular injected leptin in all BBS mice indicate that a defect in leptin transport across the blood brain barrier is not the cause of leptin resistance in BBS mice. To gain further insight into the etiology of obesity and leptin resistance in BBS mutants, we examined expression of key regulators of energy homeostasis in the hypothalamus. We found that expression of leptin receptor and melanocortin receptor 3 and 4 were unaffected. In contrast, all BBS KO models showed a slight reduction in the expression of key hypothalamic neuropeptides regulating appetite and energy homeostasis: AgRP, NPY, and POMC. Together, our results suggest that BBS proteins may be required for the normal function of the hypothalamic neuroendocrine circuit that controls appetite and energy balance.

1529/F

Functional Assessment of Variants of the Copper Transporter, ATP7B, in Wilson Disease. *S.M. Kenney, C.N. de Leeuw, L.M. Luoma, G. Macintyre, L. Prat Davies, D.W. Cox.* Medical Genetics, Univ of Alberta, Edmonton, AB, Canada.

Wilson Disease is a recessive disorder of copper transport resulting in copper accumulation in, and damage to, liver, kidney and brain. The age of onset of symptoms varies from 3 to almost 70 years, and the diagnosis for this treatable disorder is easily missed. The defective gene encodes a copper transporting membrane P-type ATPase. We have entered all reported mutations in *ATP7B* in the HUGO/HGVS Wilson Disease Database, which includes more than 500 variants (probable disease-causing and the remainder possible normal variants) from populations worldwide (www.medicalgenetics.med.ualberta.ca/wilson/index.php). Of 380 suspected disease-causing mutations in the database, 199 are missense, 114 insertions and deletions, 34 nonsense and 33 splice site mutations. A major issue to address is discrimination between disease-causing and rare normal variants. We use a transport assay in yeast, and cytotoxicity and trafficking assays in Chinese Hamster Ovary (CHO) cells, and homology modeling for functional assessment. Ccc2p, a yeast homologue of ATP7B, supplies copper to Fet3p that imports iron into yeast. *ccc2* mutant yeast do not transport copper to Fet3p, and cannot grow on iron deficient medium (IDM). Human *ATP7B* complements the *ccc2* mutant yeast defect and supports growth on IDM, mutants do not complement. We tested the copper transport ability, in yeast, of 9 ATP7B variants identified in patients. We identified 6 ATP7B variants with a severe impairment in copper transport. Three of the tested variants, and 5 additional variants were assessed in a copper cytotoxicity assay. CHO cells do not express *ATP7B* and are sensitive to copper. *ATP7B* confers protection against copper. Variants defective in trafficking are not protective. 3 variants had similar results in both assays, one severe and two mild. Of five untested variants, 4 had severe defects and were copper-sensitive. A SIFT (Sorting Intolerant From Tolerant) analysis did not distinguish normal from disease variants in 23% of cases. Identification of mutations that contribute to Wilson disease in patients will expand our understanding of the function of this copper transporter and aid in molecular diagnosis.

1530/F

Differential expression of lipid metabolism and insulin signaling genes in skeletal muscle of glycerol kinase KO mice. *L. Rahib[1], K.M Dipple[1,2].* 1) Biomedical Engineering, IDP, UCLA, Los Angeles, CA; 2) Departments of Human Genetics and Pediatrics, UCLA, Los Angeles, CA.

Glycerol kinase (GK) is at the interface of fat and carbohydrate metabolism. GK deficiency (GKD) is an X-linked inborn error of metabolism with metabolic crises as well as predisposition to obesity and type 2 diabetes mellitus (T2DM). Individuals with a GK missense mutation, N288D, are at risk for insulin resistance and T2DM (Gaudet et al., Am J Hum Genet 66:1558, 2000). The purpose of this study was to elucidate the role of GK in fat metabolism and insulin signaling in skeletal muscle (an important tissue in T2DM). To accomplish this, we performed microarray analysis (Affymetrix mouse genome 430 2.0) on a glycerol kinase (Gyk) knock out (KO) mouse model. Total RNA was extracted from muscle hindlimb from one day old Gyk KO and wildtype (WT) male mice. Microarray analysis determined that there were 525 genes that were differentially expressed (1.2 fold, p-value <0.05) between KO and WT mice. Of these 215 were up-regulated and 309 were down regulated. EASE analysis revealed that some of the most statistically significant biological groups were protein binding, ion homeostasis, growth factor binding, insulin-like growth factor binding, cell-matrix adhesion, nucleic acid binding, and regulation of cell growth. Of particular interest were the twenty genes that are involved in lipid metabolism and the ten genes involved in the insulin signaling pathway and diabetes that were differentially expressed between the KO and WT mice. Real Time-PCR confirmed the differential regulation of genes including Gyk and phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha) (Pik3r1), insulin-like growth factor 1 (Igf1), and growth factor receptor bound protein 2-associated protein 1 (Gab1). Further investigations of these genes may provide insight into the role of GK in insulin signaling, insulin resistance and type 2 diabetes mellitus in skeletal muscle. These findings support our previous studies performed in brown adipose tissue (Rahib et al., Eur J Human Genet 15:646, 2007) and further supports the role of GK in insulin sensitivity in various tissues.

1531/F

Molecular analysis of Krabbe disease in populations from Belgium and Italy: evidence for a founder mutation in late onset Krabbe disease in the Catania (Sicily, Italy) region. *W. Lissens[1], A. Arena[2], S. Seneca[1], M. Rafi[3], G. Sorge[2], L. De Meirleir[1, 4], I. Liebaers[1], D. Wenger[3], A. Fiumara[2].* 1) Dept of Medical Genetics, Univ Hosp VUB, Brussels, Belgium; 2) Dept of Pediatrics, University of Catania, Catania, Italy; 3) Dept of Neurology, Jefferson Medical College, Philadelphia, USA; 4) Pediatric Neurology, Universitair Ziekenhuis Brussel, Brussels, Belgium.

Krabbe disease is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme galactocerebrosidase. In this study, molecular defects in the GALC gene were investigated in 7 Belgian patients with the classical infantile form of the disease and 8 families with the late onset form from Sicily, Italy. Three of the Belgian patients were homozygous for a common 30kb deletion (IVS10del30kb), two were compound heterozygotes for this mutation and a novel and a previously described missense mutation. Two other unrelated Belgian patients were homozygous for the p.Tyr551Ser mutation. Five unrelated late onset patients from Sicily came from the same region north to the town of Catania. In this region, the proportion of patients with late onset Krabbe disease is high (72%), although these patients represent only 10% of all patients with Krabbe disease in other populations. Three of these patients were homozygous for a novel p.Gly41Ser mutation, the other two were compound heterozygotes for this mutation and previously described frameshift and missense mutations. The p.Gly41Ser mutation was not present in the 3 other late onset patients from other regions of Sicily, in whom known and 4 novel mutations were identified. Expression studies showed that the p.Gly41Ser mutation, that is on a polymorphic p.Thr546 allele which is known to reduce the enzyme activity by 70% relative to p.Ile 546, results in almost complete loss of enzyme activity; the mutation is also on a unique haplotype as studied by intragenic and extragenic dinucleotide polymorphisms. All these results indicate a founder effect in the patients with Krabbe disease from the Catania region. Possibly this mutation occurred in a single ancestor from that region, but was not further spread.

1532/F

Mutation analysis of the pyruvate dehydrogenase E1 α gene in 70 Japanese patients with pyruvate dehydrogenase complex deficiency. *E. Naito[1,2], K. Shinahara[1], Y. Kotani[1].* 1) Pediatrics, Inst Health Biosci, Univ of Tokushima, Tokushima, Japan; 2) Pediatrics, Tokushima Red Cross Hinomine Medical and Rehabilitation Center, Tokushima, Japan.

Defects in the pyruvate dehydrogenase (PDH) complex, an important cause of neurologic dysfunction and primary lactic acidosis, affect nearly equal numbers of men and women. Symptoms vary considerably between patients with PDH complex deficiencies, although the great majority of PDH complex deficiencies result from mutations in the X-linked pyruvate dehydrogenase (E1) α-subunit gene (PDHA1). Among 70 Japanese patients with PDH complex deficiency (36 female and 34 male) from 63 unrelated families, we identified missense/nonsense mutations of PDHA1 in 49 patients and insertion/deletion mutations in 21 patients. Six families including four with a missense mutation and two with an insertion mutation had two or more affected siblings. In four of these families, all affected individuals were male, while in two families with an N164S mutation in exon 5 both brothers and sisters were affected. Thirty-three different missense/nonsense were found in nine exons, but not exons 1 and 2; three different nonsense mutations were found. Most missense/nonsense mutations occurred in exons 3, 5, 8, and 10, while three mutations at codons N164S, R263G, and R302C accounted for one-third of patients with missense/nonsense mutations (15 of 49); R263G mutations were present in 5 male patients, while R302C mutations were found in 6 female patients. However, 12 different insertion/deletion mutations were found in exons 5, 9, 10, and 11; S388fs mutations in exon 11 were found in 8 male patients. Although total numbers of men and women affected were nearly the same, the distribution of missense/nonsense and insertion/deletion mutations differed between women and men. This difference probably reflects an effect of mutation severity as well as differential X-inactivation in women.

1533/F

Propionic acidemia: Mutation analysis of patients. *P.-W. Chiang, J.P. Kraus, S. Kopinsky, E. Spector.* Pediatrics, UCDHSC, Aurora, CO.

Propionic acidemia (PA) is an autosomal recessive disorder of organic acid metabolism caused by gene mutations in PCCA or PCCB encoding the α and β subunits of mitochondrial enzyme propionyl CoA carboxylase (PCC). PCC is a biotin-dependent heterododecamer of both subunits with a MW of ~ 800 kDa. PCC catalyzes beta-oxidation of odd-chain fatty acids and catabolism of branch-chain amino acids. PA patients suffer acute metabolic episodes that can be life-threatening, with poor feeding, vomiting, hypotonia, lethargy, hyperammonemia and ketoacidosis. Treatment aims to prevent metabolic crisis and neurological sequelae. It includes a formula restricting amino acids that feed into propionate pathways, a low protein diet, and frequent hospitalization. Biochemical testing is available for diagnosis but not for carrier testing. With tandem MS newborn screening, PA can now be diagnosed prospectively, with enzymatic or molecular confirmation. Worldwide, 48 and 55 different mutations, respectively, have been reported in PCCA and PCCB, including missense and nonsense mutations, splicing defects, insertions and deletions (www.uchsc.edu/cbs/pcc/pccmain.htm). Ethnic differences exist: in PCCB 1218del14ins12 is more common in Caucasians while p.T428I and p.R410W are more often found in East Asians. We have developed DNA testing in our clinical diagnostic laboratory to sequence the entire coding regions of PCCA and PCCB. We report here on DNA analysis of 14 patients so far, including fibroblast cell lines from patients of all complementation groups (pccA, pccB, pccC, and pccBC) with previously published Northern and Western results. We found known mutations (common and rare) and several novel mutations, including 581A>G: K194R; 996G>T: E332D; 350G>A: G117D; 1495C>T: Q499X and IVS17+1G>C in PCCA and IVS 13+1G>C in PCCB. Our synthesis of knowledge on mutations, biochemical profiles, and clinical phenotypes will make genotype-phenotype correlation studies easier and may help to predict severity of symptoms, to plan therapy, and to assess prevalence of asymptomatic homozygotes in the population. DNA sequencing will help families with reproductive planning and genetic counseling, by allowing definitive diagnosis, carrier detection and prenatal diagnosis.

1534/F

A Defect in the Thymidine Kinase 2 Gene without mtDNA Depletion. *C. Vinkler[1,3], E. Leshinsky-Silver[2,3], M. Michelson[1,3], S. Cohen[2], M. Ginzberg[3,4], M. Sadeh[5], V. Barash[6], T. Lerman-Sagie[3,4], D. Lev[1,3].* 1) Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel; 2) Molecular Genetics lab, Wolfson Medical Center, Holon, Israel; 3) Mitochondrial Disease Center, Wolfson Medical Center, Holon, Israel; 4) Pediatric Neurology Unit, Wolfson Medical Center, Holon, Israel; 5) Neurology Department, Wolfson Medical Center, Holon, Israel; 6) Metabolic Unit, Hadassah Medical Center, Jerusalem, Israel.

Isolated mitochondrial myopathies are either due to primary defects in mtDNA, or in nuclear genes that control mtDNA abundance and structure such as Thymidine kinase or due to CoQ deficiency. Defects in the thymidine kinase 2 gene have been found to be associated with mtDNA depletion attributed to a depleted mitochondrial dNTP pool in non-dividing cells. We report an unusual case of isolated mitochondrial myopathy, homozygous for the H90N mutation in the Thymidine kinase2 gene but unlike other cases with the same mutation, does not demonstrate mtDNA depletion. The patient's clinical course is relatively mild and a muscle biopsy showed ragged red muscle fibers with a decrease in complexes I and III and increased Complex IV activities. This report extends the phenotypic expression of TK2 defects and suggests that all patients who present with an isolated mitochondrial myopathy even with normal quantities of mtDNA should be screened for TK2 mutations.

1535/F

Sanfilippo syndrome type D: Natural history and identification of three novel mutations in the GNS gene. *E. Andermann[1,2], H. Cao[3], P. Kaplan[4], K. Silver[5], L. De Meirleir[6], M. Veilleux[2], F. Andermann[2], W. Lissens[7], R.A. Hegele[3], A. Jansen[6].* 1) Departments of Human Genetics, McGill University; Neurogenetics Unit, Montreal Neurological Hospital and Institute, Montreal, PQ, Canada; 2) Department of Neurology and Neurosurgery, McGill University, Montreal, Canada; 3) Robarts Research Institute, and University of Western Ontario, London, Ontario, Canada; 4) Biochemical Genetics, Children's Hospital of Philadelphia, Philadelphia, USA; 5) Paediatric Neurology, The University of Chicago Children's Hospital, Chicago, Illinois, USA; 6) Department of Pediatric Neurology, UZ Brussel, Brussels, Belgium; 7) Department of Medical Genetics, UZ Brussel, Brussels, Belgium.

Background: Mucopolysaccharidosis (MPS) IIID or Sanfilippo syndrome type D is a rare autosomal recessive lysosomal storage disorder caused by mutations in the GNS gene on chromosome 12q14. Objective: We have studied the natural history of MPS IIID in two siblings of Italian ancestry who were reported by Kaplan and Wolfe in 1987. In addition, we report the phenotype in two other unrelated families with MPS IIID and have identified three novel mutations in the GNS gene. Methods: Clinical and molecular data on three families with enzyme based diagnosis of MPS IIID were collected. Results: The course of the disease was characteristic for MPS IIID in all four patients, although survival may be longer than was previously reported. In Family 1, both siblings were homozygous for a novel nonsense mutation in the GNS gene (p.Gln390Ter). In Family 2, the proband carried a heterozygous mutation occurring in a splicing recognition site in the intron 7 boundary c.876-2A>G. The second mutation in this patient remains to be identified. In Family 3, the proband was homozygous for a novel frameshift mutation in GNS (p.Asp380GlyfsX9). Conclusions: Major issues in the care of MPS IIID patients include behavioral problems, recurrent infections and pain from orthopedic complications. To date, all mutations in the GNS gene predict premature termination of translation, and there is no obvious genotype-phenotype correlation.

1536/F

Infantile Neuronal Ceroid Lipofuscinosis (CLN8)in a child with dicentric isochromosome 8 due to a mitotic recombination event. *P. Chakraborty[1,3], L. Pham[2], D. Bulman[2], J. Michaud[1, 3], P. Humphreys[1], M.T. Geraghty[1,3].* 1) Children's Hospital of Eastern Ontario, Ottawa; 2) Ottawa Health Research Institute; 3) Dept of Pathology, University of Ottawa, Canada.

We report a girl with an isodicentric chromosome 8 [45, XX, psu dic (8;8) (p23;p23)]. She presented with global developmental delay in infancy, and developed a seizure disorder at 4 years of age and regressed developmentally in all spheres. At age 8, she had roving eye movements, continuous myoclonic jerks, and a spastic quadraparesis. CT scans showed cerebral atrophy with ventriculomegaly and she died at 11 years of age. Autopsy showed severe cerebral atrophy and storage of autofluorescent material in neurons and several extraneural organs, and membrane bound fingerprint and curvilinear inclusions. This suggested the diagnosis of a Neuronal Ceroid Lipofuscinosis (NCL). NCL's are a clincally and genetically heterogeneous group of lysosomal storage disorders causing blindness, seizures and neurodegeneration. The NCL's have been classified clinically by age at presentation, and more recently genetically by the gene involved. Northern epilepsy in the Finnish population and the Turkish variant of late infantile neuronal ceroid lipofuscinosis are known to be caused by mutations of the CLN8 gene located on the chromosome 8p23. FISH analysis using a CLN8 probe revealed homozygous deletion of this locus. Performing PCRs from the tip of chromosome 8 towards the centromere confirmed the homozygous deletion of CLN8, as well as of ZNF596 and LOC157693 (both telomeric to CLN8). MYOM2, coding for the sarcomeric myomesin M-protein 2, was absent in the patient (who did not have any phenotypic muscle defects). Microsatellite markers spanning chromosome 8 were examined in both maternal and patient DNA. Non-maternal alleles were detected suggesting that the translocation is a result of a mitotic recombination event. In conclusion, this is the first report of a dicentric chromosome 8 resulting from a post-zygotic recombination event causing homozygous deletion of CLN8 and NCL. Homozygous knockout of MYOM2 was also present without any evident muscle pathology.

1537/F

Fabry Disease: Identification and Structural Analysis of 34 Novel α-Galactosidase A Mutations Causing Fabry Disease. *D. Kwan[1], M.D. Rudelli[1], D. Germain[2], S.C. Garman[3], M.E. Grace[1], I. Nazarenko[1], R. Dobrovolny[1], M. Yasuda[1], R.J. Desnick[1].* 1) Department of Genetics & Genomic Sciences, Mount Sinai School Medicine, New York, NY 10029; 2) Assistance Publique-Hopitaux de Paris, Paris, France; 3) Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003.

Fabry disease, an X-linked recessive inborn error of glycosphingolipid metabolism, results from the deficient activity of the lysosomal enzyme, α-galactosidase A (α-Gal A). Here we report 34 novel lesions in the α-Gal A gene causing Fabry disease. These include 16 missense (M1V, M42R, G43S, E48D, W81C, A121P, V124D, G132E, K168N, Y207C, I242F, F295C, D299G, G328E, G373R, P389R), four nonsense (Q212X, Q283X, W340X, W399X), one splicing defect (IVS4-3C>G), seven small deletions (c.402delT, c.646delT, c.722delG, c.732delC, c.807delG, c.1086del13, c.1145delGCTTC), three small insertions (c. 265dupCT, c.723dupT, c.996insC), one 26 bp deletion beginning at cDNA nucleotide 57, one complex mutation (D55V/Q57L), and one complex rearrangement (c.281delG/c.283delTGGA). Of the missense mutations, K168N and Y207C occurred at the active site. Transient expression of six missense mutations revealed that E48D, V124D, Y207C, D299G, G373R, and P389R had residual activity ranging from ~6 to 17% of expressed wildtype activity. The effect of each missense mutation on the 3D structure of the enzyme was also analyzed. These studies further define the molecular heterogeneity of the α-Gal A mutations in Fabry disease and provide insight into α-Gal A structure-function relationships.

1538/F

A Block of Autophagy in Lysosomal Storage Disordersα. *C. Settembre[1], A. Fraldi[1], L. Jahreiss[2], C. Spampanato[1], C. Venturi[3], D. Medina[1], R. de Pablo[1], C. Tacchetti[3], D. Rubinsztein[2], A. Ballabio[1,4].* 1) TIGEM, Fondazione Telethon, Naples, Italy; 2) Dept. of Medical Genetics, Cambridge Institute for Medical Research, Cambridge, UK; 3) MicroSCoBiO Research Center, University of Genoa, and IFOM Center of Cell Oncology and Ultrastructure, Genoa, Italy; 4) Medical Genetics, Dept. of Pediatrics, Federico II University, Naples, Italy.

Most lysosomal storage disorders (LSDs) are caused by deficiencies of lysosomal hydrolases. While LSDs were among the first inherited diseases for which the underlying biochemical defects were identified, the mechanisms from enzyme deficiency to cell death are poorly understood. Here we show that lysosomal storage impairs autophagic delivery of bulk cytosolic contents to lysosomes. By studying the mouse models of two LSDs associated with severe neurodegeneration, Multiple Sulfatase Deficiency (MSD) and Mucopolysaccharidosis type IIIA (MPSIIIA), we observed an accumulation of autophagosomes resulting from defective autophagosome-lysosome fusion. An impairment of the autophagic pathway was demonstrated by the inefficient degradation of exogenous aggregate-prone proteins (i.e. expanded huntingtin and mutated α-synuclein) in cells from LSD mice. This impairment resulted in massive accumulation of polyubiquitinated proteins and of dysfunctional mitochondria. These data identify LSDs as "autophagy disorders" and suggest the presence of common mechanisms in the pathogenesis of these and other neurodegenerative diseases.

1539/F

Fabry Disease: Normal Renal Ultrastructure Indicates that α-Galactosidase A Variant D313Y Causes Plasma Enzyme Pseudodeficiency. *M. Yasuda[1], R.E. Gordon[2], S.H. Dikman[2], R.J. Desnick[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai Sch Medicine, New York, NY; 2) Department of Pathology, Mount Sinai Sch Medicine, New York, NY.

Fabry disease is an X-linked inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal enzyme, a-galactosidase A (α-Gal A). In affected males, the progressive lysosomal accumulation of globotriosylceramide (GL-3), particularly in the vascular endothelium, results in renal failure, cardiac and cerebrovascular disease, and early demise. While over 400 disease-causing α-Gal A mutations have been identified to date, only one pseudodeficiency allele, D313Y, has been described. Males with D313Y have markedly decreased α-Gal A activities in plasma or serum. Previous overexpression studies in COS-7 cells demonstrated that the D313Y enzyme has ~60-70% of wild-type intracellular α-Gal A activity, but was unstable in plasma at neutral pH (Yasuda et al. Hum Mutat 22:486-492, 2003). In addition, D313Y was present in ~0.45% of Caucasian individuals. However, recent studies screening for Fabry disease detected patients with deficient α-Gal A activities with only a D313Y allele in hemodialysis and hypertrophic cardiomyopathy clinics, raising concern that this mutation may cause Fabry disease. A renal biopsy was obtained from a male carrying the D313Y allele, who was being considered as a kidney donor for his nephew, who had Fabry disease (α-Gal A mutation, 895del14). The potential donor's α-Gal A enzyme level in plasma was deficient [1.6 nmol/hr/ml (normal mean ± SD: 12 ± 4.2)], while his leukocyte activity was within normal range [53 nmol/hr/ml (normal mean ± SD: 34.6 ± 14.6)]. At age 56, he did not have proteinuria or other symptoms of Fabry disease. On electron microscopy, the glomerular podocytes, mesangial and endothelial cells as well as tubular, arterial medial, endothelial, and interstitial cells all lacked the characteristic electron-dense laminated lysosomal GL-3 inclusions. These studies indicate that D313Y is a rare α-Gal A coding region sequence variant that does not cause renal pathology, and therefore, is not a disease-causing α-Gal A mutation.

1540/F

The molecular basis of Gaucher disease in black South African patients. *S. Arndt[1,2], M. Ramsay[1,2].* 1) Division of Human Genetics, National Health Laboratory Service; 2) University of the Witwatersrand, School of Pathology, Johannesburg, South Africa.

Gaucher disease (GD) is the most common lysosomal storage disease and it is caused by defects in the human glucocerebrosidase gene (GBA). The gene is located in a gene rich region on chromosome1q21,harboring 18 genes in its 200kb genomic surroundings. Two immediate neighboring genes downstream to GBA are pseudogenes resulting in this region to be prone to recombination events. GD is characterized by a high degree of heterogeneity with 266 disease causing mutations recorded in the Human Genome Mutation Database to date. The disease is panethnic in its distribution and occurs at a particularly high frequency in people of Ashkenazi Jewish descent. We studied twenty unrelated black GD patients and identified 39/40 disease causing mutations. Deletion c.222-224delTAC in exon 3 (delta T36) was found in 17/40 (0.425) alleles and 8/40 (0.2) alleles were identified as the recombinant allele RecNcil. The remaining 14/40 disease causing mutations were missense and nonsense mutations of which three are novel. Interestingly, 7/20 (0.35) black patients were compound heterozygotes for deltaT36/RecNcil, thus suggesting low genotypic heterogeneity among black South African GD patients. 66 random population-matched individuals were screened for the delta T36 mutation and a carrier frequency of 1/66 (2/132 alleles) was obtained for this variant. Haplotype studies are in progress for seven SNP markers spanning 200kb upstream and 35kb downstream of the GBA gene. Results for five SNP markers upstream of GBA (rs9628662, rs2242577, rs2361543, rs932972, rs11264372) show complete LD with the frequently observed c.222-224delTAC mutation, supporting a founder hypothesis for this allele. Ethics approval for this research project has been obtained (M030201).

1541/F

Molecular Diagnosis of Primary Carnitine Deficiency. *F.R.O. Calderon[1], L. Shwarz[1], C. Amat di San Filippo[4], M. Pasquali[1,2,3], N. Longo[1,2,3,4], R. Mao[1,2,3].* 1) ARUP Inst Clin Exp Pathology, Salt Lake City, UT; 2) Dept Pathology, Univ Utah, Salt Lake City, UT; 3) ARUP Laboratories, Salt Lake City, UT; 4) Dept Pediatrics, Univ Utah, Salt Lake City, UT.

Background: Primary carnitine deficiency is an autosomal recessive disorder of fatty acid oxidation due to defective OCTN2 carnitine transporters. Affected patients can present with hypoglycemia, liver failure and/or cardiomyopathy and diagnosis of this disorder is confirmed by measurement of carnitine transport activity in cultured skin fibroblasts. Here we evaluate full-gene sequencing of the SLC22A5 gene encoding the OCTN2 carnitine transporter as an alternative diagnostic tool. Methods: The 10 exons of the SLC22A5 gene including exon/intron boundaries were amplified by PCR and bi-directionally sequenced in 23 patients with known fibroblast transport activity. Novel missense mutations were analyzed for conservation and functionally expressed in CHO cells. Results: DNA sequencing identified 83% of mutant alleles, allowing a correct diagnosis in 9 out of 13 patients whose fibroblasts had defective carnitine transport (<10% of control). Expression of novel missense mutations (G15W, P46L, A214V, T232M, R399W) in CHO cells confirmed a pathogenic effect of the variations identified. Our results were collected into a locus-specific mutation database for primary carnitine deficiency. Conclusion: Full-gene sequencing of the SLC22A5 gene can identify causative mutations in the majority of cases of primary carnitine deficiency. Measurement of carnitine transport in fibroblasts can completely exclude primary carnitine deficiency in patients with negative DNA studies.

1542/F

Human mitochondrial microarray (h-MitoArray) and gene expression analysis in patients with mitochondrial ATP synthase deficiency. *A. Cizkova[1, 2, 5], V. Stranecky[1, 2], R. Ivanek[1, 2, 4], H. Hartmannova[1, 2], L. Noskova[2], L. Piherova[1, 2], M. Tesarova[1, 3], H. Hansikova[1, 3], T. Honzik[1, 3], J. Zeman[1, 3], J. Paul[1, 5], J. Houstek[1, 5], S. Kmoch[1, 2].* 1) Center for Applied Genomics; 2) Institute of Inherited Metabolic Disorders; 3) Department of Pediatrics, 1st Faculty of Medicine, Charles University; 4) Institute of Molecular Genetics; 5) Institute of Physiology, Academy of Science, Prague, CR.

We constructed custom microarray and analyzed gene expression of 1632 human mitochondria-related genes in 9 control and 13 fibroblast cell lines from patients with ATP synthase deficiency (2 patients with mt9205ΔTA microdeletion, MT group and 11 patients with not yet characterized nuclear defects, ND group). Principal component analysis and hierarchical clustering defined subgroup of patients with nuclear defect (ND1) which was together with (MT) group and rest of the patient (ND2) group considered in further analyses. ANOVA, functional annotation and gene enrichment analyses revealed in the ND1 group reduced expression of genes involved in cellular signaling (*FOS, NOV, CTSK, UPLC1, PIM1, NF2*), lysosomal metabolism (cathepsins, *NPC, CLN*), protein phosphorylation (*CDK5, PPAP2A*) and ROS metabolism (*GPX4, GPX5, PRDX5, SOD2*). The MT group showed reduced expression of mtDNA encoded ATPase subunits (*ATP6, ATP8*), nuclear encoded ATPase assembly factor 11, cytochrome c oxidase II and mitochondrial transcription factor B1 and elevated expression of nuclear respiratory factor 1. The ND2 group showed reduced expression of adenosin kinase, malate dehydrogenase and elevated expression of peroxisome proliferator-activated receptor gamma. Pathway analysis showed in the ND1 group elevated expression of complex I and reduced expression of complex IV and complex V subunit genes in OXPHOS system and reduced activity of several genes in MAP kinase and Jak-STAT signaling pathways. The MT group showed specific changes of genes involved in mitochondrial biogenesis regulation and retrograde signaling. No meaningful changes were detected in the ND2 group. Our analysis gave hints on potential disease causing genes and pathogenic mechanisms associated with ATPase deficiency.

1543/F

Genotype-phenotype correlation of the Phenylalanine Hydroxylase (PAH) Gene in a Multi-Origin Population. *A. Elimelech[1], Y. Anikster[4], G. Schwartz[4], S. Korem[1], T. Yardeni[1], J. Zlotogora[3], N. Gal[4], N. Goldstein[4], B. Vilensky[4], R. Segev[4], S. Avraham[4], R. Loewenthal[5], D. Bercovich[1,2].* 1) Dept Human Genetics, Kiryat-Shmona, Israel; 2) Tel Hai Academic College; 3) Hebrew University of Jerusalem; 4) Metabolic Disease Unit, Tel Hashomer; 5) 5Tissue Typing Unit, Tel Hashomer, Israel.

This study was aimed to characterize the molecular, clinical and epidemiological aspects of the Phenylketonuria (PKU), in Israel. The phenylalanine hydroxylase (PAH) gene that causes PKU, was scanned in order to define mutations, in different ethnic groups, among the Israeli populations (Jewish: Ashkenazi (AZ(/Sephardi (SF); Arabs: Muslim(MU) /Christians(CR); and Caucasian Christians). The research group consists of 180 unrelated PKU patients. The 13 fragments of the PAH gene scanned by DHPLC technology and DNA sequencing.49 different mutations were defined, which were mostly missense point mutations (61.2%). Mutations in the PAH gene were detected in 173 out of the 180 patients, which comprise 324/360 mutant alleles (90%). Nine novel mutations were identified in this study. The mutation L197F, demonstrated high significant association with the Arabs ethnic group. Six common mutations were: IVS10-11G/A, A403V, L48S, A300S, IVS4+5G/T and R408W (5-13.3%). Two mutations, A300S&L48S, demonstrated a significant correlation with ethnic groups, and were found more common in Jewish patients (20%). The IVS2+1G/A, IVS4+5G/T and F55fsX6 mutations, were mostly found in Arabs patients (33%). 17 mutations were defined as null mutations, demonstrating correlation among the in vitro and the metabolic in vivo phenotypes. Phenotype-genotype analysis revealed the effect of 14 missense mutations on the metabolic phenotype: 10 mutations, were consistency associated with the Classic PKU phenotype and 4 mutations, predict the Mild PKU phenotype. In 63.2% of the patients genotypes, the metabolic phenotype could predict the biochemical and clinical state of the patients. The mutation profile definition of PKU, enable us to construct a national database in Israel, and will be valuable for genetic consultation and prognostic evaluation of future cases of PKU.

1544/F

Compound heterozygosity of glucose-6-phosphatase results in glycogen storage disease type Ia in a Chinese family. *M.M. Gu[1], X.L. Wu[1], Y. Hu[2], D.G. Li[2], Z.G. Wang[1].* 1) Department of Medical Genetics, Shanghai Jiao Tong University School of Medicine, Shanghai, ChinaMedicine, Shanghai, China; 2) Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Glycogen storage disease type Ia (GSD Ia; MIM 232200) is an autosomal recessive inherited disorder resulting from a deficiency of glucose-6- phosphatase (G6Pase). Since the cloning of the gene coding for G6Pase, more than 80 mutations have been identified. Several common G6Pase mutations have been found in different ethnic groups. Here, we report a three-generation family with typical features of GSD Ia from Jiangsu Province, China. The genomic DNA was extracted from peripheral blood samples of two patients and 18 normal relatives. The coding regions of G6Pase were amplified and directly sequenced. The analysis revealed that the patients had a mutation of compound heterozygosity, one mutation from paternity is G to T transversion at the nucleotide 727 (727G→T), another mutation from maternity is alanine (A) to glutamic acid (E) at the amino acid 331 (A331E). Mutation of 727 G→T in exon 5 is a prevalent mutation causing glycogen storage disease Ia in Chinese population. Mutation of A331E is novel and is firstly reported. At the same time, we detect 5 relatives who carry a heterozygote mutation. This finding enrichs G6Pase mutation spectra and confirms that exon 5 may be a hot spot mutation. This character makes possible for mutation screening in Chinese population.

1545/F

Molecular spectrum of Hunter syndrome in Taiwan. *S.P. Lin[1,2,3], J.H. Chang[4], C.K. Chuang[2], G.J. Lee-Chen[4].* 1) Department of Pediatrics, Mackay Memorial Hospital, Taipei 104, Taiwan; 2) Department of Medical Research, Mackay Memorial Hospital, Taipei 251, Taiwan; 3) Department of Early Childhood Care and Education, Mackay Medicine, Nursing and Management College, Taipei 251, Taiwan; 4) Department of Life Science, National Taiwan Normal University, Taipei 117, Taiwan.

Hunter syndrome (MPS II) is a very important rare inherited disorder in East Asia. It is an X-linked recessive lysosomal storage disease caused by a defect of the iduronate-2-sulfatase (IDS) gene, and it counts for more 65% of our MPS population. Various mutations underlying Hunter syndrome have been reported worldwide. To investigate the molecular spectrum of Taiwanese MPS II to help with clinical management, probands and families were recruited and screened for IDS mutations. Expression study was also performed by transfection of COS-7 cells with the mutated cDNA. Together a total of 22 mutations, including 12 missense mutations, 4 splicing defects, 2 nonsense mutations, and 4 deletions, were characterized from 34 families. Missense single nucleotide substitutions A85T, W267C, S305P, and splicing defects G374sp, 1006+5g>c were found from 5 mild MPS II patients. P228L was identified from an intermediate form patient. Carrier detection confirmed a 3:2 ratio of inherited and de novo mutations. In transfected COS-7 cells, mutations from mild form MPS II showed 2.0-3.9% of normal IDS activity. Although they did not cause an apparent reduction in the level of IDS mRNA, the expressed IDS precursor did not show normal maturation. The characterization of gene mutations may delineate their functional consequence on IDS activity and processing, and may enable future studies of genotype-phenotype correlation to estimate prognosis and lead to better selection of MPS II patients for therapeutic intervention.

1546/F

A high frequency of acyl-CoA dehydrogenase, short chain (ACADS) variant genotypes among healthy Ashkenazi Jews. *S. Pardo, B. Kirmse, M. Wasserstein, S. Scott, R. Kornreich, R.J. Desnick, G.A. Diaz, L. Edelmann.* Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare autosomal recessive disorder of fatty acid oxidation that results from mutations in the gene encoding acyl-CoA dehydrogenase, short chain (ACADS). The biochemical phenotype includes urinary excretion of ethylmalonic (EMA) and methylsuccinic (MSA) acids, high C4 acylcarnitine levels and an elevated C4/C2 ratio. The clinical phenotype is very heterogeneous, varying from a fatal metabolic decompensation in infancy with metabolic acidosis, failure to thrive, developmental delay, hypotonia and seizures to a more subtle later-onset progressive myopathy or, most commonly, a completely asymptomatic clinical course. To date, few confirmed SCADD patients have been characterized molecularly and little is known about how genotype correlates with the biochemical and clinical phenotype. With the recent addition of tandem mass spectrometry to newborn screening programs, our Program for Inherited Metabolic Diseases has evaluated 27 newborns with a positive C4 acylcarnitine profile. Five patients of Ashkenazi Jewish (AJ) descent were found to be homozygous for the 319C>T founder mutation. None have displayed any symptoms of SCADD as yet, despite continuous excretion of EMA and MSA. To further delineate the relationship between ACADS genotypes and SCADD, we determined the population frequencies of six known pathogenic variants in ACADS in the general (AJ) population from the greater New York metropolitan area. Our screening data of 412 healthy AJ individuals indicates that the frequency of carriers for three of the variants, 511C>T, 625G>A and 319C>T, is high (0.058, 0.422 and 0.019, respectively), an unexpected finding as SCADD does not display a higher incidence in the AJ population. Biochemical analysis is underway to correlate specific genotypes with urinary concentrations of EMA and MSA. In summary, our results contribute to the understanding that pathogenic variants of ACADS do not necessarily lead to clinical manifestations of SCADD and that additional environmental and/or genetic factors may modify disease presentation.

1547/F

Maple Syrup Urine Disease: Mutation analysis in Filipino patients using the COPPER plate system. *C.L.T Silao[1], H. Samejima[2], C. Torii[2], C. Padilla[1], K. Kosaki[2], M. Matsuo[3].* 1) Institute of Human Genetics, National Institutes of Health Philippines and Department of Pediatrics, University of the Philippines-Philippine General Hospital, Manila, Philippines; 2) Division of Medical Genetics, Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; 3) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan.

Maple Syrup Urine Disease (MSUD) is a recessive metabolic disorder caused by defective function of the branched chain α-ketoacid dehydrogenase complex (BCKD). Mutation analysis of the dihydrolipoyl transacylase (E2), the alpha and beta subunits of the branched chain α-ketoacid decarboxylase (E1α, E1β) genes of the BCKD complex was done in 33 Filipino patients. A highly sensitive and specific mutation scanning method, called the COPPER (Condition-Oriented-PCR-primer-Embedded-Reactor) plate system, to analyze the entire coding regions of the E1α, E1β and the E2 genes was used. The coding regions were amplified using 31 primer pairs, all with the same cycling conditions, and aliquoted on a 96-well format polymerase chain reaction (PCR) plate. This method allowed simultaneous amplification of all coding regions of the 3 genes using a single block in a thermal cycler. Using this method, 7 novel mutations were identified - 2 missense mutations (G132S, M348K) in the E2 gene, 1 missense mutation (S339L) in the E1β gene and 2 nonsense mutations (Q157X, Q190X) in the E1α gene. We were also able to identify an A to G nucleotide substitution changing the start codon ATG to GTG in the E1β gene. Another novel deletion involving nt 788-790 (TCT) was identified in exon 6 of the E1α gene. These findings show that the COPPER plate system is an ideal tool for mutation analysis.

1548/F

Characterization of the ETHE1 protein by cellular and animal models: towards an understanding of its role in Ethylmalonic Encephalopathy. *V. Tiranti[1], R. Mineri[1], C. Viscomi[1], C. Tiveron[2], F. Forlani[3], M. Rimoldi[4], M. Zeviani[1].* 1) Molecular Neurogenetics Unit, IRCCS Foundation Neurological Institute C.Besta, Milan, Italy; 2) Foundation EBRI Rita Levi-Montalcini Disease Modelling Facility, Rome, Italy; 3) Department of Molecular and Agroalimentar Sciences, University of Milan, Italy; 4) Biochemistry and Genetics Unit, IRCCS Foundation Neurological Institute C. Besta, Milan, Italy.

The ETHE1 gene is responsible for Ethylmalonic Encephalopathy (OMIM #602473), a severe mitochondrial disorder reported in children originating from the Mediterranean area and the Middle East. We have been collecting more than 50 patients from 40 families, presenting a fairly homogeneous clinical and biochemical presentation, in spite of a wide spectrum of ETHE1 mutations. All patients showed the presence of a combination of symptoms including petechial purpura, orthostatic acrocyanosis, chronic diarrhoea, and necrotic lesions in the basal ganglia and other regions of the brain, which was associated with high levels of C4 and C5 acylcarnitines in blood, and of ethylmalonic acid in urine. An isolated defect of cytochrome c oxidase was present in skeletal muscle. The ETHE1 protein, is a cysteine-rich metallo-protein located in the mitochondrial matrix, structurally homologous to, but functionally different from, glyoxalase II, a cytosolic thioesterase involved in glutathione recycling. In silico modelling suggests that the ETHE1 protein may also be a thioesterase, acting on a still unknown substrate. By atomic-spectrometric analysis we could show that ETHE1 coordinates a single atom of iron. The possibility for ETHE1 to be a novel mitochondrial esterase is further supported by the observation that the purified wild-type (wt) recombinant protein promoted the formation of the fluorescent dye 2,7-dichlorofluorescein, by hydrolyzing its tri-acetylated precursor ester. Concordant results were also obtained using wt vs. mutant fibroblasts. We have recently produced ETHE1 knockout mice, which seem to recapitulate the main clinical and biochemical features of the human disease.

1549/F

Asian mitochondrial DNA (mtDNA) lineages are associated with altered risk of developing type 2 diabetes and metabolic syndrome. *D.C. Wallace[1], L.-M. Chuang[2], P.H. Wang[3], Y.-C. Chang[2], O. Derbeneva[1], D. Mishmar[1, 4], M.L. L'vova[1].* 1) MAMMAG, University of California, Irvine, Irvine, CA; 2) Medicine, National Taiwan University, Taipei; 3) Medicine, University of California, Irvine, Irvine, CA; 4) Biotechnology, Ben-Gurion University, Beer-Sheva, Israel.

Since we first linked mtDNA mutations to Type II diabetes (T2DM) in a family study (Ballinger, S. et al, 1992, Nat. Genet. 1:11-15), increasing evidence has accumulated implicating mtDNA variation in the etiology of T2DM and the metabolic syndrom(MS). Functional mtDNA variation includes both recent inherited mutations but also ancient adaptive polymorphisms encompassed within region-specific mtDNA lineages (haplogroups, hplgrs)(Ruiz-Pesini, E. et al, 2004, Science 303:223-6). To determine if ancient mtDNA hplgrs might also influence risk for T2DM and MS, we studied 488 subjects from Taipei which had been evaluated for T2DM & MS. Taipei was selected because it encompasses a diverse array of Asian hplgrs in a population exposed to a high calorie diet. This analysis revealed that hplgr F4 was strongly associated with obesity including increased waist circumference (wc) and body mass index (BMI)($P<0.01$), F3 with increased wc; D with elevated systolic bloop pressure (SBP) and D5 with elevated triglycerides (TGs) and SBP (all $P<0.05$). By contrast, N9a was associated with decreased fasting glucose (T2DM protective), in agreement with Fuku, N. et al (2007, AJHG 80:407-15), and low total cholesterol ($P<0.05$), associations that were particularly strong in males ($P<0.01$). Similarly, D5 and D4b were associated with reduced BMI and TG ($P<0.01$) and M10 was associated with reduced TG, SBP & diastolic BP ($P<0.05$). Hence, T2DM and MS risk can be influenced by ancient regional mtDNA polymorphisms, which migth help resolve definitional controversies of MS.

1550/F

Novel Mutations in Carnitine Palmitoyltransferase II gene. *B.Z. Yang, J.H. Ding, N. McNeill, R.J. Chai, L. Sweetman, J. Bennett-Firmin, C.R. Roe.* Inst Metabolic Disease, Baylor Research Institute, Dallas, TX.

Carnitine palmitoyltransferase II (CPT II) deficiency, one of the inherited defects of fatty acid β-oxidation, has three distinct clinical forms: the adult-onset (muscular) form, milder infantile form and severe neonatal form which may result in sudden unexplained death. In this report, a 10-month old patient with CPT II deficiency has been investigated for the molecular defects. All five CPT II exons and their flanking intronic sequences were amplified from proband's DNA. The PCR products were purified and sequenced directly. The sequencing analysis revealed that this patient was a compound heterozygous. A previous reported mutation 452 G>A (R151Q) was detected in one allele. A novel mutation 1933-34 insG was identified at exon 5 in another allele, which results in a frameshift. The mutations were also verified by DNA amplification/enzyme digestion method, but were not detected in the normal control subjects. in our group, thirty-eight unrelated families with CPT II deficiency were identified by CPT II enzyme assay and/or by Acylcarnitine levels measured by tandem mass spectrometry (MS/MS). The molecular aspects had been summarized, including two novel mutations P504L and K389fs in one Pilipino family.

1551/F

Molecular and biochemical analysis of mitochondrial respiratory chain complex I deficient patients. *V. Procaccio[1], P. Potluri[1], E. Ruiz-Pesini[2], D. Mishmar[3], A. Davila[1], K. Tien[1], R. Jimenez[1], M. Simon[1], I. Scheffler[4], D.W. Wallace[1].* 1) MAMMAG, Univ California, Irvine, Irvine, CA; 2) Biochemistry Department Zaragoza University, Spain; 3) Department of Life Sciences, Ben Gurion University, Israel; 4) Univ California, San Diego, San Diego, CA.

Mitochondrial complex I is composed of 45 subunits, 7 subunits are encoded by the mitochondrial DNA (mtDNA) and the rest by nuclear genes and accounts for most cases of respiratory chain deficiency. Mutations in these genes can affect the complex I assembly or activity. A systematic and comprehensive study of the genetic characterization of 15 isolated complex I deficient patients was performed by sequencing the entire mtDNA and all nuclear complex I subunits. Moreover, the level of complex I assembly probed for the stability of various complex I subunits and activity in these cells has been estimated through polarography and native gels and has been shown to be significantly lower than the control cells. Measurements of ATP were also performed on permeabilized cells. Pathogenic nuclear gene mutations were identified in only few patients. In one patient cell line we identified a mutation in the MWFE subunit at the highly conserved position. Complex I levels are about 50% less than normal control mitochondria. The same mutation, created through site directed mutagenesis, in the chinese hamster cell line used as a model leads to a significant decrease of complex I assembly and stability. In another patient cell line, we identified two synergistic mutations in the mitochondrially encoded subunits ND3 and ND6. Complex I assembly and activity is more than 60% reduced due to these composite mutations. In the third set of cell lines, no mutation was detected in any of the known subunits and assembly factors. In some of these extreme cases, stability of more than one subunit is drastically affected and the level of assembled complex I is almost undetectable on a native gel. Our goal is to understand the assembly of complex I through investigating the pattern of subassembly intermediates by using these cell lines and aim to find the defective genes in these cell lines involved in complex I assembly.

1552/F

Molecular screening in 45 patients with isolated COX deficiency for mutations in COX10, COX 15 and SCO1 genes. *K. Vesela, H. Hansikova, J. Zeman.* Center of Applied Genomics, Fac Med /Pediatric, Charles Univ, Prague, Czech Republic.

Cytochrome c oxidase (COX) deficiency represents a heterogeneous group of disorders which predominantly affect tissues with high energy demand, especially the brain, muscle and heart. COX is composed of 13 protein subunits. Three of them are encoded by mitochondrial DNA and the rest originate from nuclear DNA. In addition, numerous other proteins are required for efficient assembly and maintenance of the COX, these proteins originate in nuclear DNA. Currently, there is no efficient treatment, the therapy is just symptomatic; and the prognosis is unfavorable. Knowledge of the exact molecular background is very important for genetic counseling due to possible dual genomic origin of the disease. During this study, we have optimized methods for mutation screening of assembling genes COX10, COX15 and SCO1. Patients were divided according the clinical course and biochemical results. Methods: All coding regions of studied genes were amplified and sequenced. Found mutations were confirmed by RFLP. COX10 was investigated in 24 patients, COX15 in 15, and SCO1 in 6. Results: We descried a novel mutation 394G>A in SCO1 gene in homozygous form; in the other genes we did not find any new pathological mutation. Summary: There are only two patients, siblings, carrying pathological mutations (c.363_364delGA/ c.520C>T) described so far in literature. Mutation 394G>A leading to G132S was not described yet. We did not find the mutation in 200 controls. Patient, a girl, was born in 39th week of gestation (2200g/46cm); APGAR score in 1st and 5th minute was 9 and 10. During the first days of life she developed progressive central hypotony, hepatopathy and she died due to hypertrophic cardiomyopathy in the age of 6 month. Supported by GAČR 303/07/0781.

1553/F

The spectrum of mutations in the MMACHC gene in patients with cblC disease. *J.P. Lerner-Ellis[1,2,3], J. Liu[2,3], D. Coelho[1], T. Suormala[1], A.D. Loewy[2,3], D. Watkins[2,3], S. Gurd[2], C. Morel[2,3], T. Pastinen[2], M. Baumgartner[4], D.S. Rosenblatt[2,3], B. Fowler[1].* 1) Metabolic Unit, University Children's Hospital, Basel, Switzerland, CH-4005; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada, H3G 1B1; 3) Division of Medical Genetics, McGill University Health Centre, Montreal, Quebec, Canada, H3G 1A4; 4) Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland, CH-8032.

Methylmalonic aciduria and homocystinuria, cblC type (OMIM 277400) is the most common inborn error of vitamin B12 (cobalamin, Cbl) metabolism. The gene for cblC was recently identified. We sequenced MMACHC from the genomic DNA of 119 cblC patients, the second largest cohort of cblC patients in the world. Sixteen novel mutations were identified as well as 17 mutations that were observed previously bringing the total number of identified mutations to 58. Haplotype analysis suggests that several mutations have common founders whereas other mutations occurred more than once in human history. A comparison of mutations was made between 323 patients, 102 diagnosed in Europe, and 221 patients diagnosed in North America. A similar distribution of pathogenic alleles was observed for the most common mutation c.271dupA (p.R91KfsX14) and was observed primarily on one haplotype, while patients with the c.394C>T (p.R132X) mutation were twice as frequent in the European cohort; this mutation was observed on three different haplotype backgrounds. Genotype-phenotype correlations of common mutations were apparent; individuals with the c.394C>T mutation generally had late onset disease whereas patients with the c.331C>T (p.R111X) and c.271dupA mutations presented in infancy. Quantitative RT-PCR of RNA from cell lines homozygous for the c.394C>T mutation had significantly higher levels of MMACHC transcript than cell lines homozygous for c.271dupA and c.331C>T mutations as compared to controls. Clinically, individuals with the c.394C>T mutation have responded to vitamin B12 therapy with complete reversal of neurological manifestations and so these findings provide insight into disease mechanism.

1554/W

CHOPPY - A Copy Number Detection Platform Using Illumina Genotyping Data. *X. Gai, J.C. Perin, E. Rappaport, J. Glessner, S.F.A Grant, H. Harkonarson, T.H. Shaikh, P.S. White.* Children's Hospital of Philadelphia, Philadelphia, PA.

The Illumina's HumanHap550 beadchip provides an unprecedented surveillance of the human genome with an average intermarker distance of approximately 6kb. The data can also be examined for chromosomal copy number variations (CNVs), potentially allowing the detection of CNVs of only a few kb. However, identification of copy number changes with Illumina supplied BeadStudio software mostly relies upon visual inspection of the Log R Ratio plot and the B Allele Frequency plot. The process is effective only for the detection of larger CNVs and is inefficient for larger number of samples. We have developed a software platform (CHOPPY) for automatic and batch analysis of the CNVs. CHOPPY was designed for large-scale and high-throughput CNV analysis and consists of three major components: CHOPPY.R for command line data analysis, a CHOPPY database for storing CNVs, and a Web interface called Copy Number Querier (CNQ) for querying and assessing the putative CNVs. The CHOPPY web interface is integrated with a local installation of the UCSC Genome Browser. CNVs can be presented either as an annotation track or in a tabular format for a given patient or cohort, along with other relevant annotations. This provides a convenient setup for interpreting the biological and clinical significance of any observed copy number change. In general, over 90% of known CNVs 20 SNPs or larger, deletions or duplications, can be consistently detected with CHOPPY. For the cleaner data sets, the percentage can be as high as 90% for deletions spanning as few as 4 SNPs. The false discovery rate is estimated to be less than 1% for all identified CNVs that cover at least 4 SNPs. This is supported by the experimental validations of two small duplications and a 4 SNP deletion of of10kb in size. CHOPPY was used to identify 31 unique CNVs, spanning as few as 4 SNPs, in a complete trio. Out of the 15 CNVs found in the proband's genome, 13 were inherited. This high percentage argues strongly for CHOPPY's performance in terms of both the false positive rate and the false negative rate. We are currently validating performance on CNVs as small as 2 SNPs.

1555/W

Chromosomal rearrangement mechanisms underlying six terminal deletions of 1p36 detected by MLPA analyis for a panel of probes in the 1p36 region. *C. D'Angelo[1], J. da Paz[2], C. Kim[3], D. Bertola[3], C. Lourenço[4], C. Koiffmann[1].* 1) Depto Genética, USP, São Paulo, Brazil; 2) Depto Neurologia, HC-FMUSP, São Paulo, Brazil; 3) Unidade de Genética Clínica, ICr/HC-FMUSP, São Paulo, Brazil; 4) Depto Genética, HC-FMUSP, Ribeirão Preto, Brazil.

Monosomy 1p36 syndrome results from a variety of chromosomal rearrangements with scattered breakpoints on the most distal 10.5 Mb of 1p. Sequence analysis of breakpoint junctions of terminal 1p36 deletions have revealed diverse mechanisms underlying formation and/or stabilization that favors a variety of double-strand-break repair pathways competing to repair a terminally deleted chromosome. We have found evidences for telomere healing, breakage-fusion-bridge (BFB) cycles and telomere capture by performing MLPA and FISH on six patients with 1p36 monosomy. MLPA analyses with SALSA P147 and P036/B disclosed four <3 Mb simple terminal truncations and one additional 2.2-2.4 Mb terminal deletion with proximal 1p36 segments triplicated and duplicated. A sixth larger deletion (~6-7 Mb) showed duplication of sequences at 1qter, subsequently confirmed by Multiprobe® FISH to be translocated onto the 1pter. Subtelomeric FISH with the 1p probe confirmed all the deletions as terminal with de novo origin. Four patients with informative results from microsatellite analyses showed maternal inheritance. All the patients had in common the deletion of the GABRD and SKI genes. Four out of six patients presented in some period of their life obesity and/or hyperphagia, and three were initially referred for PWS testing. Our preliminary data suggests that four seemingly pure terminal deletions represent terminally deleted chromosomes repaired by telomere healing. The finding of a complex terminal deletion with a more proximal duplication and triplication is indicative of BFB cycles. We propose three BFB cycles for the formation of this chromosome before becoming structurally stable. Similarly, the observed der(1)t(1;1)(p36;q44) that resembles other three previously described rearrangements may represent a terminal deletion stabilized as a derivative chromosome by a telomere capture event. Supported by FAPESP, CEPID/FAPESP, CAPES, CNPq.

1556/W

Pallister-Killian syndrome: tetrasomy of 12pter ->12p11.22 in a boy with an analphoid, inverted duplicated marker chromosome. *X. Huang[1], M. Michelena[2], E. Leon[2], T. A Maher[1], R. McClure[1], A. Milunsky[1].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, Massachusetts USA; 2) Centro Médico Genetica and Universidad Peruana Cayetano Heredia, Lima, Peru.

Abstract Supernumerary marker chromosomes (SMCs) without detectable alphoid DNA are predicted to have a neocentromere and have been referred to as mitotically stable neocentromere marker chromosomes (NMCs). Here we report the molecular cytogenetic characterization of a new case of Pallister-Killian syndrome (PKS) in a boy with an analphoid, inverted duplicated NMC derived from 12pter→12p11.22 by using High Resolution CGH (HR-CGH), multiplex FISH and BAC-FISH mapping analyses with various alpha-satellite DNA probes, subtelomere probes, and BAC-DNA probes. Precise identification of SMCs and NMCs is of essential importance in genetic counseling. HR-CGH is a more informative and often a faster way of precisely identifying the origin of SMCs. This case is the third report of PKS with a neocentromere marker chromosome containing an inverted duplication of partial 12p with available clinical data. These observations may help to determine the critical region for PKS and the mechanisms leading to the origin of the NMC derived from 12pter→2p11.22 - a region which appears to be susceptible to the formation of neocentromeres. The use of subtelomeric probe PCP12p in buccal cells appears superior to the use of the centromere probe D12Z3 for the diagnosis of the PKS. Key Words: High Resolution CGH (HR-CGH); Neocentromere Marker Chromosomes (NMCs); Pallister-Killian Syndrome (PKS); Supernumerary Marker Chromosomes (SMCs).

1557/W

Effect of chloroquine on human lymphocytes, *in vitro*: Micronucleus assay. *I.P. Aranha[1], C.L.R. Chagas[1], I.C.D. Silva[2], M.Q. Monteiro[2], E.C.M. Passos[2].* 1) Inst. de Biologia, Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; 2) Fac. de Ciências Médicas, Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

Chloroquine was initially used for the treatment of malaria but due to its antiinflamatory characteristics, it has also been successfully used in the treatment of reumathoid arthritis, of cutaneous porphyria and of lupus erithematosus. In this work, the micronucleus assay in human lymphocytes was used to observe the effect of chloroquine on these cells. Micronuclei appear during cell division as a result of acentric chromosome fragments or whole chromosome, outside the nucleus. Peripheral whole blood cells were collected from healthy donors 18 to 30 years old. Cells were incubated at 37ºC for 72 hours in enriched RPMI 1640 medium in the presence of chloroquine (15ng/ml). Cells not exposed to the drug served as control for the experiment. Lymphocytes were exposed to cytochalasin B (4μg/ml) 44 h postinitiation. Following fixation, cells were stained with Gurr's Giemsa (2%) and were analyzed under the optical microscope. In the test group, from 11742 binucleated cells observed, 57 micronuclei were found while in the control group from 11001 cells analyzed, 4 micronuclei were observed. The chi-square test with Yates correction showed that our results were extremely significant ($p<0.0001$) indicating an association of chloroquine and the occurrence of micronuclei.

1558/W

A novel presentation of twins with an interstitial 11q deletion and discordant phenotype. *R. Arvon[1], S. Madan-Kheterpal[2], S. Emery[1], U. Surti[1].* 1) Magee Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, Pa; 2) The Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, Pa.

We report a case of twins who both inherited an unbalanced rearrangement from their father, a balanced insertional carrier, and who exhibit clearly different phenotypes. A 32 year old G 2 P 1 with a dichorionic/diamniotic twin pregnancy presented at 32 wks after twin A was diagnosed with a congenital diaphragmatic hernia (CDH). The father of the subjects mentioned that he had a "balanced chromosomal rearrangement" found when his mother underwent an amniocentesis for advanced maternal age in 1977. Both the ultrasound exam and fetal MRI of twin A confirmed a left-sided CDH as well as a single umbilical artery (SUA) and micrognathia. The ultrasound exam of twin B revealed normal anatomy. We obtained a blood sample from the father and an amniocentesis was performed on each twin. The father was found to be a balanced carrier of an insertion of 11q into 5p 46,XY,ins(5;11)(p13.1;q14.2q23.1) assumed to be de novo because he reported that his parents had normal karyotypes. Chromosomal analysis of the amniotic fluid of both twins revealed partial monosomy of 11q 46,XY,del(1-1)(q14.2q23.1)pat at 650 bands. The mother went into premature labor and delivered both twins at 32 wks . Twin A expired within a few hours after birth and autopsy confirmed a left-sided CDH with abdominal organs in the left chest as well as a SUA and micrognathia. At birth, twin B was noted to have bilateral talipes equinovarus and appeared dysmorphic. The prominent features were micrognathia, down slanting palpebral fissures, mild hypertelorism and posteriorly rotated and simple ears. Chromosomal analysis at 650 bands performed post-natally confirmed the karyotype in both twins 46,XY,del(11)(q14.2q23.1)pat. At present, zygosity testing of both subjects to help determine the reason for the discrepancy in the phenotypes is pending. Partial monosomy of 11q has been previously reported, however not with the same break points as our subjects and the discrepancy in the phenotypes of the twins was unexpected, making this case unique.

1559/W

Detections of genomic alterations of congenital diseases using BAC microarray. *S. Asakawa[1], Y. Murayama[1, 3], T. Yamamoto[3], Y. Furutani[3], R. Matsuoka[3], N. Shimizu[1, 2].* 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) GSP center, The Leading Institute of Keio University, Tsukuba, Japan; 3) The International Research and Educational Institute for Integrated Medical Sciences, Tokyo Women Medical University.

As reported at the last ASHG meeting, we established BAC microarray on which 7718 Keio BAC-DNAs are spotted in triplicate. Using these arrays (GSPArray7700TM), we are examining copy number changes of various cancer tissues (Murayama et al. this meeting). Also, we employed this aCGH system to investigate genomic copy number changes in various congenital diseases and we detected copy number changes of DiGeorge syndrome and Down syndrome patients. We have established about 4,000 cell lines from various congenital diseases including Down syndrome, DiGeorge syndrome, and Williams syndrome patients. Majority of the cases are accompanied by heart abnormalities, but causative genomic alterations of many cases remain unknown. We chose eleven cell lines that showed no known genomic alterations and no point mutations in the selected genes. Of these, one case that exhibited epilepsy, mental retardation, and dysmorphic facial expression showed two large deletions in 7q: One corresponded to Williams syndrome causative region, but the other was a novel deletion. At this meeting, we demonstrate the results of these analyses.

1560/W

Partial trisomy 16 and genitourinary anomalies. *W.A.R. Baratela[2], L. Martelli[1,2], J.A. Squire[3], C.C. Rebelo[2], J. Huber[1,2], L.A.F. Laureano[2], E.S. Ramos[1,2].* 1) Dept. Genetics, Medical School, Ribeirao Preto, University of Sao Paulo, Brazil; 2) Clinical Hospital of Ribeirao Preto, HCFMRP-USP; 3) University Health Network, Toronto, Canada.

Trisomy 16 is the most common autosomal trisomy in spontaneous abortions. The small number of live births reported are mosaic with multiple malformations. The proband was born to a 35 years old G2P0 woman at 35 weeks gestation after an uncomplicated pregnancy. Prenatal ultrasound revealed intrauterine growth restriction. He was delivered by cesarean section due to fetal distress, weighting 1270g, head circumference 29cm, Apgar scores 3 and 7. The physical examination showed macrocephaly, systolic murmur (3+/6) without facial dysmorphic features, ambiguous genitalia described as reduced phallus, penoscrotal hypospadia, bifid scrotum, with one palpable gonad. Echocardiogram was compatible with Tetralogy of Fallot. CT scan of the brain demonstrated bilateral ventricles dilatation and occipital extra dural hematoma. Abdominal ultrasound was normal and vesicoureteral reflux was diagnosed by cystography. Endocrinologic evaluation with full hormone pathways tests suggested bilateral functional testicles. Cytogenetic analysis of 100 metaphases by GTG banding showed 47,XY with a marker chromosome in all cells. Parental chromosome analysis showed that the patient's mother carried a 46,XX,t(15;16)(p11;p12) balanced translocation. Fluorescence *in situ* hybridization studies and spectral karyotype (SKY) confirmed the cytogenetic diagnosis 47,XY,+der(16)t(15;16)(p11;p12)mat. At 6 months of age the proband has failure to thrive with significant developmental delay. The identification and characterization of patients who have inherited chromosomal rearrengements may help to clarify the genomic origin and molecular consequences of partial trisomies. Our study demonstrates the efficiency of the molecular cytogenetic techniques in the analysis of marker chromosomes and expands the phenotypic presentations of chromosome 16 trisomy.

1561/W

Unbalanced derivative chromosome 7 with mild phenotypic features. *S.A. Berend[1], J.B. Ravnan[2], R.E. Bruce[3], M.J. Sutcliffe[4, 5], M.L. Loscalzo[4, 5].* 1) Genzyme Genetics, Tampa, FL; 2) Genzyme Genetics, Santa Fe, NM; 3) Florida Perinatal Associates, Tampa, FL; 4) All Children's Hospital, St. Petersburg, FL; 5) University of South Florida, Tampa, FL.

Cytogenetically visible unbalanced chromosome rearrangements involving the euchromatic regions most often result in relatively severe phenotypic features. We present an unbalanced chromosome rearrangement resulting in mild phenotypic features in a family. The patient was referred for an amniocentesis due to Tetralogy of Fallot with pulmonary atresia seen on ultrasound. Cytogenetic analysis revealed an abnormal chromosome 7 with additional material of unknown origin located on the long arm. Fluorescence in situ hybridization (FISH) analysis with chromosome 7 specific probes showed that the subtelomere region of the long arm of chromosome 7 was deleted. There was a portion of the long arm that did not hybridize with the whole chromosome painting probe for chromosome 7, indicating that there was material present from an additional chromosome. The banding pattern suggested involvement of chromosome 18. Chromosome 18 specific probes confirmed that the material had originated from the long arm of chromosome 18. This rearrangement results in monosomy for the terminal region of chromosome 7 (appears to only involve the subtelomere region), and trisomy for the distal region of the long arm of chromosome 18, from 18q21.3--qter. Cytogenetic and FISH analysis on the father of this fetus indicated he had the same unbalanced derivative chromosome 7. He had mild phenotypic features and normal mental capacity. Previous reports of similar deletions of chromosome 7 report severe mental retardation with short stature and other minor anomalies. Previous reports of similar duplications of chromosome 18 also suggest a more involved phenotype, reporting intrauterine growth retardation, dysmorphic features, and severe to profound mental retardation. The mild phenotype associated with the derivative chromosome 7 in this family is unusual and appears to be discordant with what is reported in the literature.

1562/W

Familial 15qtel trisomy detected by FISH. *V. Catala[1], E. Geán[2], C. Garrido[1], D. Velasco[3], E. Cuatrecasas[1], P. Poo[3], A. Serés[1].* 1) Molecular Cytogenetics, Prenatal Genetics, Barcelona, Catalonia, Spain; 2) Genetics Unit, Hospital Sant Joan de Deu, Esplugues, Spain; 3) Neurology Service, Hospital de Sant Joan de Deu, Esplugues, Spain.

An 8 years old boy from young and healthy parents, with severe behavior problems and mental retardation, and apparently normal karyotype, was studied with a subtelomeric probes panel (Totelvysion, Vysis Abbot). Three signals for 15qtel probe was observed in all metaphases and nuclei analyzed. The third signal was observed in 15p, over satellites. Chromosomes were revised and big satellites in one chromosome 15 were observed. A family study was carried out and the same result was observed in the younger sister. The girl showed a milder phenotype. The father showed normal subtelomeric regions, and a mosaicism with a normal line and a trisomic line for 15qtel was detected in the mother. These findings are very important for the family, because a prenatal or a preimplantation genetic diagnosis are feasible in future pregnancies.

1563/W

Trisomy 5qter (Hunter-McAlpine syndrome) and monosomy 10qter syndrome occurring simultaneously as a result of inheritance of a der(10)t(5;10)(q35.3;q26.1). *P.L. Crotwell, B.M. Hannan, P.R. Delk, W. Torres-Martinez, D.D. Weaver, V.C. Thurston, G.H. Vance.* Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202.

The proband is a 9-month-old female referred to our clinic for counseling and evaluation of an abnormal karyotype of 46,XX,der(10)t(5;10)(q35.3;q26.1), which was discovered via conventional cytogenetics and subtelomeric FISH analysis, and confirmed by CGH. She presented with speech and developmental delay, and dysmorphic features including upslanting palpebral fissures, posteriorly rotated ears, micrognathia, a short nose, and genital hypoplasia. Cytogenetic and CGH results obtained on the proband's parents showed that the mother carries a balanced t(5;10) translocation. A family history disclosed that the proband's maternal uncle has mental retardation and dysmorphic features. By CGH analysis, this latter individual has the same unbalanced rearrangement as the proband. We conclude that the dysmorphic features and developmental delay and mental retardation of the proband and her uncle, respectively, are a result of their partial monosomy of 10q26.1-qter and partial trisomy 5q35.3-qter. Monosomy 10qter syndrome and trisomy 5qter (Hunter-McAlpine syndrome) each have been described in the literature, but to our knowledge, have not been described as occurring simultaneously in the same individual. The syndromes have overlapping features including microcephaly, congenital heart defects, strabismus, and mental retardation (1,2). Our proband has microcephaly and developmental delay and her uncle has microcephaly, strabismus, and mental retardation, but neither has had a congenital heart defect. Further analysis will be performed and a summary of the clinical and laboratory findings will be presented. [1. Hunter et al., 2005. Clin Genet 67:53-60. 2. Leonard et al., 1999. AJMG 86:115-7.].

1564/W

Complete skewed X-inactivation pattern in a patient carrying a Xq28 deletion. *R. Della Casa[1], R. Taurisano[1], D. Melis[1], F. D'Elia[1], C. Figliuolo[2], R. Genesio[2], A. Conti[2], F. Fabbrini[2], L. Nitsch[2], G. Sebastio[1], G. Andria[1].* 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Dept Biology and Cellular and Molecular Pathologye, Federico II Univ, Naples, Italy.

We describe a patient carrying a duplication of Xp22.3 and a deletion of Xq28 band in one of two X chromosomes. The patient, a female, was born at term by cesarean section. Intrauterine growth retardation was registered during pregnancy and microcephaly was observed at birth. When the patient was 5 year-old, the phenotype included: short stature, microcephaly, dysmorphic features (small forehead, slightly deep-set eyes, apparent hypotelorism, relatively large ears, a prominent nasal bridge, prognathism), small hands and feet, proximal implant of thumbs bilaterally. Brain Magnetic Resonance Imaging showed thinning of corpus callosum; Psychometric tests demonstrated an IQ of 60; ABR, ophthalmology examination, abdominal ultrasound showed no abnormalities. Karyotype analysis was normal. The study of subtelomeric regions revealed a "de novo" duplication of Xp22.3 and a deletion of Xq28 band in one of two X chromosomes. The duplication and the deletion interested whole par1 and par2 regions, respectively. The study of chromosome X inactivation pattern showed a complete (100% analyzed lymphocytes) skewed X inactivation of the chromosome carrying the alteration. The present case confirm the hypothesis, already reported in the literature, that genes, involved in the regulation of skewed X inactivation, map in Xq28.

1565/W

46,XY/46,X,del(Yq) mosaicism ascertained in a normospermic man by cytogenetic assessment of an early pregnancy loss and a late termination of an anencephaly foetus. *R. Frikha[1], R. Rekik[2], M. Meddeb[2], T. Rebai[1], N.B. Abdelmoula[1].* 1) Histology Laboratory, University of Medicine, Sfax, Tunisia; 2) Private Sector.

Balanced chromosomal rearrangements have been found at an increased frequency in couples with recurrent spontaneous abortions.Most of them are reciprocal translocations. Robertsonian translocations, autosomal inversions and X chromosome mosaicism are less frequent. Structural Y chromosomal abnormalities are exceptional since they are responsible of infertility, low sperm counts or sexual ambiguity. Here, we report an exceptional observation of a normospermic man for who a 46,XY/46,X,del(Yq) mosaicism was detected by cytogenetic assessment of two early pregnancy losses and one late termination of an anencephaly fetus. The patient was a healthy 45 year old man married since 2005. His wife was a 39 year old woman. No familial history of infertility was recorded but the patient had two half brothers affected by neurofibromatosis. The non consanguineous couple has been explored at 2005 and normal reproductive function was recorded for both. After 8 months, two pregnancy losses takes place: At the first pregnancy with a positive hCG test, pelvic ultrasound reported a pregnancy sac but failed to record any heart activity until 10 week's gestation and pregnancy was ended. The second pregnancy, 3 months later, progressed normally since 18 week's gestation when, elective termination was decided. In fact, the fetus had major anencephaly. Chromosome analysis revealed a 4,XX karyotype for the wife and two cell lines for the husband. The first cell line (21 cells) was 46,XY while the second cell line (9 cells) was of 46 chromosomes with a small size rearranged Y chromosome with a centromere. It was interpreted as a deleted Yq chromosome but others types of rearrangement as dicentric Y chromosome cannot be excluded. FISH analysis using X/Y centromeres, SRY and Yqh probes will be conducted to assess more precisely mosaicism level and Y chromosome structure. Y chromosome AZF regions will also be explored by multiplex PCR. Our study suggests that there is a potential liaison between Y long arm chromosome and embryo development and that Y chromosome may play a role in pregnancy losses.

1566/W

Inter-and intrachromosomal distribution of 13 different types of structural chromosome aberrations localized on acrocentric chromosomes. *L. Kalz, G. Schwanitz.* Institute of Human Genetics, University of Bonn, Germany.

Chromosome investigations were performed in 532 probands, 432 showing constitutional aberrations and 100 with heterochromatic variants in the short arm regions of the acrocentrics. The constitutional aberrations comprised Y-autosome translocations, intrachromosomal abnormalities (del, dup, r, der, i, inv) and interchromosomal rearrangements (rob, rcp, der, CCR) with breakpoints in heterochromatin, euchromatin, and a combination of both. The 13 different types of aberrations each showed an aberration- and chromosome- specific pattern of the abnormalities. Hot spots of aberration and specific frequencies of combination of the chromosomes involved could be delineated. The relation of de novo and familial aberrations was different for the 5 chromosomes investigated. Our results were compared to a large study from literature. We found comparable results for some peculiarities but also differences in frequencies of aberrations, which can be explained by the different ways of documentation. The polymorphisms analyzed were stable in the chromosomes with rearrangements, and in Robertsonian translocations a selection of specific polymorphisms could be excluded.

1567/W

Possible post-meiotic origin of the constitutional t(11;22). *T. Kato[1], H. Inagaki[1], H. Kogo[1], T. Ohye[1], M. Tong[1], B.S. Emanuel[2], H. Kurahashi[1].* 1) Division of Molecular Genetics, Fujita Health University, Toyoake, Japan; 2) Division of Human Genetics, Children's Hospital Philadelphia, Philadelphia, PA.

The constitutional t(11;22) is the only known recurrent non-Robertsonian translocation in humans. The translocation breakpoints occur within palindromic AT-rich repeats on chromosomes 11q23 and 22q11. In our previous studies, we established translocation-specific PCR by using the sequence of the translocation junction fragments from both derivative translocation chromosomes. Using this method, we successfully detected *de novo* t(11;22)s in sperm samples from normal healthy males, but not in lymphoblasts or fibroblasts. To understand how this translocation occurs during spermatogenesis, we divided sperm samples into small aliquots prior to DNA extraction and directly performed translocation-specific PCR. Multiplex PCR allowed us to detect der(11) and der(22)-specific PCR products of *de novo* origin, which were amplified concomitantly from the same aliquots. This result suggests that the *de novo* t(11;22) occurs as a reciprocal translocation. Further, we changed the combinations of primer pairs, which allowed us to identify dicentric and acentric translocation derivative chromosomes. Interestingly, these two unusual derivative chromosomes also appear concomitantly in the same aliquots. Based on the fact that no unbalanced translocation products were identified, we speculate that *de novo* t(11;22) translocations are likely to arise at post-meiotic stages of spermatogenesis.

1568/W

Segregation and pathogenesis of balanced/unbalanced homologous Robertsonian translocations, t(13;13), t(14;14); t(15;15), t(21;21) and t(22;22) - Case reports and review. *D.S. KrishnaMurthy[1], F.M. Al Kandari[1,2], M.A. Redha[1], K.K. Naguib[1], L.A. Bastaki[1], S.A. Al-Awadi[1].* 1) Cytogenetics Laboratory, Kuwait Medical Genetics Centre, Al Sabah, Kuwait; 2) Department of Allied Health, Kuwait University, KUWAIT.

One in 900 humans is born with a Robertsonian translocation. The most frequent forms of Robertsonian translocations are between chromosomes 13 and 14, 13 and 21, and 21 and 22. Robertsonian translocations (balanced or unbalanced) involving acrocentric chromosomes, 13,14,15 and 21, 22 are well known chromosomal abnormalities leading to multiple congenital anomalies, infertility, repeated fetal loss, dysmorphism and mental retardation. However, homologous Robertsonaian translocations, t(13;13q),t(14;14),t(15;15) and t(22;22) are relatively rare. Carriers of balanced ROBs are at an increased risk of having chromosomally unbalanced, phenotypically abnormal offspring. These individuals are trisomic for one of the chromosomes involved in the translocation, with three copies instead of the normal complement of two. Carriers of ROBs are also at an increased risk of uniparental disomy (UPD), the inheritance of both chromosome copies from a single parent. Uniparental inheritance of some chromosomes has been shown to be deleterious due to the effects of imprinting (the differential expression of genes depending on the parent of origin). Risk estimates vary depending on the type of rearrangement. Carriers of homologous acrocentric rearrangements are at very high risk of having multiple spontaneous abortions and chromosomally abnormal offspring. Parents of fetuses and children with unbalanced homologous acrocentric rearrangements are rarely found to be carriers or mosaic for the same rearrangement.

1569/W

Chromosome 10(q23-qter) Deletion and Pericentric Inversion 9(p13-q12) in Congenital Lower Lid Entropion. *M. Kumar[1], R. Kumar[2], P. Gupta[3], R. Dada[2], N. Pushker[3], J. Kaur[1].* 1) Ocular Biochemistry, RP Center, All India Institute of Medical Sciences, New Delhiø; 2) Department of Anatomy, All India Institute of Medical Sciences, New Delhiä; 3) Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhiê(E-mail: kaurjasbir@rediffmail.com).

Entropion is an inversion of the eyelid (inward turning of the eyelid margin) toward the globe. The lower eyelid is more frequently affected and depending on the underlying disorder, the entropion may be either unilateral or bilateral. Congenital entropion is an extremely rare disorder and usually involves the lower eyelids. It is often familial and is seen more frequently in Asians. The possible causes for this condition include structural tarsal plate defects (horizontal tarsal kink syndrome) and shortened posterior lamella (tarsus, conjunctiva, eyelid retractors). It has been reported that congenital entropion is a part of a syndrome involving multiple systemic anomalies. A case of primary congenital upper eyelid entropion with cardiovascular, musculoskeletal, and central nervous system abnormalities and another with congenital heart defect has been reported. But, to the best of our knowledge there is no report describing the genetic background of the disease. We report a patient of congenital lower lid entropion and corneal opacity with the help of conventional cytogenetics. GTG-banding revealed an interstitial deletion in chromosome 10 and pericentric inversion of chromosome 9. Chromosomal analysis shows 46,XX,del(10q23-qter)/46,XX,inv(9p13-q12) karyotype. Most publications suggest that pericentric inversion of chromosome 9 is a polymorphic variation and its clinical significance is uncertain. Thus our finding raises the possibility that the congenital lower lid entropion locus may be located on chromosome 10. This represents a more severe manifestation of the disease. Finally, a workup of this finding is suggested and more cases of congenital lower lid entropion needs to be screened using cytogenetics.

1570/W

Complex telomeric imbalances uncovered by array CGH: Is there a common mechanism for some telomeric rearrangements? *J. Lee, Z. Nawaz, E.J. Wallace, D.H. Ledbetter, C.L. Martin.* Dept Human Genetics, Emory Univ, Atlanta, GA.

Several platforms utilizing array-based comparative genomic hybridization (aCGH) have been applied to assess individuals with unexplained mental retardation/developmental delay and have confirmed that telomere imbalances are overrepresented compared to "average" chromosomal regions. We designed a custom oligonucleotide microarray consisting of high-density coverage for all telomere regions, as well as a whole-genome backbone, to develop a more efficient and comprehensive method for characterizing telomere imbalances. Our custom array has been used to calibrate the size and gene content in 44 samples with cryptic pathogenic subtelomeric imbalances. In five of these cases, three of which were originally detected as pure deletions and two which were structural rearrangements, aCGH detected additional interstitial imbalances adjacent to the telomere imbalance. Two cases, involving the telomeric regions of 2q and 22q, showed duplication juxtaposed immediately adjacent to the breakpoint of a terminal deletion. One case, involving 6q, also showed duplication proximal to the telomeric deletion, however a ~1.5 Mb gap was present between the deletion and duplication. An unbalanced translocation between 9p and 20p, that resulted in partial monosomy 9p and partial trisomy 20p, showed an additional duplication next to the breakpoint of the 9p deletion. Finally, in a case with a ring chromosome 22, duplication was identified adjacent to a terminal deletion. In all of these cases, the duplicated material was derived from the same chromosome as the deletion. Two other recent aCGH studies have also observed inverted duplications adjacent to telomere deletions in cases with a 1p terminal deletion and a ring chromosome 14. The possible mechanism proposed for these rearrangements is pre-meiotic breakage-fusion-bridge cycles after random breakage. Our data provides further evidence for a common mechanism that involves duplication-deletion in cryptic telomeric rearrangements. Further detailed studies, such as FISH and sequence analysis of this breakpoint, will help to interpret this complexity and understand the mechanism underlying some terminal telomere imbalances.

1571/W

Characterization of a novel asymmetrical isodicentric chromosome 18. *C.C. Lin[1,4], P.P. Liu[2], Y.C. Li[3], L.J. Hsieh[1], Y.C. Liu[1], Y.M. Cheng[1], S.L. Shi[4], C.H. Tsai[4], F.J. Tsai[4].* 1) Lab for Chromosome Research, China Medical Univ & Hosp, Taichung, Taiwan; 2) Women's Clinic, Taichung, Taiwan; 3) Dept. of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan; 4) Dept. of Medical Genetics and Pediatrics, China Medical University Hospital, Taichung, Taiwan.

Molecular cytogenetic analysis identified a new type of isodicentric chromosome involving different breakpoints at 18q in a female fetus; the anomaly was termed asymmetrical pseudoisodicentric chromosome 18 [46,XX,dic(18)(pter→q11.2:: q21.3→pter)]. A series of BAC clones for 18q11.2 and q21.3 regions were used to identify one breakpoint within the region q11.2 between 19.8Mb and 21.6 Mb from the telomere of 18p and another breakpoint within q21.3 between 55.4 Mb and 56.9 Mb from the telomere of 18p by FISH analysis. Real-time quantitative PCR and microsatellite analysis further verified that the dicentric chromosome was maternal in origin and resulted from break-a reunion between sister chromatids of a single maternal chromosome. We propose that a loop-type configuration of sister chromatids took place and that the break-reunion occurred at cross sites of the loop to form an asymmetrical isodicentric during either in mitosis or meiosis. In this case, the asymmetrical pseudoisodicentrics resulted in an 18pter→q11.2 duplication and an 18q21.3→qter deletion, which could lead to certain dysmorphic features of 18q- syndrome in the fetus. Particularly, we presented here a severe form of congenital aural atresia (common feature of 18q- syndrome), anotia associated with an 18q terminal deletion and identified the breakpoint occurred at 18q somewhere between 55.6 Mb and 56.9 Mb from the 18p telomere.

1572/W

Inherited homozygous paracentric inversion affecting both arms of chromosome 12. *L. Martelli[1, 2], I. Gomy[2], L.A.F. Laureano[2], M. Yoshimoto[3], E.S. Ramos[1, 2], M.S.J. deVozzi[1], J.A. Squire[3].* 1) Department of Genetics, University of Sao Paulo, Ribeirao Preto, SP, Brazil; 2) Medical Genetics Division, Clinical Hospital of Ribeirao Preto, SP, Brazil; 3) University Health Network, Toronto, Canada.

In general, carriers of paracentric inversions are phenotypically normal, although individual reports have described occurrence of infertility, miscarriages and mental retardation in inversion carriers. Inversions involving chromosome 12 are rare, not correlated to phenotypic findings and have been recognized in some benign tumors. The proband was born to a 30 years old G4P2A2 woman at term, after an uncomplicated pregnancy. The parents are consanguineous, second degree cousins. He was delivered by cesarean section due to cephalopelvic desproportion, weighting 4000g. The boy was referred to the Medical Genetics Division at 9yo due to developmental delay and dysmorphic features. Physical examination showed macrossomy with obesity, H=151cm (p>97) and W=58400g (p>97), brachycephaly, facial dysmorphism characterized by hypertelorism, downward slanting palpebral fissures, broad nasal bridge, large ears (7.5cm), preauricular pit, tapering phalanges, toe position anomaly and unilateral cryptorchidism. Delayed developmental milestones were evident, presenting hyperactivity and mild mental retardation. Conventional cytogenetic analysis by GTG banding showed 46,XY chromosomes and paracentric inversion affecting both arms of the pair 12. M-band results confirmed that both chromosomes 12 were identical, with the same rearrangements and final karyotype 46,XY,der(12) inv(12)(p11.2p12.3)inv(12)(q21.1q24.1)x2. Parental chromosome analysis showed that the patient's mother carried an identical karyotype and his father was inversion heterozygote. The aberrations in the child implicate that subtle genomic alterations resulting from the inversion may have contributed to his phenotype. Supported by CAPES and FAEPA, HCFMRP-USP.

1573/W

Interstitial 6q25 deletion accompanied by an unexpected *STS* (Xp22.31) microdeletion. *F.M. Mikhail, E.J. Lose, K. Goodin, A.J. Carroll.* Dept. of Genetics, Univ. of Alabama at Birmingham, Birmingham, AL.

At least 60 cases with deletions of the long (q) arm of chromosome 6 have been reported to date. To correlate phenotype with genotype; 6q deletions have been classified into three groups: group A with del(6)(q11q16), group B with del(6)(q15q25), and group C with del(6)(q25-qter). Interstitial deletions in the distal region of 6q are relatively rare, and have been shown to cluster in band 6q25. Here we report an 11.5-year-old boy with an interstitial distal 6q deletion who was seen in our clinic because of history of mental retardation, developmental delay, and dysmorphic features. He displayed some overlapping features of group B and C 6q deletions. These included IUGR, mental retardation, developmental delay, autism, no speech, eye anomalies in the form of exophthalmos, ear anomalies in the form of small low set ears with a preauricular pit on the left ear, hand anomalies in the form of bilateral single transverse palmar creases with short fingers, and foot anomalies in the form of short widely spaced toes with narrow nails. HRB chromosome analysis revealed an interstitial deletion on 6q [del(6)(q25.2q25.3)]. Using the 32k BAC tiling path array CGH chip, we were able to precisely map the breakpoints of the deletion, which was estimated to be ~6.3 Mb in size. Unexpectedly however, array CGH analysis also demonstrated that the patient carries an ~1.5 Mb *STS* gene microdeletion on the short (p) arm of chromosome X, which was confirmed by FISH analysis using the *STS* probe. Nullisomy of the *STS* gene is consistent with the clinical diagnosis of X-linked ichthyosis. Clinically, the boy had bilateral congenital cataracts and his skin was scaly and dry. The patient's final karyotype was 46,XY,del(6)(q25.2q25.3).ish del(X)(p22.31p22.31)(*STS*-). In conclusion, our patient represents a perfect example for the clinical usefulness of whole genome array CGH analysis, which was able not only to map the breakpoints of the distal 6q deletion but also revealed the *STS* microdeletion. Parental chromosome and FISH analyses are underway. Detailed description of our patient's clinical features and comparison with previously reported distal 6q deletions will be presented.

1574/W

A complex chromosome rearrangement in three generations : reproductive risk, meiotic pairing and phenotype-karyotype correlation. *J. Puechberty, A. Schneider, A.M. Chaze, P. Sarda, G. Lefort, P. Blanchet.* Service de génétique médicale, Hôpital Arnaud de Villeneuve CHU Montpellier , Montpellier, France.

Complex chromosome rearrangements (CCRs) are uncommon events generally defined as involving two or more chromosomes and at least three breakpoints. Familial forms are rare and usually associated with a history of infertility, recurrent miscarriage and abnormal phenotype. The advent of multicolor FISH studies and more recently microarray technologies has greatly aided the characterization of CCRs. We report on three generations of relatives with a familial CCR. The rearrangement was ascertained through an unbalanced product during the first pregnancy of a clinically healthy mother. Initial chromosome studies with classical RHG banding techniques suggested that the healthy mother was the carrier of an apparently balanced simple three-way exchange translocation t(7q;12q;17q). This rearrangement was later found to be inherited from her mother. Multicolor FISH studies subsequently identified an additional event : chromosome painting revealed that the interstitial segment between 12q21and 12q22 was translocated onto 7q34, thus modifying the initial karyotype interpretation. The healthy mother has a brother with mental retardation and an unbalanced karyotype. She has a history of 3 pregnancies terminated because of unbalanced karyotypes as well as one spontaneous abortion. She gave birth to a healthy girl with a normal 46,XX karyotype and is presently pregnant for the 6th time. Amniocentesis was performed and the female fetal karyotype shows the apparently balanced maternal rearrangement. We discuss the reproductive risk, pachytene configuration and phenotype-karyotype correlation of this CCR.

1575/W

Identification of multiple cell lines in a female patient suggests a biological mechanism underlying cell line mosaicism. *S.C. Reshmi[1,2], L.J. Henderson[2], J. Miller[3], D. Deplewski[3], D.J. Waggoner[2,4], S. Schwartz[1,2].* 1) University of Chicago, Chicago, IL. Department of Medicine; 2) University of Chicago, Chicago, IL. Department of Human Genetics; 3) University of Chicago, Chicago, IL. Department of Endocrinology; 4) University of Chicago, Chicago, IL. Department of Pediatrics.

Sex chromosome abnormalities account for close to 0.5% of live births. Of these, many phenotypes are commonly known to be associated with a specific karyotype. However, individuals with mosaic cell lines that include a structurally rearranged sex chromosome cell line appear to have a much less predictable phenotype, and often present with ambiguous genitalia. In this study we report a 2.5 year old female with phenotypic features of Turner syndrome including webbing of the neck, wide spaced nipples, no uterus, and no normal testes or ovaries. Cytogenetic analysis revealed an 45,X/46,X,idic(Y)(p11.2) karyotype. Fluorescence *in situ* hybridization (FISH) mapping of the Y chromosome with probes localized the Yp11.2 breakpoint to a region just distal to *SRY*. We also, however, observed the presence of an additional cell line with a deleted (Y)(p11.2) that was detected mainly in interphase cells. To date, no reports have identified the presence of an 46,XY cell line or 46,X,del(Y) cell line in persons with an 45,X/46,X,idic(Y)(p11.2) karyotype. Our findings suggest a possible mechanism for cell line mosaicism: 1) Sister chromatid breakage during spermatogenesis, resulting in a deletion of the Yp11.2 - Ypter region; 2)Subsequent misdivision of the deleted Y during mitosis resulting in both 45,X and 45,X,del(Y)(p11.2) cell lines, in which the dicentric Y chromosome forms from an attempt to repair double stranded breaks.

1576/W

Identification of chromosomal rearrangements in children with mental retardation by CGH and FISH. *R. Ruiz-Esparza, A.C. Velazquez-Wong, C. Hernandez-Huerta, M.C. Palacios-Reyes, D. Arenas-Aranda, M.A. Araujo-Solis, F. Salamanca-Gomez.* Unit of Investigation In Human Genetics, Instituto Mexicano del Seguro Social, Mexico City, Mexico.

Introduction: Mental retardation (MR) is the most common handicap in childhood, affecting about 3% of the general population. The etiology of MR is unexplained in 30-50% of all cases. Researchers have identified multiple causes including genetic disorders, environmental factors, traumatic accidents, prenatal events such as maternal infection or exposure to alcohol. It has been estimated that chromosomal anomalies account for 4-28% of cases of MR. Recent advances in cytogenetics have shown that subtelomeric rearrangements are involved in 5-7.4% of cases of MR, being deletion 1p36 the most frequent chromosomal rearrangement. In this work, children with idiopathic mental retardation were studied utilizing a FISH assay to detect deletion 1p36 and CGH to identify chromosomal rearrangements. Methods: 55 children with MR were screened using a multicolor FISH assay using probe 1p36 (D1Z2) developed at LLNL, and 11 of them were also analyzed by CGH. A peripheral blood sample was obtained from every patient and lymphocytes cultures were used to obtain metaphase chromosome spreads to perform the FISH assay. A blood sample was also used to obtain total genomic DNA to carry out CGH. At least 25 metaphases were analyzed for each patient for each methodology. Results: CGH results revealed one patient with a deletion in the region 8q24.3. Interestingly, FISH assay did not showed any patients with deletion 1p36. Discussion: These results confirm that the application of molecular cytogenetic methods opens up a promising way to identify chromosomal rearrangements and, therefore genes related to the etiology of MR. This work was conducted with support from the Instituto Mexicano del Seguro Social and Conacyt 2005-01-13947.

1577/W

Micronuclei in human lymphocytes exposed to sodium pertechnetate, in vitro: Preliminary results. *M.B. Santana[1], C.M. dos Santos[2], I.P. Aranha[3].* 1) Fac. Ciências Médicas, Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; 2) Inst. de Ciências Biológicas e Ambientais, Univ. Santa Úrsula, Rio de Janeiro, Brazil; 3) Inst. de Biologia, Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

Since its humble beginning in 1958, technetium-99m (^{99m}Tc)has become the most widely used radioisotope in the detection of inflamatory sites as well as in the diagnosis of transplanted tissues. The goal of the present work is to study the effect of sodium pertechnetate on human lymphocytes *in vitro*, using the micronucleus assay. Peripheral whole blood cells collected from healthy donors, 18 to 30 years old, were incubated at 37°C for 48 hours in the presence of ^{99m}Tc (3.7 MBq/100µl). Cells not exposed to the radionuclide served as control for the experiment. Cytochalasin B (4µg/ml) was added to the cultures 20 h postinitiation. After fixation, cells were stained with Gurr's Giemsa (2%) and were observed under optical microscope. In the test group, 3573 binucleated cells were studied and 143 micronuclei were found. In the control group, 4845 cells were observed and 3 micronuclei were seen. The chi-square test with Yates correction indicated that the results were extremely significant ($p<0.0001$) suggesting that sodium pertechnetate was responsible for the micronuclei observed.

1578/W

Breaking chromosomes and rules: Phenotypic abnormalities in a family with a seemingly balanced 11,22 translocation. *N. Shur[1], R. Marion[1], J. Greally[2].* 1) Montefiore Medical Center, Bronx, NY; 2) Albert Einstein College of Medicine, Bronx, NY.

Case report: The most common balanced translocation described in humans involves chromosome 11 and 22: t(11;22)(q23;q11). Previous molecular studies have shown that the breakpoints of the translocation are conserved among carriers, who are described as phenotypically normal and often remain undetected until they present with fertility problems. Balanced carriers incur the major risk of future progeny born with Emanuel syndrome, the result of abnormal segregation of the derivative chromosome 22, which leads to supernumerary der (22)t(11;22) syndrome. Clinical features include developmental disability (DD), malformed ears, and congenital heart defects. We report the case of a phenotypically normal female carrier whose son had DD and dysmorphic features: molecular testing confirmed that he is a carrier of the same translocation. Case report: A 37-year-old woman with known(t11;22)(q22.3q11.2) was counseled after amniocentesis that the fetus had her same translocation but was "normal." She continued the pregnancy. During the first year of life significant hypotonia, dysmorphic features, and DD occurred, prompting referral for genetics evaluation. High-resolution chromosome analysis and comparative genomic hybdridization (CGH) failed to reveal additional abnormalities. An extensive work-up including Fragile X was negative. Discussion: Although the balanced translocation might be a serendipitous finding, this case raises the possibility that breaks in chromosomes, even those that are seemingly balanced, may cause disruptions through a variety of mechanisms, including break points on a critical gene, uniparental disomy, microdeletions, mosaicism, and epigenetic effects. Pinpointing whether subtle molecular variations among carriers exist proves difficult, although in the future improved CGH resolution and molecular diagnostics will certainly provide more complete translocation characterization. In the interim, families with 11,22 balanced translocations may benefit from counseling that even seemingly balanced translocations carry risk of phenotypic abnormalities.

1579/W

LCR22-B - mediated chromosome translocation t(4;22)(q11.2;q21.22): A search for the AT-rich cruciform structures. *M. Smyk[1], J. Pietrzak[1], M. Lisik[2], E. Obersztyn[1], E. Bocian[1], P. Stankiewicz[1,3].* 1) Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 2) Department of Medical Genetics, Silesian University School of Medicine, Katowice, Poland; 3) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston TX.

Chromosome 22q11.2 is an unstable genomic region associated with a number of common genomic disorders such as DiGeorge/Velocardiofacial syndrome (deletion 22q11.2), microduplication dup(22)(q11.2q11.2), cat-eye syndrome and der(22) syndrome. The increased instability of this region is related to the presence of several highly homologous low-copy repeats. The der(22) syndrome results from an unbalanced product of the most frequent non-Robertsonian recurrent translocation in humans, t(11;22)(q11.2;q23.3). The breakpoints of this translocation have been mapped within the center of the AT-rich cruciform structures also known as the Palindromic AT-Rich Repeat or PATRR. The chromosome 22q11.2 palindrome is located within the LCR22-B copy, is 595 bp in size and has been found to harbor the breakpoints of a few other non-recurrent translocations: t(17;22)(q11.2;q11.2) (two cases), t(1;22)(p21.2;q11.2), t(4;22)(q35.1;q11.2), and t(8;22)(q24.13;q11.21). Interestingly, the partner chromosome breakpoints have been mapped also within palindromic sequences, most of which are AT-rich. We present an apparently balanced translocation t(4;22)(q11.2;q21.22) identified in a phenotypically normal girl and her mother, who had several miscarriages. Using FISH with BAC clones, we have mapped the chromosome 22q breakpoint within the LCR22-B copy and the chromosome 4q21.22 breakpoint within an ~50 kb fragment of a BAC clone RP11-51G24. Using computer analyses of the DNA sequence of this DNA segment, we have identified three AT-rich palindromes. We hypothesize that one of them, together with the 595 bp palindrome in LCR22-B, might have mediated the formation of the described translocation t(4;22)(q11.2;q21.22). Our data identify a novel potentially unstable region in the human genome.

1580/W

A dysmorphic newborn with partial monosomy of 7q36-->qter and partial trisomy of p24-->pter. *M. SOYLEMEZ, G. TOKSOY, C. SAYAR, A. GIRAY, T. YARDIMCI, B. TURKOVER.* DEPARTMENT OF GENETICS, ZEYNEP KAMIL WOMAN AND CHILDREN HOSPITAL, ISTANBUL, Turkey.

We report on a 3 month-old male presenting with intrauterin growth retardation, facial dysmorphic features such as "premature craniosynostosis, microphthalmia, blepharophimosis, narrow forehead, bitemporal narrowing, large ears", mental retardation, cardiac defects, microcephaly and hypotonia. Cytogenetic studies revealed an apparent robertsonian translocation between chromosome 15 and 22. Additional material on chromosome 7q was identified and determined to be from chromosome 3p by analysis with fluorescence in situ hybridization (FISH). The karyotype is 45,XY,der(15;22)(q10;q10),add(7)(q36).ish t(3;7)(p24;q36) de novo. His father and mother had a normal karyotype. The robertsonian translocation seen in all metaphases between 15 and 22 chromosomes was not expected to explain dysmorphic features. It was concluded that the phenotypic features were due to partial monosomy of 7q36-->qter and partial trisomy of 3p24-->pter.

1581/W

An unusual marker resulting in partial tetrasomy 12p in a patient with multiple congenital anomalies. *J.F. Turocy[1], U. Surti[1], S. Madan-Khetarpal[2], E. James[2].* 1) Dept Clinical Genetics, Magee-Womens Hosp of University of Pittsburgh, Pittsburgh, PA; 2) Dept Clinical Genetics, Childrens Hospital of Pittsburgh of University of Pittsburgh, Pittsburgh,PA.

A female infant was first seen at age 3 weeks. Prenatal amniocentesis (maternal age 38y) showed a marker chromosome determined to be partial chromosome 12. After birth, she was determined to have partial small bowel malrotation, hydrocephalus with macrocephaly requiring VP shunt, infantile spasms, cortical visual impairment, a nasal dermoid of the forehead, and bifid uvula. At age 3 years, she had hypertelorism, downslanting palpebral fissure, normal hair and eyelashes, short webbed neck, polythelia, 5th digit clinodactyly, hypotonia, and inability to sit up without support. OFC was 25th percentile. She was profoundly developmentally delayed, and had limited use of her hands. Karyotype of peripheral lymphocytes showed 47,XX,+mar in all cells. FISH analysis showed that the marker contained 2 copies of the 12p telomeric region, was positive for 12CEP centromeric probe, and was C-Band positive. Microarray analysis indicated a 2-copy gain of 23 BACS measuring 5.1Mb from 12p. Two previous reports described a marker 12 containing a partial distal 12p including 12pter that had a phenotype similar to or milder than that of Tetrasomy 12p Pallister-Killian Syndrome (PKS). Both of these markers were analphoid, with a neocentromere. This appears to be a first case of a similar marker with the centromeric region containing a chromosome 12 α-satellite sequence. In comparison to the other two reported cases our patient has a pericentromeric fragment (containing α-satellite DNA in the 12p11.22-p10 region), and a similar phenotype. All three patients have a somewhat milder phenotype than typical isochromosome 12p PKS. Also, unlike PKS, the marker chromosome in our case was present in 100% of the peripheral blood metaphases analyzed. The use of microarray analysis of partial tetrasomy 12p may help narrow the critical PKS region(s) responsible for the phenotype and the well documented loss of the complete isochromosome 12p from peripheral blood cultures in vitro.

1582/W

Molecular cytogenetic investigation of two patients with Y chromosome rearrangements and intellectual disability. *C. Tyson[1], A.J. Dawson[2, 3, 4], S. Bal[2], M. Tomiuk[2], T. Anderson[2], D. Tucker[2], D. Riordan[2], I. Chudoba[5], B. Morash[2, 3, 4], A. Mhanni[3, 4], A.E. Chudley[3, 4], B. McGillivray[6], M. Parslow[8], G. Rappold[7], R. Roeth[7], C. Fawcett[1], Y. Qiao[1], C. Harvard[1], E. Rajcan-Separovic[1].* 1) Dept Pathology, University of British Columbia, Vancouver, Canada; 2) Div Lab Medicine and Pathology, Health Sciences Centre, Winnipeg, Canada; 3) Dept Pediatrics and Child Health, Health Sciences Centre, Winnipeg, Canada; 4) Dept of Biochemistry and Medical Genetics, Univ Manitoba, Winnipeg, Canada; 5) Metasystems, Germany; 6) Dept of Medical Genetics, Univ BC, Vancouver, Canada; 7) Dept Human Molecular Genetics, Univ Heidelberg, Heidelberg, Germany; 8) Cytogenetics Lab, Victoria General Hospital, Victoria, Canada.

The human Y chromosome has been extensively studied because of its primary function in sex determination and male fertility. Although structural abnormalities of the Y chromosome can explain conditions such as loss of fertility or short stature, the significance of Y chromosome rearrangements in some patients with intellectual disability (ID) is hard to establish, due to a lack of correlated genes identified on the Y. Here we describe two patients with ID and facial dysmorphism, both of whom have non-mosaic Y chromosome rearrangements resulting in deletions of large portions of the Y chromosome. Patient A, who also presented with speech delay, developmental delay, Duane's anomaly of the eye, hypermetropia and mild conductive hearing loss, has 2 derivative Y chromosomes, both of which have p and q arm terminal deletions. Patient B, also with speech and language delay, developmental delay and short stature, has an interstitial deletion of Yq11.21-11.23. The deleted regions for both patients include many genes involved in spermatogenesis and fertility, although the presence of genes involved in physical and intellectual development is questionable. As there were no other imbalances in the genomes of our patients, as investigated by 1 Mb resolution array-CGH, which could be responsible for their clinical picture, then a review of similar cases in the literature was performed, and the significance of Y chromosome rearrangements in ID is discussed.

1583/W

Molecular cytogenetic characterization of a unique and complex de novo 8p rearrangement. *G. Velagaleti[1], S.L. Cooke[2], J.K. Northup[3], N.L. Champaigne[1], W. Zinser[1], P.A.W. Edwards[2], L.H. Lockhart[1].* 1) Dept Pediatrics, Univ Texas Medical Branch, Galveston, TX; 2) Dept Pathology, Cambridge University, Cambridge, UK; 3) Dept Pathology, Univ Texas Medical Branch, Galveston, TX.

Human chromosome 8p is prone to recurrent rearrangements with inv dup del(8p) being most common. Each of these recurrent rearrangements is associated with different clinical manifestations. Some of these recurrent rearrangements at 8p occur as a consequence of an 8p submicroscopic paracentric inversion between the olfactory (OR) gene clusters in one of the parents. Recent reports have shown that some of the rearrangements are unique and complex and are mediated by other repetitive elements within 8p. Here, we report a complex 8p rearrangement with seizures as the major presenting feature. Extensive fluorescence in situ and microarray analyses with tiling path 8p array showed that the rearrangement consists of a terminal deletion of 6.3 Mb from pter to 8p23.1; followed by a single copy segment of 5.3 Mb spanning the 8p23.1 and a duplication of 12 Mb extending from 8p23.1-8p21 region. FISH analyses with BAC clones showed that the rearrangement is unique in that the 8p duplication is a direct tandem duplication and, unlike the more common inv dup del(8p), is not derived from parental submicroscopic inversion. Also unlike the inv dup del(8p), the phenotype in our case is milder with no central nervous system malformations or cardiac defects. Similar to the common inv dup del(8p) our rearrangement also appears to be mediated by the OR repeat clusters at 8p23.1. We propose a mechanism similar to the one proposed to explain the PLP1 duplications in Pelizaues-Marzbacher disease. As per our model, the OR repeats behave similar to the LCRs and cause inherent instability leading to a double strand break (DSB) in the proximity of these OR repeats. This is followed by a strand invasion and copying of the sister chromatid and completion of the event by non homologous end joining (NHEJ) repair. A second DSB in trans near another OR repeat followed by transposition to another site of the duplicated segment and repair by NHEJ leading to interrupted direct duplication with terminal deletion.

1584/W

Duplication 22q11.2: Clinical and Cytogenetic Findings in 4 Families. *B.T. Wang, T.A. Boomer, F.Z. Boyar, X.J. Yang, M.M. El Naggar, C. Zapata, P.H. Kohn, B.J. White, A. Anguiano.* Cytogenetics Dept, Quest Diagnostics,Nichols Inst, San Juan Capistrano, CA.

Duplication 22q11.2 syndrome has been identified relatively recently compared with 22q11.2 microdeletions. The phenotype can be mild to severe, with some overlaps with DiGeorge/velocardiofacial syndrome (DG/VCFS). We present the clinical and cytogenetic findings of 4 families with duplication 22q11.2.Blood samples from the 4 families were processed for standard chromosome analysis. FISH was performed using probes for the DG/VCFS critical region (TUPLE1). Routine karyotype analysis revealed no chromosome 22 abnormalities in families 1 to 3 and an apparent duplication 22q in family 4. Phenotypes ranged from mild to severe. The first proband was a 2-year-old boy with cleft palate and mild developmental and speech delay. Parental FISH studies showed that the duplication was inherited from the mother, who had mild mental retardation but no apparent physical manifestations. The second proband was an 18-month-old boy with hypotonia and developmental delay. Parental FISH studies indicated that the duplication was de novo. The third proband was a 20-month-old boy with developmental delay, ventricular septal defect, and dysmorphic features characteristic of DG/VCFS. FISH analysis indicated that the duplication was inherited from the mother, whose IQ was considered low-normal; the father did not have the duplication but had features of cleft lip and cleft palate. The last subject was a 22-year-old woman with mild mental retardation referred because her 2 children (one boy and one girl) had a dup(22q). In conclusion, all familial duplications were inherited maternally, consistent with the high ratio of maternal duplication reported previously. Duplication size may contribute to the phenotype but does not explain the wide phenotypic diversity observed within families, the high frequency of maternal inheritance, or the milder phenotype observed in females. Analysis of the duplication size with comparative genomic hybridization might provide a better understanding of the genotype-phenotype correlations in these families.

1585/W

Distinguishing inversions from insertions: balanced paracentric rearrangement of chromosome 9 (q32q34.3) capable of producing a viable monocentric recombinant dup(q21.3q31)/del(q32q33). *S.P. Yang[1], S.T. South[2,3], A.R. Brothman[2,3].* 1) U.C. Davis Medical Center, Sacramento, CA; 2) University of Utah, Salt Lake City, UT; 3) ARUP Laboratories, Salt Lake City, UT.

J.R. presented at birth with IUGR, microcephaly, dysmorphic facies, bilateral hip dislocations and foot deformities. High resolution karyotype showed an apparently balanced inv(9)(q32q34.3)mat. Subtelomere FISH and chromosome 9 painting probes indicated no cryptic terminal or interstitial translocation. The mother had some mild learning disabilities but her CGH microarray result was completely normal (Spectral Genomics, Inc. - 1 Mb Chip). The same CGH microarray study in J.R. revealed duplication of 14 BAC clones spanning 9q21.3 to 9q31 (11.9-13.4 Mb), and deletion of 4 BAC clones spanning 9q32 to 9q33 (4.0-6.4 Mb). These abnormalities were confirmed by specific FISH probes mapping to those regions of chromosome 9 (q21.3 and q32, respectively). Studies are underway to see if recombination occurred within a paracentric inversion, as opposed to a cryptic within-arm intra-chromosomal insertion. One “rule-of-thumb” to help distinguish between inversions and insertions is that the latter will always produce interstitial aneuploidy bracketed by the breakpoints in the rearranged parental chromosome. This case probably represents a paracentric inversion, based on the interstitial duplication/deletion stretching proximally beyond the 9q32 breakpoint. The mechanism of “U-loop recombination” (Chia et al.,1992) is possible if the inversion breakpoints are actually at q21.3 and q33. The recurrence risk for viable aneuploid offspring in the relatively common paracentric inversion carriers is very low, in contrast to the 15% or higher risk for those rare carriers of a cryptic within-arm intra-chromosomal insertion. These two alternatives are not easily separated even after extensive high resolution karyotype, FISH, CGH microarray, and haplotype analyses. Ascertainment of a familial paracentric rearrangement through the birth of an abnormal child challenges the clinician and the laboratory to define the correct underlying mechanism. Such parents are likely to be at significantly greater than background risk.

1586/W

Presence of a familial translocation t(7;15)(p22;q14) and de novo deletion of the 15q11-q13 region in a 12 month old girl with Angelman syndrome. *L. Jenkins[1], D. Delgado[1], R. Roshan[2], J. Kobori[1].* 1) The Permanente Medical Group, Inc. Genetics Department, San Jose, CA; 2) The Permanente Medical Group, Inc. Department of Pediatric Neurology, San Francisco, CA.

A 12 month old girl with developmental delay, hypotonia, happy demeanor, and delayed speech was identified prenatally to have a maternally inherited balanced translocation: t(7;15)(p22;q14). Postnatal DNA methylation studies confirmed the clinical suspicion of Angelman syndrome. Further diagnostic testing by FISH methods detected a de novo chromosomal deletion in the 15q11q13 region in the der(15) chromosome, with the loss of the SNRPN and UBE3A locus. A human oligo-based array was used to refine the size and the location of the deletion. The translocation breakpoints map to a newly described breakpoint cluster region at 15q14 and the distal subtelomeric end of the short arm of chromosome 7. Similar rearrangements involving 15q14 and the terminal ends of the recipient chromosome have been reported to result in an unbalanced karyotype of 45 chromosomes due to the loss of the der(15). This is the first reported case of Angelman syndrome in which the 15q11q13 de novo deletion is found in a familial balanced translocation involving 15q14 in a “balanced” complement of 46 chromosomes. Non-allelic homologous recombination between 15q-specific low copy repeats (LCRs) could result in the deletion observed in the der(15), and the reciprocal duplication of this region in the “normal” 15 homolog during maternal meiosis. The mother has a history of multiple miscarriages with no other significant clinical findings. Risk assessment for a de novo deletion of the 15q11q13 region in carriers of a balanced reciprocal translocation involving chromosome 15 is limited due to few reported cases. These findings have important implications for prenatal diagnosis. When a fetus from a carrier parent is found to have the same balanced translocation or normal appearing 15 homologs, FISH analysis should be considered to rule-out a submicroscopic deletion or duplication of the 15q11q13 region. Depending on the parental origin, the defect could lead to Angelman syndrome, Prader Willi syndrome, or dup(15)(q11q13).

1587/W

Clinical diagnostic testing of 450 patients with mental retardation or developmental delay by whole genome array CGH. *D.T. Miller[1,2,4], Y. Shen[1,3], V. Lip[1], X. Sheng[1], K. Tomaszewicz[1], H. Shao[1], H. Fang[1], H. Tang[1], M. Irons[2,4], C.A. Walsh[2,4,5], O. Platt[1,4], J.F. Gusella[3,4], B.L. Wu[1,4].* 1) Laboratory Medicine, Children’s Hospital, Boston, MA; 2) Genetics, Children’s Hospital, Boston, MA; 3) Center for Human Genetics, Massachusetts General Hospital, Boston, MA; 4) Harvard Medical School, Boston, MA; 5) Howard Hughes Medical Institute.

Array comparative genomic hybridization (aCGH) targeted for known microdeletions and subtelomeric regions is an important but limited approach to the clinical diagnosis of mental retardation (MR). High resolution whole genome coverage improves clinical sensitivity, but enthusiasm is tempered by concerns about interpretation of copy number variants (CNVs). Whole genome aCGH (Agilent 244K) was performed on peripheral blood DNA from 450 individuals with MR or developmental delay. CNVs less than 150kb (~10 consecutive array features) were excluded from analysis. Retained CNVs were compared to the Database for Genomic Variants (as of 3/29/07). Previously unreported CNVs were evaluated using an algorithm based on gene content, OMIM citation, copy number databases, and parental testing. Previously unreported CNVs were identified in 138 of 450 samples. Among samples with unreported CNVs, 47 of 450 samples (10.4%) had a genomic imbalance region greater than 500kb, and 91 of 450 samples (20.2%) had genomic imbalance between 150-500kb. In each of these two groups, a disease-causing gene deletion was identified in 6 cases. High-density whole genome aCGH identifies small and large clinically significant genomic imbalance events in regions that may not be represented on a targeted array. Although whole genome coverage identifies many CNVs that are not clearly clinically relevant, this method significantly improves the yield of aCGH in the clinical setting.

1588/W

Defining neocentromere identity: role of L1 retroelements in the epigenetic formation of ectopic centromere chromatin. *A.C. Chueh, K.H. Brettingham-Moore, E.L. Northrop, L.H. Wong, K.H.A. Choo.* Murdoch Childrens Res Inst, Royal Children’s Hosp, Parkville, Australia.

Human neocentromeres are fully functional centromeres that arise epigenetically from non-centromeric precursor sequences that are devoid of alpha-satellite DNA. Centromere Protein A is a centromere-specific histone H3 variant, which serves as the epigenetic mark for defining the centromere core. Using ChIP-PCR array analysis, we have recently described a hierarchical and symmetrical interspersion pattern of CENP-A-associated nucleosomal blocks, found within a 330 kb domain of 10q25 neocentromere. Here, we investigated the possible DNA motifs or sequence properties that enable or favor the nucleation of neocentromeres. Bioinformatic analysis was performed to make direct comparisons for the percentage of AT content and prevalence of intersperse repeats between CENP-A-associated clusters and non-CENP-A-binding regions. While no difference was found for AT content and the majority of the interspersed repeats analyzed, we observed a 2.5-fold increase in the prevalence of underlying sequences corresponding to L1 LINE retroelements and a cluster of 4 full-length L1s concentrated around 330-kb CENP-A domain. Although most L1s possess 5’ end-truncations and are generally not transcribed, recent reports showed that RNA transcripts from full-length L1P elements (~ 6 kb) can be detected in various human cell lines. For transcription studies, we designed RT-PCR primers that specifically target each of the four CENP-A-associated FL-L1 elements (FL-L1a-d) in CHO-human monochromosomal hybrid lines, containing the neocentric mardel(10) marker or the progenitor chromosome 10. Interestingly, transcripts from only one of the four FL-L1s (FL-L1b) were detected and that the FL-L1b locus is actively transcribed both before and following neocentromere formation. Furthermore, RNA-ChIP-qPCR analysis was also performed showing an enrichment of FL-L1b RNA in the CENP-A-associated chromatin. In summary, we hypothesize that FL-L1 retroelements, acting via an RNA intermediate, may be important for the establishment or maintenance of the neocentromere chromatin through currently unknown mechanisms.

1589/W

The distribution of a human specific interstitial telomere like sequence at 22q11.2 in normal population. *O. Samassekou, J. Yan.* Dept Medical Genetics, Sherbrooke Univ (CHUS), Sherbrooke, PQ, Canada.

Interstitial telomeric sequences (ITSs) and telomere-like repeats at intrachromosomal sites are common in mammals. We previously reported the presence of ITS at 22q11.2. This sequence is constituted of tandem repetitive 9-base monomers, TTAGGGAGG or TTATGGAGG, covering 909 bp. The proximity of this ITS to the common rearrangements region of multiple disorders such as DiGeorge syndrome and chronic myeloid leukemia, and the instability of ITSs may suggest the involvement of ITS at 22q11.2 in the pathogenesis of these disorders. Before scrutinizing its status in these different pathologies, we studied its distribution in normal population. We studied 50 normal people including members of 10 distinct families. The use of the primed in situ labeling (PRINS) technique with primers specific to ITS at 22q11.2 enabled us to detetect this ITS with high frequency. Moreover, we noticed different patterns of distribution from subject to subject. The use of the PCR technique by using primers flanking the ITS at 22q11.2 confirmed this result. We unexpectedly found different patterns ranging from 1 Kb to 4 Kb, and 90% of these patterns are more than 1Kb (the expected size of PCR product). We concluded that this sequence is highly polymorphic. The linkage analysis study of ITS at 22q11.2 in members of the 10 different families has showed a strong relation between offspring and parents. This result opens an avenue for the use of this sequence as an allelic marker. All these results can serve as foundation for the study ITS at 22q11.2 in relation with the genomic instability.

1590/W

Complex balanced translocation t(1;5;7)(p32.1;q14.3;p21.3) and two microdeletions del(1)(p31.1p31.1) and del(7)(p14.1p14.1) in a patient with features of Greig cephalopolysyndactyly and mental retardation. *E. Bocian[1], K. Borg[1], B. Nowakowska[1,2], E. Obersztyn[1], S.W. Cheung[2], L. Korniszewski[3], T. Mazurczak[1], B. Wiśniowiecka-Kowalnik[1], P. Stankiewicz[1,2].* 1) Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, USA; 3) Institute of Physiology and Pathology of Hearing Outpatient Genetic Clinic, Warsaw, Poland.

Complex chromosome rearrangements (CCRs) are rare structural abnormalities that involve at least two chromosomes and more than two breakpoints and are often associated with developmental delay, mental retardation and congenital anomalies. Additional microdeletions localized on derivative translocation chromosomes yet not directly at the translocation breakpoints have been described very rarely. We report a de novo, apparently balanced translocation t(1;5;7)(p32.1;q14.3;p21.3) in a 7-year-old boy with severe psychomotor retardation, neonatal muscular hypertonia, congenital heart defect, polysyndactyly of hands and feet, and dysmorphic features resembling Greig cephalopolysyndactyly syndrome (GCS). Analysis of the chromosome breakpoints using FISH with locus-specific BAC clones and long-range PCR products did not identify chromosome imbalance at the interrogated regions. High-resolution comparative genomic hybridization (HR-CGH) and array CGH (aCGH) revealed two additional cryptic de novo deletions del(1)(p31.1p31.1) and del(7)(p14.1p14.1) that are not associated with the translocation breakpoints. FISH and polymorphic marker analyses showed that both deletions are located on the derivative chromosomes, are 4.2-6.1 Mb and 5.1 Mb in size, respectively, and are paternal in origin, suggesting that the described CCR arose during spermatogenesis. The deletion on chromosome 7p encompasses the GLI3 gene that is causative for the GCS, Pallister-Hall and Acrocallosal syndrome. We hypothesize that the intellectual disability and cardiac defects in our patient may be due to deletion or disruption of other genes localized either at the translocation breakpoints regions or within the deletions. We discuss the potential mechanisms of formation of the described CCR.

1591/W

Molecular cytogenetic characterization of an 11q23.3q24.2 duplication in a child with growth/developmental delay. *R.D. Burnside, F.M. Mikhail, E.J. Lose, A.J. Carroll.* Dept Genetics, Univ Alabama at Birmingham, Birmingham, AL.

Duplications of the distal part of the long (q) arm of chromosome 11 have been well described, and certain phenotypic features such as growth delay, mental retardation, and microcephaly are consistently seen. However, 11q duplication often results from an unbalanced translocation with another chromosome, making it difficult to determine whether other phenotypic features that may present are the result of the 11q duplication. Here, we present a case with an interstitial insertion resulting in duplication of part of distal 11q in a two-and-a-half year old male child. This patient was referred to our genetics clinic due to dysmorphic features and developmental delay including speech delay. These features alone without addititonal physical findings have a very broad differential diagnosis. Chromosome analysis was performed on GTG banded chromosomes from cultured lymphocytes and showed a darker-staining band in the light-staining 11q13.1 region. In order to determine whether this band was chromosome 11 material, whole chromosome painting was performed on metaphase chromosomes. The result indicated that the extra band was chromosome 11 material. Using a 32K BAC tiling path array CGH chip, we demonstrated an ~8.8 Mb duplication within the 11q23.3q24.2 region. Our patient therefore carries an unbalanced insertion of the 11q23.3q24.2 region into band 11q13.1. These results were confirmed by metaphase FISH using the *MLL* (11q23.3) probe. The patient's final karyotype is 46,XY,der(11)ins(11;11)(q13.1;q23.3q24.2).ish der(11)ins(11;11)(q13.1;q23.3q23.3)(*MLL*++). In conclusion, our patient demonstrates the clinical usefulness of whole genome high resolution array CGH analysis as a powerful molecular cytogenetic tool capable of detecting genomic imbalances due to cytogenetically visible but uncertain rearrangements. Parental chromosome and FISH analyses are underway. A detailed description of the patient's clinical features and comparison with previously reported distal 11q duplications will be presented.

.

1592/W

Report of a prenatally detected XX/XY chimera with true hermaphroditism. *T.L. Gillan[1], R.L. Sparkes[2,3], J.L. Lauzon[2,3], A.M. Innes[2,3], S. Shetty[1,2], P.J. Bridge[2,4], J.E. Chernos[1,2].* 1) Cytogenetics Laboratory, Alberta Children's Hosp, Calgary, AB, Canada; 2) Department of Medical Genetics, University of Calgary; 3) Clinical Genetics Unit, Alberta Children's Hospital; 4) Molecular Diagnostic Laboratory, Alberta Children's Hospital.

We report a case of an XX/XY chimera, first ascertained prenatally following chromosome analysis for a positive first trimester screen. On chorionic villus sampling (CVS) four distinct cell lines were identified: two predominant diploid cell lines (46,XX and 46,XY), seen with a frequency of 70% and 30% respectively, and a smaller proportion of tetraploid cells (92,XXXX and 92,XXYY). Subsequent amniocentesis demonstrated the presence of both 46,XX and 46,XY cell lines in a ratio similar to that in CVS. Second trimester ultrasonography and echocardiography were normal. Postnatally, the infant showed ambiguous genitalia: prominent labioscrotal folds, 2 cm phallus with a single opening at the base, left inguinal hernia and the absence of palpable gonads. Further investigations confirmed that both Mullerian and Wolffian structures were present. Pathology on the single gonad showed distinct regions of both ovarian and testicular tissue, consistent with an ovotestis. Interphase FISH on blood lymphocytes confirmed the presence of XX and XY cell lines in a ratio similar to that detected prenatally. Molecular analysis of 15 polymorphic markers in all tissues revealed a single maternal allelic contribution at all loci and two paternal contributions at some loci. The establishment of individual XX and XY clones to determine if one or both cell populations have biparental contributions is currently underway. Molecular studies of these cell lines will provide evidence as to the mechanism underlying true hermaphroditism in this individual. True hermaphroditism in humans is a rare condition phenotypically characterized by the presence of both testicular and ovarian tissues in the same individual often resulting in the formation of ambiguous genitalia. Approximately 10% of hermaphroditic individuals are mosaic for a 46,XX/46,XY karyotype. Several proposed mechanisms to explain chimerism for 46,XX/46,XY will be discussed.

1593/W

Partial trisomy 2q: Report of a de novo inv dup(2)(q35-qter). *Y.F. Li[1], E. Roeder[2], B. Nowakowska[3], M.L. Cooper[1], A. Patel[1], W.W. Cai[1], S.W. Cheung[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Genetics and Metabolic Disorders, Department of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, TX; 3) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.

The partial trisomy 2q (q35-qter) phenotype has been well described in the literature. Many cases of 2q duplication result from familial translocation, and concomitant monosomy for other chromosome segments obscures the genotype-phenotype correlation of the 2q duplication. Pure duplications of the distal part of chromosome 2q are rare. We describe a female infant with a de novo inverted duplication involving the distal long arm of one chromosome 2. The clinical findings of this patient include congenital heart defect, hypothyroidism , hypotonia, nystagmus, dysmorphic features, and developmental delay. Initial chromosome analysis by GTG-banding analysis revealed an additional chromosome material of unknown origin on the terminal long arm of chromosome 2. To identify the origin of this material, a genome-wide BAC clone array with a set of 21,658 RP11 BAC clones with 7 BAC clones per 1 Mb was used. This set of clones was selected by a computer program with unique sequences at both ends and tightly distributed insert size that completely cover the entire human genome. An interstitial duplication of 21.2 Mb in size between 2q35-qter was identified. This duplication was subsequently confirmed by fluorescence in situ hybridization analysis and was determined that it is an inverted duplication. Thus, the proband's karyotype was interpreted as 46,XX,inv dup(2)(q35-qter). Both parental chromosome studies were normal. These findings provide further evidence for a recognizable facial appearance associated with duplication of 2q35-qter. The phenotype-genotype correlation as compared to other case reports of partial trisomy 2q will be reviewed.

1594/W

Chromosome 15q11.2 copy number variants (CNV): population frequency and clinical implications. *M.C. Seleme[1], T.H. Shaikh[2], M. Lincicum[3], M. Sathanoori[4], U. Surti[4], H. Hakonarson[2], L.G. Shaffer[3], R.D. Nicholls[1].* 1) Children's Hospital of Pittsburgh, Pittsburgh, PA; 2) Children's Hospital of Philadelphia, Philadelphia, PA; 3) Signature Genomic Laboratories, Spokane, WA; 4) University of Pittsburgh, Pittsburgh, PA.

Genome studies have unveiled an important role for CNV in shaping the normal human genetic landscape. At the extreme of CNV, genomic disorders arise from deletion or duplication of dosage-sensitive genes. Genomic disorders and many CNV appear to have endpoints associated with segmental duplications (SDs). However, the exact relationships between CNV and SDs, or the frequency of CNV associated with disease is unknown for most chromosome regions. This study examines these questions for proximal chromosome 15, a region known to be meiotically unstable resulting in a number of genomic disorders (Prader-Willi and Angelman syndromes, autism, and others). For 15q11-q13 genomic disorders, at least six breakpoint (BP) hotspots, each composed of related SDs, contain the rearrangement endpoints. We describe here CNV limited to the BP1-BP2 region of 15q11.2, which include a unique segment of ~ 250-kb containing four highly conserved genes. From a total study population of 1981 normal individuals using oligonucleotide (ON) array-CGH, QPCR, and mining of published databases, we estimate at 1% the population frequency of CNV for BP1-BP2 deletions and duplications. By comparison, using BAC and ON array-CGH, we estimate a 1.2% frequency for BP1-BP2 CNV in two samples totaling 8750 clinical cases tested because of mental retardation and/or MCA. In several tested cases, the CNV was inherited from an unaffected parent. Based on the equivalent frequency in normal and clinical populations, and heritability, we conclude that most BP1-BP2 CNV are not linked to disease and represent a copy number polymorphism. Conversely, due to the presence of 4 functional genes, CNV within BP1-BP2 could unmask recessive mutations or represent disease susceptibility alleles due to gene dosage alterations. Moreover, CNV in an unstable genomic region could predispose to further chromosomal instability leading to genomic disorders in offspring.

1595/W

Robertsonian translocation in infertile North Indian population. *M. Jena[1], D. Pathak[1], M. Tanwar[1], R. Kumar[1], R. Kumar[2], M.B. Shamsi[1], R. Dada[1].* 1) Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India- 110029; 2) Department of Urology, All India Institute of Medical Sciences, New Delhi, India- 110029.

Robertsonian translocations (RT) are the most common structural chromosomal abnormalities observed in humans with a total frequency of 1.23 per thousand. Among them translocation (13q;14q) and the translocation (14q;21q) are the most common, with an estimated frequency of 0.97 and 0.20 respectively. It is well known that in infertile male cases, the frequency of chromosomal aberrations is increased and varies from 1.9-4.0% among them, Robertsonian translocations and numerical sex chromosomal aberrations are the most frequent. Of these, 60% inherit the rearrangement from one of their parents and 40% occur denovo. This study was planned with the aim to determine the incidence of RT in men with spermatogenic arrest and to corelate if cases with such structural aberrations lead to recurrent ART failure. Our finding of chromosomal aberrations (3.7%) in the infertile males are in good agreement with literature of 3.3%. In this study of infertile men with non-obstructive azoospermia and oligozoospermia, three cases had 13q14q fusion. Thus the frequency of robertsonian translocation in our study was nearly 30 fold higher than in general population (0.1%). Although robertsonian translocation is likely to be found in chromosomes investigation of infertile men, their role in oligospermia is not clear. The testicular histology of the men carrying such a rearrangement shows a variable picture, ranging from severe impairment to near normality. Individuals carrying each of the ten possible nonhomologous robertsonian translocations of the five humans acrocentric chromosomes (13,14,15,21, & 22) have been reported, but two combinations, rob(13;14) & rob (14;21) are observed at a greater frequency than the rest (73% & 10)%,respectively. Since there is a high frequency of robertsonian translocations in infertile men drawing a genotype and phenotype correlation in these studies will help to acess the severity of spermatogenic arrest in these cases.

1596/W

Exploration of genes related to X-linked mental retardation (XLMR) by MCG X-tiling array. *S. Honda[1,2,3], S. Hayashi[1,2], I. Imoto[1,2], E. Nakagawa[4,5], Y. Goto[4,5], J. Inazawa[1,2,3].* 1) Dept. of Mol. Cytogenet., Med. Res. Inst., Tokyo Med. and Dent. Univ; 2) Core Res. for Evolutional Science and Technology, Japan Science and Technology Agency; 3) 21st Century Center of Excellence Program for the Frontier Res. on Mol. Destruction and Reconstruction of Tooth and Bone; 4) Div. of Child Neurol., Musashi Hosp., Natl. Center of Neurol. and Psychiat; 5) Dept. of Mental Retardation and Birth Defect Res., Natl. Inst. of Neurosci., Natl. Center of Neurol. and Psychiat.

An estimated 13-15% of mental retardation (MR) is caused by mutation on the chromosome X. Although 59 XLMR genes have been identified to date, many genes involved in XLMR remain to be identified. Known XLMR genes have been identified by conventional positional-cloning strategies, but cryptic copy number aberrations (CNA) cannot be detected by routine karyotyping due to its limited resolutions. Since array-based comparative genomic hybridization (array-CGH) can detect such cryptic CNAs, we constructed a high-density and high-resolution chromosome X array (MCG X-tiling array) for array-CGH, which contains a total of 1001 bacterial artificial chromosome (BACs) throughout chromosome X except pseudoautosomal regions. To identify novel XLMR-associated genes, we have screened 73 families with XLMR or probable XLMR by MCG X-tiling array and detected CNAs related to MR in 5 families (7%). Among these 5 CNAs, 2 had duplication of known XLMR-associated genes which have already been reported, whereas, in other 3 families, the genomic materials involved in CNAs have never been reported so far: (1) duplication containing 2 genes completely, (2) duplication harboring 2 genes partially, and (3) deletion involving no known protein-coding gene. Since all CNAs detected were not de novo and also observed in females in some families, the pattern of X-chromosome inactivation in a female was evaluated by FISH with metaphase chromosome using BrdU-labeling technique in late S-phase. MR phenotype in female was correlated with preferential activation of chromosome X with each of those CNAs. Our results demonstrate that array-CGH using X-tiling array is the powerful tool to explore novel CNAs related to XLMR.

1597/W

Benefit of Whole-Genome 500K SNP Microarray in Clinical Practice. *J.M. Milunsky[1, 2, 3], M. Ito[1, 2], T.A. Maher[1], A. Milunsky[1, 2].* 1) Center for Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Dept Pediatrics, Boston Univ Sch Medicine, Boston, MA; 3) Dept Genetics and Genomics, Boston Univ Sch Medicine, Boston, MA.

Chromosomal deletions and duplications are a major cause of developmental disabilities including mental retardation (MR), developmental delay (DD), and autistic spectrum disorder (ASD), as well as multiple congenital anomalies (MCA). Targeted and whole-genome arrays are now clinically available to detect these genomic imbalances. We have utilized the previously validated 500K Affymetrix SNP microarray (two 250K arrays) to clinically evaluate over 100 patients with several different postnatal indications. The first group were those with known or suspected unbalanced chromosomal rearrangements to further refine their deletion/duplication. The second group were those with apparently balanced karyotypes who had abnormal phenotypes. The third group were those with mental retardation/developmental delay/ASD/MCA. Abnormal findings from the first array were confirmed and further refined with the second array and FISH studies. When available, parental samples were obtained to determine if the rearrangement was familial vs. de novo or to assess the significance of a possible variant. Several unexpected findings were revealed including more complex chromosomal imbalance (chromosome 8 deletion in addition to the known duplication), an unsuspected diagnosis (chromosome 7q11.22-7q11.23 duplication), and an unrelated diagnosis (HNPP in a child with a balanced 5/6 translocation and developmental delay). An interstitial deletion was detected in a child with MR/MCA and his normal mother in the paternally imprinted chromosome 1p31.3 region adding further complexity to the interpretation of abnormal findings. Multiple additional chromosomal deletions and duplications (several atypical involving known loci) were found. Further refinement of genomic imbalance by SNP microarrays may eventually optimize anticipatory guidance and also may allow better genotype/phenotype correlations. Whole genome SNP microarrays are a valuable tool to determine genomic imbalance in patients with MR, DD, ASD and/or MCA.

1598/W

Mosaicism of a duplication 1q due to de-novo translocation 1/14: Studies on origin and development of the pathologic cell line. *G. Schwanitz[1], D. Hansmann[2], U. Gamerdinger[3], U. Paetzold[1], N. Schönherr[4], G. Knöpfle[5], T. Eggermann[4].* 1) Institute of Human Genetics, University of Bonn, Germany; 2) Institute of Prenatal Medicine and Genetics, Meckenheim - Bonn, Germany; 3) Institute of Pathology, University Medical Center of Giessen and Marburg, Germany; 4) Institute of Human Genetics, RWTH Aachen, Germany; 5) Department of Pathology, University of Bonn,Germany.

Pure duplications of 1q are extremely rare. We report for the first time mosaicism for duplication 1p11 to 1qter in a malformed fetus. An additional long arm of chromosome 1 was translocated onto the constitutive heterochromatin of chromosome 14p (karyotype: mos46,-XY,der(14)t(1;14)(p11;p11.2)/46,XY). Mosaic formation in the partial trisomy 1 (duplication 1q) was investigated in different somatic tissues of first and second trimester pregnancy. The phenotype of the fetus was in good accordance with findings from the literature. The distribution of the pathologic cells was unequal, ranging from 4 to 93%. To exclude an in-vitro effect which leads partially to an increase of the cells with normal karyotype we compared the results of chromosome analyses with molecular results of the different tissues. The duplicated region could be delineated as paternal in origin. We present a proposal for the complex formation mechanism and the development of the pathologic cell line in this rare type of chromosome disorder.

1599/W

Shorter telomere length in women who experienced multiple trisomic pregnancies. *C.W. Hanna[1], K.L. Bretherick[1], J.L. Gair[2], M.D. Stephenson[3], W.P. Robinson[1].* 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Island Medical Program, University of Victoria, Victoria, BC, Canada; 3) Recurrent Pregnancy Loss Program, University of Chicago, Chicago, IL, USA.

As women approach menopause they experience a decrease in oocyte quality leading to an increased rate of miscarriage due to aneuploidy. We previously found an increase in skewed X-chromosome inactivation (XCI) in women who have recurrent miscarriage with at least one trisomic pregnancy, which was greatest among women who had experienced multiple trisomic pregnancies. Skewed XCI may be a consequence of accelerated stem cell depletion, possibly reflecting a biological (and reproductive) age that is older than chronological age. Thus, we hypothesized that these women will also tend to have shorter mean telomere lengths, as this also reflects age-related stem cell turnover. DNA was extracted from peripheral blood from mothers of a single trisomy (ST) (N=68), mothers with multiple trisomies (MT) (N=22), control women between ages 20-55 with no history of miscarriage (C1) (N=86), and women who had a healthy pregnancy after age 37 with no miscarriages (C2) (N=41). Quantitative PCR was used to measure average telomere length. The age adjusted mean relative telomere length for ST, MT, C1, and C2 were 0.89, 0.82, 0.89, and 0.93 respectively (p=0.03, ANCOVA). The ST were not significantly different from either control group; however, the MT had smaller telomeres than C1 (p=0.05) and C2 (p=0.01). Because most of the MT women were ascertained through recurrent miscarriage, shorter telomere length may alternatively reflect an association with recurrent miscarriage. There was no association between telomere length and XCI skewing within all the samples or in the MT specifically. This suggests that reduced telomere length occurs independently of the mechanism for increased skewed XCI. Telomere lengths may be shorter due to a variety of mechanisms including: 1) a direct effect, such that women with multiple trisomic pregnancy losses have shorter telomere lengths from conception; 2) increased exposure to oxidative stress; 3) stem cell turnover independent of the mechanism for skewed XCI.

1600/T

qPCR quality assessment of whole genome amplified FFPE DNA samples and comparison of their use on BAC and oligo array platforms. *C. Williams[1], S. Michalik[2].* 1) PerkinElmer Life and Analytical Sciences, Waltham, MA; 2) Sigma-Aldrich, St. Louis, MO.

Archival, formalin-fixed, paraffin-embedded (FFPE) tissues are an invaluable source of material for molecular genetic studies linked to patient history. Unfortunately FFPE samples are regarded to be poor resources for such applications since they tend to contain small amounts of highly fragmented and damaged nucleic acids. Array-based comparative genomic hybridization (aCGH) is a relatively new technology used to assess chromosomal aberrations, particularly in evaluation of cancer samples. A limitation of aCGH to FFPE applications is that it typically requires microgram quantities of high quality input DNA. Typically the DNA yields of FFPE samples are less than the microgram range, so aCGH application to FFPE tissues has yet to be reliably established. Recent reports suggest that FFPE DNA can be directly labeled for oligo-based aCGH, however the high DNA input requirement (5 μg) is unreasonable for most situations. A number of studies have shown the successful use of whole genome amplified (WGA) FFPE DNA in aCGH applications, but none have directly compared the performance of WGA product on an oligo-array and BAC array platforms. Here we show that as little as 10ng FFPE genomic DNA or ~1mg FFPE tissue can be amplified directly with GenomePlex WGA and analyzed via PerkinElmer SpectralChip 2600 BAC arrays to generate high quality aCGH data from archival samples. We surmise that BAC aCGH is more reliable than oligo aCGH platforms for determining chromosomal gains or losses when using GenomePlex WGA FFPE DNA due to lower signal-to-noise that likely arises due to oligo aCGH probe length (~25 - 70 mer) as opposed to BAC aCGH probe length (~100Kb).

In addition, we show that the assessment of DNA quality is a crucial step in acquiring meaningful data from FFPE tissues, and other sources of damaged DNA. Determining the quality of FFPE DNA via PCR strategies prior to downstream applications is key for successful analysis of chromosomal aberrations (e.g. aCGH, SNP analysis, LOH, etc.).

1601/T

Wolf-Hirschhorn: from syndrome to phenotypic spectrum. *N.P. Rao[1], M. Mulatinho[1,2], J. Llerena[2], F. Quintero-Rivera[1].* 1) Department of Pathology , David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) IFF/FIOCRUZ, Departamento de Genetica Medica, Rio de Janeiro, RJ, Brazil.

Wolf-Hirschhorn syndrome (WHS) is a complex genetic disorder that presents with mental retardation, epilepsy, severe growth delay, and cranio-facial dysgenesis. The severity of its core characteristics is highly variable. The critical region is located in 4p16.3, where WHSC1 is always deleted. However, recent mouse model studies indicate that deletion of WHSC1 alone does not account for the full phenotypic spectrum observed in WHS. Here we present two patients who have a common deletion of WHSC1, and present with a different phenotype and genotype. The first is an infant with significant multi-systemic involvement including seizures, bilateral dysplastic kidneys, hearing loss, branchial cleft cyst, bilateral colobomas, cleft lip/palate, hypospadias and multiple dysmorphic features. The karyotype showed 46,XY,del(4)(p14)dn. FISH with the 4pter subtelomeric and WHS probes confirmed the deletion of WHSC1 and of an additional 35Mb. This deletion involves several other genes telomeric (WHSC2, SLBP, MSX1, FGFR3, LETM1, TACC3) and centromeric (SLIT2, TAPT1) to the critical region. The second patient was referred for developmental delay and dysmorphic features including microcephaly, retrognathia, low set ears, wide tip nose, macrostomia, nasal voice, and cubitus valgus. Pitt-Rogers-Danks syndrome (PRDS) was suspected clinically. Karyotype was normal. Subtelomeric FISH was performed and found to be abnormal for 4pter. Further analysis confirmed a deletion of WHSC1 and of an additional 2.1Mb. aCGH studies are ongoing. In conclusion these two cases confirm the model in which deletion of WHSC1 is essential for the pathogenesis of WH, but deletion of surrounding genes contributes to both the severity of the core characteristics and the presence of additional manifestations. PRDS should be considered a part of the milder end of the WHS spectrum generated by its phenotypic variability. We suggest that abnormal 4pter subtelomeric FISH in a patient with subtle dysmorphism and/or PRDS should prompt an evaluation of the 4p16.3 critical region.

1602/T

Array Comparative Genomic Hybridization (a-CGH) in clinical practice: new syndromes identified, known syndromes redefined, variants and unknowns uncovered. *D.A.S. Batista[1,2], S. Morsey[1], J. Biscoe[1], E.C. Lisi[2], T. Wang[2], J. Hoover-Fong[2], A. Hamosh[2].* 1) Kennedy Krieger Inst, Baltimore, MD; 2) Johns Hopkins Univ, Baltimore, MD.

The use of a-CGH is expanding the phenotypic spectrums of known syndromes and allowing identification of new microdeletions and microduplications. We performed a-CGH with the BAC constitutional array from Spectral Genomics in 116 cases with a normal high-resolution karyotype (≥ 550 bands). A total of 7 abnormalities were detected by array (6%), all confirmed by FISH: two deletions of the Williams region on 7q11.23; an interstitial duplication at 10p15.3; a partial duplication of the Smith-Magenis region on 17p11.2; two deletions that included the NF1 gene on 17q11.2; and one duplication of the DiGeorge region at 22q11.2. Prior to a-CGH only one case of Williams syndrome and the cases with NF were suspected. The second patient with Williams had significant developmental delay, autistic behavior and no cardiac anomalies. Duplication 10p15.3 was seen in a boy with developmental delay, seizures, asymmetric face, prominent incisors and dysrhythmia with hx of supraventricular tachycardia; parental analysis to determine if this duplication is de novo is pending. The patient with partial duplication of the Smith-Magenis region had several brain anomalies, hypotonia and hypertelorism and the smallest duplication described for the dup 17p11.2 syndrome. Both patients with NF1 deletion were atypical: one patient had vascular ring and developmental delay more severe than expected; the other had pulmonic stenosis, segmental hyperpigmentation, vascular malformations and facial dysmorphic features. Dup 22q11.2 was found in a patient with failure to thrive, trigonocephaly, high arched palate and micrognathia. Several others copy number changes (n=12) were also detected, most within regions of known segmental duplication and in 3/3 cases tested were also present in a normal parent, thus possibly normal variants. In summary, using a-CGH we have observed unusual clinical presentations of known syndromes, described the smallest duplication in 17p11.2 thus far reported and discovered a possible new syndrome at 10p15.3.

1603/T

Oligo array-CGH analysis as a tool for discovering disease mechanisms, atypical phenotype in known syndromes and novel deletion syndromes. *F. Mari, R. Caselli, F.T. Papa, M.A. Mencarelli, V. Uliana, E. Katzaki, K. Sampieri, M. Pollazzon, F. Ariani, I. Meloni, I. Longo, A. Renieri.* Medical Genetics, University of Siena, Siena, Italy.

A group of 70 MCA/MR pts has been analyzed by 44K Agilent oligo array-CGH. A first group (45) was selected for having MCA/MR not recognizable on clinical ground and normal karyotype. In this group we have identified 4 novel de novo del in 2q24, 2q32, 6q25, 7q36, 5 known del in atypical cases (4p16[2Mb], 15q11, 17p11, 22q11), 2 known rearrangements difficult to recognize on clinical ground (22q13del, 17p11dup) and 3 novel inherited rearrangements (7q11del, 17q12dup, Xq25del). Excluding the last 3, the mutation detection rate was 24%(11/45). A recognizable phenotype for the novel del could be traced: long and broad alluces, untreatable seizures for 2q24; sleep disturbance, behavioural problems, bifid nasal tip, micrognatia for 2q32; septal heart defect, Williams-like upper face, dysmorphic ears, short stature for 6q25; fetal phenytoin like-face, renal hypoplasia, long QT for 7q36. The inherited 17q12dup (reciprocal of renal cyst-diabetes del syndrome) was identified in a sex reversal male with MR-Peters' anomaly and in his healthy father. Since the dup includes TCF2 and it is located 4Mb apart to HSD17B1 low penetrance may be considered. A second group (25) was selected for having defined clinical diagnosis and array-CGH was used to clarify disease mechanism. Deletion mapping in 3 retinoblastoma pts with or without MR pinpointed the MR critical region on 13q14. Analysis of 10 Rett pts ruled out a 16p11 dup polymorphism, probably responsible for the modulation phenotype. Analysis of a pt with Alport and leiomyomatosis (ATS-DL) and Xq23del allowed to confirm the mechanism that only smaller del cause DL in ATS. Analysis of a family including ichthyosis pts with or without MR revealed that pts with MR have a smaller deletion (Xp22[1Mb]) than those without (Xp22[2.8Mb]) pinpointing to ATS-DL similar mechanism. Among the above 70 pts, 24 were analyzed by 105K oligo array and 39 known polymorphisms, 1 novel polymorphism in 1q21 and 4 putative pathogenic variants in 1q44, 7q31, 10q24, Xq21 were identified.

1604/T

8q24 deletion identified by a-CGH without the Langer-Giedion phenotype. *M. Mulatinho[1,2], J. Llerena Jr.[1,3], N. Rao[2].* 1) UFRJ, RJ, Brazil; 2) UCLA, CA; 3) FIOCRUZ, RJ, Brazil.

We describe a case of a girl with large and malpositioned central incisors, partial cutaneous syndactyly in hands (interdigital webs), small hands and feet, centripetal obesity, preterm, small stature, retarded intra-uterine growth, severe myopia, genu valgum and developmental delay. A 450 G-band karyotype was 46,XX,t(5;6)(q35.1; p22.2) de novo. Since this translocation was de novo, it was suggested that a submicroscopic/molecular loss of DNA could be causing the phenotypic abnormality. Array-CGH (a-CGH) with 6kb resolution (Nimblegen) was performed with the intention of uncovering the genes disrupted in the translocation. There was no loss of DNA material at the translocation breakpoints on chromosomes 5 and 6. However, a 12.3 Mb deletion at 8q24.12-q24.22 was observed. A retrospective HR G-band analysis (550 bands) showed a suspicious 8q deletion. This region of 8q24 region is associated to Langer-Giedion Syndrome (LGS) or Trichorhinophalangeal Syndrome, Type II (TRPS). It's a contiguous gene syndrome, in which affected individuals present with multiple dysmorphic facial features and exostoses due to loss of function of TRPS1 and EXT1 genes. Mental retardation is not necessarily associated with LGS, an important feature for genetic counseling. In our case, a-CGH showed that the EXT1 and TRPS1 genes were present, which is consistent with an absence of the expected LGS phenotype. On the other hand, the genes MYC associated with hematopoietic tumor activity, and KCNQ3, linked to benign familial neonatal convulsions/epilepsy (BFNC2) were deleted. The proband has not exhibited any features of malignancy yet, and did not present with any neonatal seizures or epilepsy in childhood. To our knowledge, this is the second such case to have a balanced translocation in addition to a deletion in the region 8q24. Bowen et al (1985) described a small deletion on chromosome 8 in addition to an apparently balanced translocation (2;9)(q21;q13) (PubMed ID: 3879433). In our study, a-CGH helped to delineate a detailed karyotype for proper clinical diagnosis and genotype-phenotype correlation. Detailed results and the reported LGS variants will be presented.

1605/T

Evaluation of the limitations of detection of BAC aCGH using quantitative PCR and oligo array hybridization. *W. Bi[1], K. Li[2], C. Chen[2], S.W. Cheung[1].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Applied Biosystems, 850 Lincoln Center Dr., Foster City, CA 94404.

Array-based comparative genomic hybridization (aCGH) is a powerful tool for detection of genomic imbalance and has been successfully implemented in molecular cytogenetic evaluation of genetic diseases. Targeted BAC/PAC arrays have been developed for clinical diagnosis at Baylor College of Medicine. As a standard practice, the copy number changes are identified by aCGH and subsequent confirmatory FISH studies are performed. The routine cutoff is set at +/- 0.2 for the log ratio and < 0.05 for the T-statistics permutation based p-value; however, there are occasional data points near this value whose significance is ambiguous. We have examined five such cases with potential changes by BAC arrays not confirmed by FISH. Three Taqman quantitative PCR assays were designed for each BAC with RNaseP as an active reference and the copy numbers were determined by relative quantification. These cases were further studied using a focused 44K oligonucleotide-based BAC emulation microarray with 20-30 oligos for each BAC clone as a second method for comparison. The results of qPCR analysis for all 15 assays were consistent with the results of the oligo aCGH analysis. Case 1 had a putative gain detected by a single clone in BAC aCGH. A duplication was detected by about 60% of the BAC clone; this partial coverage likely contributed to no changes detected in FISH. Case 2 had a potential deletion indicated by two adjacent clones in BAC aCGH. We found a deletion detected by 40% of one of the clones; the second clone included a copy number variation within an 80 kb region, which may have contributed to the marginal hybridization value. No changes were observed in the remaining three cases, suggesting that the potential gains with a single clone in BAC aCGH were weak false positive signals. We conclude that the causes for the inconsistency between BAC aCGH and FISH analysis include gains or losses detected by part of a BAC clone or other technical limitations of BAC aCGH. Our data also indicate that the focused oligo array enables a higher resolution and better accuracy than the BAC array.

1606/T

Clinical Study on 137 Patients with Subtelomeric FISH Analysis: Comparaison of two Checklists. *C. Brunel-Guitton, E. Lemyre.* Medical Genetics, CHU Ste-Justine, University of Montreal, Montreal, PQ.

Detection rate of subtelomeric abnormalities in patients with mental retardation varies from 3-6%. Discordance between studies reflects differences in inclusion criteria. Clinical checklists have been proposed to improve the diagnostic yield. Here, we present a study where two checklists (de Vries, 2001; Walter, 2004) were compared on 137 children with mental retardation: 25 with a subtelomeric defect and 112 controls. The 4 main clinical criteria in both checklists (MR, dysmorphism, growth & pedigree anomaly) were compared between cases and controls for their diagnostic yield and use in clinical preselection. 48% of cases had 4 criteria, 48% had 3 criteria. Controls showed a wider distribution range: 3.7% 1 criteria, 34% 2 criteria, 41% 3 criteria and 21% 4 criteria. Overall, patients with subtelomeric abnormalities had more malformations with a predominance of cardiac, ophthalmologic and genital malformations, hearing impairment and cleft palate. 96% vs. 63% of cases vs. controls tested positive for Walter's checklist. 96% vs. 71% of cases vs. controls tested positive for de Vries's checklist. One patient scored negative for both checklists. In contrast to previous studies, prenatal onset growth restriction was not discriminant, 8% vs. 11.6% in controls. Positive predictive value was 25% for Walter's checklist and 23% for de Vries's; results correlate with the detection yield identified previously. Sensitivity was 96% for both. Specificity was slightly higher with Walter's checklist 37% vs. 29% (p=0.0001). Conclusions: Patients with subtelomeric defects are more dysmorphic and possess a contributive family history in a greater proportion. Frequency of pre and postnatal growth anomalies does not appear to differ from controls. 96% of patients with subtelomeric abnormalities had 3-4 clinical criteria on both checklists. Diagnostic yield for subtelomeric abnormalities triples when a checklist is used. However, these checklists can miss cases and cannot be the only indicators for subtelomere testing. The checklist proposed by Walter seems more specific and could help to prioritize testing in patients fulfilling these criteria.

1607/T

High resolution array comparative genomic hybridization (aCGH) in individuals with Prader-Willi syndrome. *M.G. Butler, N. Kibiryeva, W. Fischer, D.C. Bittel.* Children's Mercy Hospital and University of Missouri-Kansas City, MO.

Prader-Willi syndrome (PWS) is a neurodevelopmental obesity disorder caused by a loss of expression of imprinted genes from the paternal 15q11-q13 region usually due to a deletion. The proximal deletion breakpoint occurs at one of two sites located within either of two large duplicons. The larger type I (TI) deletion involving breakpoint 1 (BP1) is nearer to the centromere and located proximal to D15S1035 while the smaller type II (TII) deletion involves breakpoint 2 (BP2) and distal to D15S1035. Breakpoint 3 (BP3) is located at the distal end of 15q11-q13 and common to both typical deletion subgroups. Using high resolution oligonucleotide aCGH analysis (244K DNA microarray, Agilent Technologies), we examined the position of the chromosome 15 breakpoints in 12 PWS subjects with TI deletions (mean age 24.7y) and 13 with TII deletions (mean age 18.6y). BP1 spanned a region from 18.68 to 20.20 Mb (mean 19.89) from the p terminus while BP2 spanned from 20.81 to 21.36 Mb (mean 21.19). BP3 spanned the region from 25.94 to 27.29 Mb for both deletion subgroups; however, a bimodal distribution of breakpoints was observed. The size of the TI deletion ranged from 5.72 to 8.15 Mb (mean 6.58) and 4.77 to 6.40 Mb (mean 5.31) for TII deletions. The BP1 region (1.52 Mb) was larger than BP2 (0.55 Mb). The BP3 region was 1.35 Mb for the combined TI and TII subgroups. A subset of the TI subjects (e.g., breakpoint at 18.68 Mb) includes the loss of three genes/transcripts (i.e., *LOC283755, POTE5, OR4N4*) in addition to the four genes previously recognized between BP1 and BP2 (i.e., *GCP5, CYFIP1, NIPA1, NIPA2*). Thirteen of the 25 PWS subjects showed copy number variation of other chromosomes particularly deletions and duplications of chromosome 8p (clustered at approximately 39 Mb from the p terminus). Additional studies are required to further characterize the regions of copy number variation among PWS and control subjects. The use of high resolution oligonucleotide microarrays should allow for more detailed genomic data for genotype - phenotype correlations and more precise breakpoint location and assignment of duplications and deletions.

1608/T

De novo interstitial inverted duplication 1q41-q42 delineated by molecular cytogenetic techniques. *S. Chantot-Bastaraud[1,2,5], K. Krabchi[1,2], E. Pipiras[3], A. Guet[4], A. Afenjar[4], K. McElreavey[5], B. Benzacken[3], JP. Siffroi[1,2].* 1) AP-HP, Hopital Tenon, Service d'Histologie, Biologie de la Reproduction et Cytogenetique - Universite Pierre et Marie Curie, Paris6, France; 2) Universite Pierre et Marie Curie- Paris 6, EA 1533, Paris, F75020, France; 3) AP-HP, Hopital Jean Verdier, Service d'Histologie-Embryologie et Cytogenetique - UFR-SMBH, Bondy, France; 4) AP-HP - Hopital Trousseau- Service de Neuropediatrie- Universite Pierre et Marie Curie, Paris6, France; 5) Unite de Reproduction, Fertilite et Developpement, Departement de Biologie du Developpement, Institut Pasteur, Paris, France.

Partial duplications of the long arm of chromosome 1 are uncommon cytogenetic anomalies. Most of them arise from de novo unbalanced translocations or from the unbalanced inheritance of a parental balanced rearrangement. However, involvement of other chromosomes may confound the phenotype of trisomy 1q. Thus, pure trisomy are particularly useful for establishing a karyotype-phenotype correlation . We report the case of a 10 month-old male who was referred to clinic because of dysmorphic features and psychomotor retardation. Traditional G-band chromosome studies of the patient was interpreted as 46,XY,dup(1)(q42q41) and subsequently confirmed by fluorescence in situ hybridization using whole chromosome paint 1. No terminal deletion was found. To further evaluate the extent of the chromosome 1 duplication, a series of fluorescence in situ hybridization probes were used . The characterization by multiple locus specific FISH probes allowed a more refined delineation of the phenotypic findings and clinical significance associated with this rare partial trisomy 1q with inverted duplication 1q41-q42. The clinical similarities and differences between previously reported cases with trisomies of the long arm of chromosome 1 are discussed.

1609/T

Are we missing low level chromosomal mosaicism? Mosaic Trisomy 9 identified via Comparative Genomic Hybridization. *K.A. Chapman[1], D.M. McDonald-McGinn[1], R. Jethva[1], L. Campbell[1], M. Falk[1], S. Spinner[1], L. Shaffer[2], E.H. Zackai[1].* 1) Clinical Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Signature Genomic Laboratories, LLC, Spokane, WA.

Standard cytogenetic studies routinely include a 20 cell count which excludes 14% mosaicism with 95% confidence. We report three patients with mosaic trisomy 9 identified using commercially available comparative genome hybridization (CGH) and confirmed by interphase FISH following normal chromosomal analysis. All three patients have features consistent with mosaic trisomy 9: Patient 1 presented at 2 months of age with upslanting palpebral fissures, overfolded helices, a cleft palate, micrognathia and significant failure to thrive; Patient 2 was evaluated at birth with overfolded helices, micrognathia and hydronephrosis and again at 14 months with failure to thrive, recurrent URI's and developmental delay; and Patient 3 was seen at 21-months of age due to recurrent URI's, dysphagia, kyphoscoliosis, low-set ears with overfolded helices and developmental delay. Standard karyotypes, counting 20 cells, were reportedly normal in all three patients. However, CGH identified mosaic trisomy 9, confirmed via interphase FISH in 13%, 18% and 8% of cells respectively. Subsequently, the original cytogenetic metaphase slides for patient 2 were re-reviewed and found to have 3/30 cells with trisomy 9 mosaicism therefore independently corroborating the CGH results. Thus, these findings suggest that we may in fact have been missing low-level cytogenetic mosaicism in metaphase cells and highlights the utility of CGH for identifying such abnormalities followed by confirmatory interphase FISH .

1610/T

63 copy number variants (CNVs) identified in 400 autism spectrum disorder patients using a novel 19K whole genome tiling path BAC microarray. *S. Christian[1], C. Brune[2], J. Sudi[1], R. Kumar[1], J. Badner[3], J. Conroy[4], D. McQuaid[4], E. Hatchwell[5], S. KaraMohamed[1], C. Gilliam[1], N. Nowak[4], W. Dobyns[1], E. Cook[2].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept of Psychiatry, Univ of Illinois at Chicago, Chicago, IL; 3) Dept of Psychiatry, Univ of Chicago, Chicago, IL; 4) Roswell Park Cancer Institute, Dept of Cancer Genetics; 5) SUNY at Stony Brook, Stony Brook, New York.

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterized by qualitative impairment of reciprocal social interaction and communicative development, restricted interests and repetitive behaviors. The prevalence of ASDs is ~1:160 with a 4:1 male to female ratio. One genetic mechanism that is associated with ASDs is chromosomal abnormalities. We have performed array comparative genomic hybridization (aCGH) on 400 ASD patients from the AGRE families using a novel 19K whole genome tiling path BAC microarray to identify copy number variants (CNVs) associated with ASD. 260 individuals from the NIMH Genetics Initiative control samples comprised of 160 Caucasians and 100 African-Americans were also analyzed to exclude common CNVs. We have identified 63 CNVs not present in the control samples in these 400 ASD patients using a threshold of 2 contiguous BACs to define a CNV. Currently, 38 CNVs have been confirmed using fluorescence in situ hybridization (FISH), microsatellite and/or quantitative PCR analyses. Four recurrent loci were identified at 11p11.2, 15q11-q13, 16p11 and 22q11. The other 49 CNVs were present in only a single patient. The sizes of the CNVs ranged from 185 kb to 6.1 Mb and included 0-45 Refseq genes. 18 loci overlapped regions with linkage to autism. These CNVs associated with ASDs will allow identification of recurrent chromosomal abnormalities, as well as, individual candidate genes that may either be causative or contributory to the ASD phenotype.

1611/T

Clinical application of whole genome oligonucleotide array CGH demonstrates markedly improved sensitivity in detecting unbalanced chromosomal anomalies involved in human disease. *J. Compton, S. Bale, Y. Shevchenko, G. Richard.* GeneDx, Gaithersburg, MD.

Oligonucleotide array comparative genomic hybridization (oligo aCGH) in microarray format has been used previously only in the research setting to detect unbalanced chromosomal anomalies. Encouraged by research results, we have tested the hypothesis that whole genome oligo aCGH analysis can significantly improve detection of disease-related anomalies as implemented in a clinical diagnostic laboratory. Here we report outcomes using a custom-designed 44,000 probe microarray with a near-uniform probe spacing of 80 kb across the genome except in large repetitive regions, and only 5 kb spacing in over 100 clinically significant regions or other locations of interest (e.g. sub-telomeres). Likely clinically-relevant gains or losses were confirmed by qPCR, microsatellite marker, or FISH analysis. At the time of writing, 119 specimens have been analyzed, of which 21 were positive; an overall positive yield of 17.6%. Among the 35 cases reported with normal G-band karyotype, 7 had positive findings (20%). Interestingly, 3 positive results were also obtained among the 35 cases submitted with previous negative findings from BAC-based targeted aCGH analysis, which suggests improved detection sensitivity of 10% using oligo aCGH compared to BAC-based aCGH. One patient with two separate balanced translocations by G-banding harbored a large interstitial deletion on another chromosome. In counterpoint, two patients with normal aCGH results were subsequently found to have mutations in single genes by sequence analysis, illustrating that clinical pathology and mutational spectrum of known disease genes should be used to guide the choice of diagnostic methodology. Our present data demonstrate that oligo aCGH will provide a significantly higher diagnostic yield compared to other cytogenetic methods. The high resolution possible with oligo aCGH provided a marked improvement in the detection of clinically-relevant chromosomal anomalies, yielded a more precise definition of breakpoints and boundaries, and refined interpretation of translocations that appeared balanced by karyotype analysis.

1612/T

Utility of the targeted Array-based Comparative Genomic Hybridization (aCGH) in detection of copy number changes in conjunction with traditional cytogenetic analysis-Experience at Pittsburgh Cytogenetics Laboratory using 670 clinical samples. *L. Gole[1], S. Madan-Khetarpal[2], N. Powell[2], R. Avron[2], K. Schmidt[2], J. Hu[1], S. Mann[1], U. Surti[1].* 1) Reproductive Genetics, Magee Womens Hospital and University of Pittsburgh, Pittsburgh, PA; 2) Department of Medical Genetics, Children's Hospital of Pittsburgh, 3520 Fifth Avenue, Pittsburgh, PA.

We present a retrospective analysis of targeted aCGH and traditional cytogenetic analysis performed from May, 2006 to June, 2007 on 670 mostly pediatric samples with abnormal phenotype and/or developmental delay and mental retardation. Majority of the samples showed a normal karyotype and were subsequently processed for aCGH at Signature Genomic Laboratory using 1887 BAC clones representing 622 loci. Eighty six cases gave abnormal aCGH results (12.8%). These included 11 *de novo* deletions, 6 *de novo* duplications, 4 familial abnormalities, 23 polymorphic variants and 42 cases that are awaiting parental investigation. Four balanced chromosome abnormalities and 3 cytogenetic abnormalities from the regions not represented on the array resulted in normal microarray results as expected. At present we process the sample for traditional cytogenetics before considering microarray analysis. This technology has almost replaced laborious all telomere FISH testing in our laboratory and quick turn around time is a distinct advantage. In addition to the targeted aCGH analysis, 4 cases with known complex structural abnormalities involving chromosomes 8, 15, 20 and X were analyzed using the GeneDX 44K oligo-array to refine the breakpoints.

Our experience with microarray testing - the advantages and drawbacks - has been an ongoing learning process enabling us to evaluate the needs of each patient and order an appropriate test platform which would yield maximum useful information and minimize complications due to structural variants.

1613/T

Whole Genome 500K SNP Microarray Analysis for Evaluation of Patients with Mental Retardation, Developmental Delay, Autistic Spectrum Disorder and/or Multiple Congenital Malformations. *M. Ito[1,2], J.M. Milunsky[1,2,3], T.A. Maher[1], A. Milunsky[1,2].* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Whole genome SNP (Single Nucleotide Polymorphism) microarray copy number analysis is a powerful tool for detection of cryptic unbalanced chromosomal abnormalities, providing detailed insights of chromosomal aberrations such as small duplications and deletions, and defining breakpoints of unbalanced rearrangements often not detected by standard cytogenetic analyses. We have utilized the previously validated 500K Affymetrix SNP microarray (combination of 250K NspI and 250K StyI chips) with Copy Number Analysis Tool (CNAT) software. The average distance between one SNP to another is approximate 5.8kb in these chips. The average call rates were greater than 97%. Abnormal findings from the first array were confirmed and further refined with the second array and FISH studies. In 115 patients with mental retardation (MR), developmental delay (DD) and autistic spectrum disorder (ASD), and/or multiple congenital anomalies (MCA), after normal karyotype with or without CGH or subtelomeric FISH analyses, we have found ten patients with clinically significant structural chromosomal aberrations by this analysis (8%; nine deletions and one duplication). The average size of such aberrations is approximate 2Mb, ranging from 0.8Mb to 3.8Mb. In addition, approximately 30% of patients have smaller aberrations less than 0.4Mb. Two thirds of them are reported variants found in normal individuals, however, others require parental samples to clarify the pathogenicity of such aberrations. Caution is necessary in reporting these results. Since resolution is much finer, we propose using actual physical positions in conjunction with cytogenetic banding terminology to indicate exact locations of aberrations to avoid confusion. This experience underwrites the value of copy number analysis using the 500K SNP microarray which clearly is a powerful tool for identifying chromosomal aberrations and should routinely be included in the genetic analysis of those with MR, DD, ASD, and/or MCA.

1614/T

Duplication 17p13.3 detected by array CGH. *V. Jaswaney, J. Tepperberg, P. Papenhausen, B. Wilford, I. Gadi.* Lab Dir/Cytogenetics, Laboratory Corp America, Res Triangle Park, NC.

We report 4 patients with an apparent 17p13.3 duplication and three patients with a 17p13.3 deletion using a PerkElmer constitutional BAC array. These patients were submitted for array CGH analysis for developmental delay, learning difficulties, mental retardation or other birth defects. It is reported that many of the microduplications are not associated with a severe phenotype as compared to microdeletions which are well characterized in literature. Duplications in "normal" individuals appear to represent a benign variant. Three of the four patients had duplications of multiple bac clones and 1 was a duplication of a single bac. Theoretically, an unbalanced meiotic recombination should result in a deletion or duplication with an equal frequency. FISH is an excellent targeted tool to identify specific microdeletions however, microduplications may not be apparent by G-banding or by interphase FISH. Confirmation of a bac microdeletion or microduplication by FISH may not be necessary and can be identified by the increased sensitivity of the array, and the density and redundancy of the bac clones within the constitutional array. Microduplications detected by the recent advances in genome analysis using array based techniques will increase our understanding of the clinical pathology associated with specific duplications. Parental studies are pending to determine whether the origin of the duplication is de novo or familial. The apparent extent of DNA duplication observed and details of clinical presentation of each of these patients will be presented.

1615/T

Development and validation of a focused oligonucleotide-based BAC emulation microarray for clinical array-CGH analysis. *S.H.L. Kang[1], Z. Ou[1], C. Carmack[2], L. White[1], J.R. Lupski[1], A. Patel[1], A.L. Beaudet[1], S.W. Cheung[1], A.C. Chinault[1].* 1) Baylor College of Medicine, Dept. of Molecular and Human Genetics, Houston, TX; 2) Agilent Technologies, Santa Clara, CA.

Array comparative genomic hybridization (array-CGH) allows genome-wide screening for copy number changes that is limited only by the probes that are present on the array. Recently the trend has been toward the use of commercially manufactured high-density oligonucleotide microarrays to detect these changes. However, major hurdles in transitioning this technology to the clinic include the need to establish a reliable and cost-effective method to 1) confirm copy number changes, and 2) determine whether the observed change is a normal variant or pathological. To overcome this, we have developed and validated an oligo-based BAC emulation array using the Agilent platform for use in clinical practice. This array contains ~44,000 oligos grouped into the same BAC sequences that have been previously used on our array-CGH assay. The microarray was developed by initially selecting 105,000 oligos covering the regions of interest and using empirical hybridization data and oligo distribution analysis to select an optimized 44K oligo array. Therefore, this 44K oligo array remains primarily focused on known disease regions and telomeres combined with backbone coverage of the entire genome and emulates the BAC arrays with which we have generated a valuable database with results from over 8000 patients. Additionally, by maintaining a correlation with BAC sized sequences, FISH analysis with verified clones can still be utilized to confirm the array-CGH results. From parallel analyses on BAC and OLIGO arrays we found that the OLIGO version is more reliable and more sensitive/robust (up to 2-fold) than the BAC version because of improved dynamic range. The increased reliability of the OLIGO data negates the need for parallel dye-swap experiments. The increased sensitivity of the OLIGO data as well as advances in the software used to calculate and display copy number changes permits more accurate determination of smaller genomic imbalances that are not statistically significant on the BAC versions.

1616/T

Paving the Way to Accurate Genotype-Phenotype Predictions Using High Resolution Whole Genome SNP Oligonucleotide Microarray Analysis (SOMA) in the Clinical Cytogenetics Laboratory. *B. Levy[1], V. Jobanputra[1], O. Nahum[1], W. Chung[1], A. Shanske[2], K.A. Yeboa[1], L.G. Shaffer[3], D. Warburton[1].* 1) Columbia University Medical Cntr, New York, NY; 2) Children's Hosp Montefiore, Albert Einstein College of Med, Bronx NY; 3) Signature Genomic Laboratories, Spokane WA.

Constitutional chromosomal abnormalities are often associated with a spectrum of clinical abnormalities. The phenotypic consequences of the anomaly vary considerably and depend on the nature and chromosomal origin of the apparent imbalance, as well as precisely which genes are involved in the aberrant region. In many cases, prognostic information is solely derived empirically by reviewing apparently similar cases in the literature. High resolution SNP oligonucleotide microarray analysis (SOMA) allows for the identification of visible and submicroscopic cytogenetic imbalances by scanning the entire genome in a single step. A major advantage of SOMA is its ability to more precisely define the boundaries and nature of the region of imbalance, especially with respect to the gene content. We have performed studies using the Affymetrix 500K array to study: [1] cases with well characterized cytogenetic aberrations, [2] cases containing a variety of previously uncharacterized chromosomal imbalances and [3] cases containing genomic imbalances involving single gene regions. We also performed a blinded study of coded specimens with cytogenetic aberrations, including cryptic subtelomeric unbalanced rearrangements and diseases caused by single gene abnormalities. In all studies, we reliably detected the various chromosomal imbalances whose sizes ranged from single genes to megabases. In this report we highlight the potential diagnostic scope of SOMA with particular attention to the benefits of characterizing the gene content together with accurate clinical information. In clinical cytogenetics, the precise identification of the origin of the additional or missing chromosomal material is a key factor when considering genotype-phenotype correlations and may ultimately lead to the discovery of the genes that are responsible for the clinical features that present in such patients.

1617/T

Identification of marker chromosomes using FISH-based technology and DNA polymorphic marker. *Y.C. Li[1], L.J. Hsieh[2], C.P. Chen[3], F.J. Tsai[4], C.C. Lin[2].* 1) Dept. of Biomedical Sci, Chung Shan Medical Univ, Taichung, Taiwan; 2) Dept. of Medical Research, China Medical University and Hospital, Taichung, Taiwan; 3) Dept. of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan; 4) Dept. of Medical Genetics and Pediatrics, China Medical University Hospital, Taichung, Taiwan.

Marker chromosomes are chromosome or chromosome fragment which their origin can not be resolved by conventional cytogenetic analysis. Marker chromosome account for about 1:1,100 cases at amniocentesis, 1:4,000 in live born and approximately 1:400 in mentally retarded patients. We performed 24 colors spectral karyotyping (SKY) analysis and/or FISH study with chromosome region specific probes to identify 33 cases of marker chromosome (19 cases were prenatal). All but 3 cases, the origin of marker chromosome can be unequivocally identified (success rate: 90.9%). Among those marker identified, 10 cases were derived from chromosome 15 (33%), 6 cases involved chromosome 22 (20%), 3 cases originated from chromosome 9 (10%), 3 cases involved chromosome 18 (10%), 3 cases were derived from X chromosome (10%) and 2 cases from Y chromosome. The remaining 3 cases were each from chromosome 2, 3 and 14 respectively. In some of the cases, QF-PCR assays with STR markers on specific chromosome regions were also conducted to reveal the paternal or maternal origin of the marker chromosomes. Three of 33 cases had been reported as case reports (Chen et al. 2004; Lin et al. 2006; Chen et al. 2006). The study demonstrated the importance of clinical cytogenetic and molecular analysis for diagnosis of marker chromosome and showed how crucial it was for prenatal counseling and management of the pregnancies. The study was supported by grants from the National Health Research Institute (NHRI-EX92-9207SI) and from the National Science Council (NSC95-2311-B-040-001), Taiwan.

1618/T

Whole genome 500K SNP microarray delineates duplication/deletion of 8p in a child with MR/MCA. *S. Newton[1], M. Ito[1,2], X.L. Huang[1], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics, BUSM, Boston, MA; 2) Department of Pediatrics, BUSM, Boston, MA; 3) Department of Genetics and Genomics, BUSM, Boston, MA.

Whole genome microarray is a useful method for detecting cryptic unbalanced chromosomal abnormalities. This technique has proven valuable in evaluation of patients with mental retardation, developmental delay, autism, and/or congenital malformations. We report a 9 year old Brazilian male who was evaluated due to mental retardation, agenesis of corpus callosum, dysmorphic features, scoliosis, absent language, and limited mobility. Hypotonia and delay at 4 months of age prompted an MRI revealing corpus callosum agenesis. He has no reported history of cardiac problems or seizures. He demonstrates bruxism and midline hand-wringing. An upper GI series revealed congential malrotation. He has scoliosis (25º). OFC is in the 50-75th centile, weight and height are <3rd centile. Multiple dysmorphic features include: long eyelashes, arched eyebrows, elongated face, bulbous nasal tip, anteverted nares, and smooth, prominent philtrum. He has a high arched palate, micrognathia, and widely spaced teeth. He has tapered digits and fifth finger clinodactyly. High resolution chromosome analysis in addition to whole chromosome 8 painting revealed an apparent interstitial inverted duplication in the 8p21.3-23.1 region. 500K SNP microarray analysis was performed to further delineate the duplication. Analysis revealed a 31.2 Mb duplication at 8p22-11.21. In addition, a 6.8 Mb deletion at 8p23.3-23.1 was revealed that was confirmed by FISH. Parental studies have been requested. Inv dup/del 8p has been well documented in the literature with a clinical picture consisting of agenesis of the corpus callosum, hypotonia, M/R, dysmorphic features, orthopedic abnormalities, and heart defects. Given our patient's clinical findings and the reported literature on inv dup/del 8p, this finding likely explains his phenotype. SNP microarray analysis proved helpful in this case, revealing a 6.8 Mb cryptic deletion not detected by high resolution chromosome analysis, and clarifying the size of the duplication. This additional information may potentially lead to more optimal anticipatory guidance in the future.

1619/T

Two patients with unusual chromosomal anomalies detected by consecutive BAC and SNP based microarrays. *P. Papenhausen, J. Tepperberg, V. Jaswaney, I. Gadi.* Dept Cytogenetics, Labcorp of America, Res Triangle Park, NC.

Two probands are reported at 6.8 (case 1) and 0.2 (case 2) years of age which were referred for a BAC based microarray analysis due to MR with aphasia and growth retardation/contractures, respectively. Case one was known to have a chromosome 15 derived marker and case 2 had not been previously studied cytogenetically. Case 1 revealed very significant gains at the 5 most distal 15q BACS from the 90.4Mb linear position (15q26.1) to the most distal BAC at 100.1MB. Chromosome G-bands showed a small symmetrical marker chromosome in all metaphases with a slightly off center constriction. Subsequent FISH confirmed an analphoid marker positive with a subtelomere 15q at each end and presumptive tetrasomy for 15q26.1>qter. Subsequent analysis by high resolution SNPs revealed a proximal region of apparent trisomy from 82.8 to 84 linear Mb that stepped up to tetrasomy from 84 Mb to the 100 linear Mb telomere. Thus, apparently the marker single copy increase was near the off center constriction. Case 2 revealed a significant three BAC loss at 13q at 65.5Mb to 68Mb (13q21.1-q21.3) combined with a single BAC gain at 97.46Mb (13q32.3). Cytogenetics revealed a highly abnormal 13q G-band pattern. High resolution SNPs revealed alternating regions of single and double copy gain from 55.3 to 64.9 Mb and 4.8 Mb loss from 64.9 to 69.7 Mb. A second region of loss from 83.2 to 91.2Mb and gain from 93.5 to 104 Mb was also found. The genes in these copy number variations will be compared to the resulting phenotype. These cases demonstrate the high precision at which high resolution arrays can elucidate the underlying genomic imbalance in children with genetic disorders. Additionally, case one data suggest that asymmetric constrictions frequently reported in terminal analphoid inverted duplications may correlate with regions of proximal single copy number gain, rather than the duplicate copies in remainder of the chromosome.

1620/T

Identification by array-CGH of new candidate regions for utero-vaginal defects. *C. Rosenberg, C. Cheroki, A.C. Krepischi-Santos, P.A. Otto.* Dept Genet. Evol Biology, Univ Sao Paulo, Sao Paulo, Brazil.

Failure in fusion of müllerian ducts has an incidence of about 1/5000 newborn females and results in defects of the genital tract ranging from upper vaginal atresia to total absence of fallopian tubes, uterus and upper vagina. It might occur in otherwise phenotypically normal females (Mayer-Rokitansky-Küster-Hauser anomaly - MRKH [OMIM 277000]), but it is often associated with other malformations involving kidneys, skeleton, extremities and hearing defects (known as the MURCS association), suggesting the involvement of major developmental genes. Although a mutation in the WNT4 gene has been identified in one atypical patient and chromosomal alterations have been sporadically reported, the etiology of mullerian anomalies remain poorly understood. Array-based comparative genomic hybridization (array-CGH) allows ascertaining cryptic chromosomal imbalances that escape detection by routine chromosome analysis. It provides a genome-wide screening by hybridizing differentially labeled test and reference DNAs to arrays consisting of thousands of genomic clones. This approach has proved useful in determining the etiology of 15-20% of mental retardation of previously unknown cause, and lead to the identification of novel genes involved in malformation syndromes. We investigated by array-CGH fifteen syndromic females with uterus-vaginal defects, but normal G-banded. Cryptic imbalances were detected in five patients (33%). Relatives carrying the imbalances showed variable penetrance and expressivity, including renal defect in a proband's son. The results point to 1q21.1, 17q12, 22q11.21-q11.22 and Xq21.31 as relevant regions for urogenital tract development.

1621/T

Higher Incidence of Chromosome Deletions and Duplications Identified by Array CGH. *J.H. Tepperberg, I. Gadi, B. Williford, D. Fuentes, J. Whaley-Davis, C. Legacki, C. Bullen, J. Kesler, N. Elliott, P. Papenhausen.* Cytogenetics, LabCorp, RTP, NC.

Genetic imbalances are generally associated with multiple birth defects, developmental delay, growth retardation, and dysmorphic features. The incidence of targeted chromosome microdeletion syndromes is estimated to be 1 in 1000-2000 while the detection rate of clinically significant subtelomere abnormalities was recently shown to be approximately 2.5%; (Ravnan et. al. 2005). Array based Comparative Genomic Hybridization (aCGH) is used as an adjunct to cytogenetics and FISH to detect unbalanced chromosome alterations (aneuploidy, microdeletions, duplications, and unbalanced subtelomere rearrangements) associated with developmental delay and mental retardation. Array CGH analysis of 2484 clinical cases submitted for aCGH (PerkinElmer targeted constitutional bac array) showed 6.03% (150/2484) cases with clinically significant unbalanced rearrangements. The most common chromosomes (7, 15, 17 and 22) identified in microdeletion syndromes were also the most common identified by the array. Seventy-two abnormal cases (47.3%) showed apparent terminal deletions, fifty-two abnormal cases (34.2%) showed interstitial duplications, four cases (19.0%) were unbalanced derivative chromosome rearrangements, and nine cases (5.9%) were unbalanced structural abnormalities (e.g., inv, dups, isochromosomes and markers). Five cases with mosaicism were observed with the lowest threshold of 23.0%. Twelve percent trisomy 9 mosaicism, confirmed by chromosomes, was observed retrospectively at the threshold level. The constitutional bac array was not designed to diagnosis single clone alterations in the backbone region however it did uncover true alterations. Twenty cases of a single bac copy gain or loss confirmed by FISH were detected in the non-targeted backbone region of the array. This data adds to the growing body of literature indicating that chromosome deletions and duplications are more prevalent among patients with unexplained MR and developmental delay.

1622/T

Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MLPA) Analysis of Subjects with Chromosome 15 Abnormalities. *M.F. Theodoro, D.C. Bittel, N. Kibiryeva, M.G. Butler.* Section of Medical Genetics, Children's Mercy Hospital and Clinics and University of Missouri-Kansas City, MO.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are neurodevelopmental disorders caused by loss of expression of imprinted genes from the 15q11-q13 region. They arise from similar defects in the 15q11-q13 region but originate on the paternal or maternal chromosome 15, respectively. There are two recognized typical 15q11-q13 deletions depending on size identified in the majority of PWS and AS subjects. Several diagnostic assays are available for identifying genetic subtypes in PWS and AS. However, each has limitations due to cost, time or reliability which make them less than ideal. We evaluated the usefulness of methylation specific multiplex ligation-dependent probe amplification (MLPA) in 95 subjects with chromosome 15 abnormalities (62 PWS, 10 AS, 10 individuals with other chromosome 15 abnormalities [e.g., markers, rings, duplications, translocations, distal deletions] and 13 cytogenetically normal individuals). A commercially available MLPA kit (MRC-Holland; Amsterdam) was used to detect copy number changes as well as to analyze CpG island methylation in the 15q11-q13 region. It contains 25 probes specific for sequences in or near the PWS /AS critical region. Five of these probes contain a HhaI recognition site and are specific for imprinted sequences. Eighteen probes for genes located outside the PWS/AS region are used as controls for copy number changes. Three of these probes contain a HhaI site and unmethylated in control DNA samples used to check for completeness of digestion. We developed an algorithm for MLPA probe analysis which correctly identified methylation abnormalities associated with PWS and AS and accurately determined copy number to correctly assign genetic subtype status including microdeletions of the imprinting center. Furthermore, MLPA analysis identified copy number changes in those with distal 15q deletions and ring 15s. MLPA is a relatively simple, reliable and cost-effective technique which is useful and accurate for analysis of methylation status, copy number and genetic subtype in PWS and AS as well as other chromosome 15 abnormalities.

1623/T

The European Cytogenetic Initiative: Molecular karyotyping of 120 patients with unexplained mental retardation by Mapping 500K SNP arrays. *J.A. Veltman[1], A. Dufke[2], D.J. McMullan[3], B.B.A. de Vries[1], E.C. Rattenberry[3], M. Bonin[2], S. Jacobs[4], M. Steehouwer[1], R. Pfundt[1], N. de Leeuw[1], A. Riess[2], O. Altug-Teber[2], H. Enders[2], E.V. Davison[3], O. Riess[2], L. Brueton[3].* 1) Dept. Human Genetics, UMC Nijmegen, Nijmegen, The Netherlands; 2) Dept. Medical Genetics, University of Tuebingen, Tübingen, Germany; 3) West Midlands Regional Genetics Laboratory & Clinical Genetics Unit, Birmingham Womens Hospital, Birmingham, United Kingdom; 4) Affymetrix, Inc, Santa Clara, USA.

The underlying genetic defect remains difficult to diagnose in the majority of patients suffering from mental retardation. Recent developments in genomic microarray technology now allow for the genomewide detection of subtle chromosomal alterations, and this has been demonstrated to significantly improve the diagnostic yield in this patient group. This technology has rapidly matured and now appears ready for widespread introduction in routine diagnostics. We hypothesize that it is best to use commercially available microarrays as these provide the highest genome coverage, they can be produced according to industrial quality standards, are available to all diagnostic laboratories, and their widespread use will generate large reference datasets. In this study three European diagnostic centres assessed the use of Mapping 500k SNP arrays for molecular karyotyping in patients with mental retardation. Each centre tested DNA from 40 patient-parent trios. In addition, 40 known submicroscopic copy number variations (CNVs) were run for validation purposes and for optimizing data-analysis. All known CNVs were unequivocally detected on the Mapping 500K arrays. In the cohort of 120 patients with unexplained MR a total of 17 de novo CNVs were identified, varying in size from 700 kb to 12 Mb. In addition numerous inherited CNVs were detected, including a large maternally transmitted deletion on 16p13.11-p12.3. In conclusion, we show that Mapping 500K SNP arrays can be used to reliable detect and characterize subtelomeric and interstitial CNVs. The diagnostic yield of this approach is significant and warrants a rapid diagnostic implementation of these microarrays in mental retardation.

1624/T

Microarray CGH analysis and genotype-phenotype correlation in a patient with subtelomeric 9q deletion syndrome. *T.J. Chen, Y. Wang, J. Chaplin, C.M. Tuck-Muller, W. Wertelecki, J.E. Martinez.* Dept Medical Genetics, Univ South Alabama, Mobile, AL.

Subtelomeric deletion of chromosome 9q is a newly recognized microdeletion syndrome. Most patients have a submicroscopic deletion of the gene-rich critical region, which is about 700 kb distal to 9qter but less than 30 cases of this condition have been described so far and most deleted regions have been studied by FISH and/or STR analysis. Therefore, a correlation between the size of the deletion and the severity of clinical manifestations has not yet been determined. We performed microarray analysis on a black female infant with developmental delay and a subtelomeric 9q deletion using high resolution oligo CGH array. She was born with a birth weight of 3.1 kg at term to the first pregnancy of young non-consanguineous parents. Congenital heart anomalies were diagnosed at birth including a VSD, a PDA and at the age of 22 month, she was noted to be developmentally delayed, small and microcephalic, and had the following measurements: OFC 41.5 cm (<2nd%), Height 73.5 cm (<5th%) and Weight 8.6 kg (<5th%). Craniofacial dysmorphism included coarse facial features, prominent forehead, hypotonic face and large mouth. She was also noted to have tracheomalacia and gastro-esophageal reflux and a gastrostomy tube was placed for supplemental feedings. Cytogenetic studies of the patient and her parents were normal. The subtelomeric deletion 9q was first detected by multiple ligation-dependent probe amplification (MLPA) and array CGH analysis revealed a 3.0 MB deletion on 9q34.3, from 137.2 MB to 140.2 MB, which is smaller than previously reported cases. The patient's phenotype is consistent with the clinical manifestations reported in cases with deletions in the critical region but our patient did not have brain abnormalities, seizures, joint laxity, trigonocephaly, or furrowed palate, anomalies described in patients with a deletion size larger than 1.2 MB. Thus, a more precise determination of deletion size and molecular location will be needed to clarify the genotype-phonotype correlation in this syndrome.

1625/T

Improved detection of 22q11 rearrangements with a high density MLPA probe set. *G. Jalali[1], J.A.S Vorstman[1], A. Errami[2], R. Vijzelaar[2], J. Biegel[1], T. Shaikh[1], B.S. Emanuel[1].* 1) Dept Genetics, CHOP, Philadelphia, PA; 2) MRC-Holland, Netherlands.

Chromosome-specific low copy repeats or segmental duplications predisposes chromosome 22 to deletions and duplications. The current diagnostic procedure for detection of deletions and duplications at 22q11.2 is chromosomal analysis coupled with fluorescence in situ hybridization (FISH). Recently, a PCR based multiplex ligation dependent probe amplification (MLPA) method has been used for this purpose. However, there are copy number variations in 22q11.2 that are only detected by high throughput platforms such as array CGH and not the current FISH probes or MLPA kit. Here we report on development of a high density MLPA (MLPA-HD) kit capable of detecting aberrations of chromosome 22. The MLPA-HD probe set detects copy number changes at 37 loci on the long arm of chromosome 22. These include the 3Mb region commonly deleted in DiGeorge/Velocardiofacial Syndrome (DGS/VCFS), the Cat Eye Syndrome (CES) region and more distal regions in 22q11 recently shown to be deleted in patient samples. We have used this HDMLPA probe set to analyze 363 previously well-characterized samples with a variety of different rearrangements at 22q11. We demonstrate that the HDMLPA kit can detect copy number alterations with excellent sensitivity and specificity. In addition to detection of the common recurrent deletions associated with DGS/VCFS, variant chromosome 22 aberrations that are distal to this region and duplications within this region have been detected. Further, the HDMLPA detects deletion endpoint differences between patients with the common 3 Mb deletion. Thus, the HDMLPA set allows for detection of aberrations that would not have been disclosed by either diagnosticFISH probes or the currently available MLPA kit. Based on these findings, the HDMLPA kit is proposed as a cost effective alternative to the currently available detection methods for individuals with features of the 22q11.aberrations. The HDMLPA probe set could replace FISH with N25 or TUPLE1 probes for the clinical diagnosis of 22q11.2 deletions and duplications in patients with the relevant phenotypic characteristics.

1626/T

Fine breakpoint mapping using Affymetrix Human Mapping 500K Array of two unrelated patients with rare de novo overlapping interstitial deletions of chromosome 9q. *A.S. Kulharya[1,2], D.B. Flannery[1], K. Norris[2], C.M. Lovell[1], B. Levy[3], G.V.N. Velagaleti[4].* 1) Pediatrics, Medical College of Georgia, Augusta, GA; 2) Pathology, Medical College of Georgia, Augusta, GA; 3) Pathology,Columbia University, New York, NY; 4) Pediatrics, University Texas Medical Branch, Galveston, TX.

Chromosome 9 comprises 5% of the total human genome with several clinically significant genes. Approximately, 20 cases of interstitial deletions of 9q have been reported in the literature spanning the breakpoints from 9q21 to 9q34. Unlike the 9q subtelomeric deletions, the interstitial deletions do not demonstrate a specific recognizable phenotype, although up to half of the patients had microcephaly. Lack of precise molecular delineation of the extent of deletions in the published cases makes it difficult to develop an accurate genotype phenotype correlation. We report fine mapping of breakpoints using the Affymetrix Human Mapping 500K Array Set in two unrelated female patients with an overlapping de novo deletion in 9q presented here previously (A902; 2004). SNP Oligonucleotide Microarray Analysis (SOMA) indicated these to be large deletions with patient 1 having a 9.7Mb deletion (>60 genes) spanning 9q31.3-q33.1 and patient 2 having a 6.6Mb deletion (>20 genes) localized to 9q32-q33.1. In patient 1 the proximal breakpoint appears to map to a region where the cytoskeletal protein tyrosine phosphatase gene, PTPN3 is localized and the distal breakpoint appears to interrupt the DBC1 gene. For patient 2, the proximal break maps to a region where the RGS3 gene, a regulator of G-protein signaling, is localized and the distal break appears to map to the region bordering the microcephaly gene, CDK5RAP2. FISH analysis with BAC clones containing these genes is underway. The interruption/deletion of these genes might explain some of the phenotypic features seen in these patients (particularly microcephaly) and may also provide a guideline for clinical management.

1627/T

Clinical and Molecular Characterization of a patient with Langer-Giedion syndrome mosaic for del(8)(q22.3q24.13). *A.L. Shanske[1], A. Patel[2], S. Saukam[2], O. Nahum[4], H.J. Luedecke[3], B. Levy[4].* 1) Children's Hosp Montefiore, Albert Einstein College of Med,Bronx, NY; 2) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Institut fur Humangenetik, Universitatsklinikum, Essen, Germany; 4) Dept of Pathology, Columbia U College of Physicians & Surgeons, New York, NY.

The tricho-rhino-phalangeal syndrome type II(TRPS II)is characterized by sparse scalp hair,a long nose with a bulbous tip, a long flat philtrum, cone-shaped epiphyses, and multiple cartilaginous exostoses. Almost all patients have a hemizygous deletion on chromosome 8q24.1 affecting at least the contiguous *TRPS1* and *EXT1* genes. Here we describe a mentally retarded 14 year old girl with features of TRPS II who is mosaic for an interstitial deletion in 8q24.1. Her birth weight,length, and head circumference were all 2-4 SDs beneath the mean. Her height, weight, and HC were all 2-4 SDs beneath the mean. She had brachycephaly, thick brows with a synophrys and a bulbous nasal tip. She had a duplicated right lower lateral incisor. A bone age done at chronological age 9 years and 4/12 years showed a bone age 6-7 SDs beneath the mean. Cone-shaped epiphyses were present at many phalanges. She had multiple exostoses of the humeri,femora,tibia and the scapula. A WISC-R administered when she was 6 and ½ years of age revealed a full-scale IQ of 62. She has always attended a special education program and is now able to converse in complete sentences in English. A female karyotype with two cell lines was found in blood and skin. Seven of 105 peripheral blood cells exhibited an interstitial deletion in the long arm of chromosome 8 and the remaining cells yielded a normal karyotype, 46,XX,del(8)(q22.3q24.13)[7]/46,XX [98]. The del(8) cell line was present in a much higher percentage (71%) in skin fibroblasts. Deletion of the *TRPS1* and *EXT1* genes was confirmed by FISH. SNP oligonucleotide microarray analysis indicated the deleted region to be 19.59Mb in size with over 50 genes including *TRPS1*, *EXT1* and a number of disease genes on either side of the *TRPS1-EXT1* interval. Mosaicism as this case illustrates, must be considered in patients with TRPS II.

1628/T

Prenatal diagnosis of a 9q34.3 microdeletion by array-CGH in a fetus with an apparently balanced translocation. *S.A. Yatsenko[1], M.J. Simovich[1], M.E. Dudek[4], A. Pursley[1], P.A. Ward[1], S.W. Cheung[1], E.K. Brundage[1], C.A. Chinault[1], A. Patel[1], J.R. Lupski[1,2,3].* 1) Dept Molec & Human Genetics; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children Hospital, Houston, TX; 4) Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN.

The 9q34.3 microdeletion syndrome is a recently identified condition and only after molecular technologies were applied in chromosome studies. Patients with 9q34.3 terminal deletion exhibit a clinically identifiable phenotype characterized by specific craniofacial features, hypotonia, childhood obesity, microcephaly, and substantial speech delay (CHOMS). It has been recognized as one of the most frequent subtelomeric aberrations identified in live births. However, little is known about the impact of such rearrangements for pregnancy outcome and prenatal course. We report the first prenatally detected case of the 9q34.3 microdeletion syndrome. A 42-year-old pregnant female was referred at 14 weeks of gestation for genetic counseling due to an abnormal ultrasound with increased nuchal translucency. G-banded chromosome analysis was performed on chorionic villus sample (CVS), and showed an apparently balanced *de novo* translocation: 46,XY,t(2;9)(q11.2;q34). Using targeted array-CGH we identified a submicroscopic 9q34.3 deletion in a fetus, revealing the unbalanced nature of the rearrangement. Deletion of the 9q34.3 region was studied further by implementing a custom 9q34.3 tiling path fosmid-based and oligonucleotide-based array-CGH analyses to enable higher resolution genome investigation. The deletion was delimited to 2.7 Mb in size encompassing at least 98 genes, and includes the *EHMT1* gene located within the 700 kb critical interval. The identification of cryptic 9q34.3 microdeletion in our case illustrates the importance and clinical relevance of high resolution genome analysis by array-CGH in prenatal diagnosis. Precise molecular characterization is essential for further prenatal and postnatal clinical management, and informed decision making.

1629/T

The role of fluorescence in situ hybridization technique (FISH) in the clinical cytogenetics: 10 years experience of Kuwait Medical Genetic Centre (1995-2006). *S. Abulhasan, A. Al-Adwani, H. Al-Shememaimry, Z. Mohammad, A. Al-Autaiby, K. Al-Kherainej, K. Algareeb, Y. Alfaily, S. Al-Awadi.* Molecular Cytogenetics, Kuwait Med Genetics Ctr, Mansauria, 35652, Kuwait.

Fluorescence in situ hybridization (FISH) technique is a direct way and simple procedure for mapping genes and other DNA sequences which enable for rapid detection and reliable assessment of numerical and structural aberrations including markers and fragments. In addition, using appropriate labeled DNA probes FISH technique has the advantages of providing rapid results which can be scored conveniently by eye and precise diagnostic using an epi-fluorescent microscopy. The purpose of this molecular cytogenetics study to analyze the data from 1995-2006, show the importance of FISH technique and other molecular cytogenetics tool in clinical cytogenetics, and how it is usefulness in speeding the diagnosis and prognosis especially in sex determination and cancer genetics conditions. During the period from September 1995 to December 2006 molecular cytogenetics lab at Kuwait Medical Genetics Centre (KMGC) have recieved up to 1431 samples for investigation of different genetics disorders. All samples were referred by government hospitals and health centres, and private hospitals and clinics. All data will be presented later.

1630/T

Frequent pathogenic and apparently benign *de novo* copy number variants detected by 500K GeneChip® array genomic hybridization in children with idiopathic mental retardation. *J.M. Friedman[1,2], S. Adam[1], L. Armstrong[3], C. Boerkoel[3], S. Chan[4], D. Chai[3], A.D. Delaney[4], W.T. Gibson[3], S. Langlois[2,3], E. Lemyre[5], B. McGillivray[2,3], J. Michaud[5], M. Patel[3], H. Qian[4], G. Rouleau[5], M. Van Allen[3], S.-L. Yong[3], F. Zahir[1,2], P. Eydoux[3], M. Marra[2,4].* 1) Medical Genetics Research Unit, Child & Family Res Inst, Vancouver, Canada; 2) Dept of Medical Genetics, U of British Columbia, Vancouver, Canada; 3) Children's & Women's Hosp, Vancouver, Canada; 4) Genome Sciences Ctr, BC Cancer Agency, Vancouver, Canada; 5) Centre de Recherche, CHU Sainte-Justine, Montréal, Canada.

We are using 500K GeneChip® array genomic hybridization to screen for novel pathogenic copy number variants (CNVs) in children with mental retardation (MR) and normal cytogenetic studies. We found *de novo* CNVs in at least 16 (18%) of 90 children with idiopathic MR studied. There were 7 duplications and 11 deletions, including two unbalanced translocations, ranging in size from 89 kb to 11.1 Mb.

At least 3 of the *de novo* CNVs found in these children are unlikely to be pathogenic for their MR. One child had a 107 kb deletion of 14q11.2, but fosmid FISH showed some cells with 2 copies, some cells with 1 copy, and some cells with no copies of the affected region. This genomic region contains many T-cell receptor alpha V genes, and it is likely that the deletion occurred somatically. A second child had a 166 kb duplication of a different genomic segment in 14q11.2. The region contains multiple olfactory receptor loci but no other annotated genes and is frequently involved in polymorphic (presumably benign) gain CNVs. A third child had an 89 kb deletion of 6p21.33→p22.1 within the HLA region. Polymorphic loss CNVs of this genomic segment are frequently observed in normal populations.

Array genomic hybridization with high-density oligonucleotide chips can detect CNVs smaller than 100 kb in children with idiopathic MR, but many of these genomic alterations may not be pathogenic even if they have arisen *de novo*.

1631/T

Assessment of copy number variants (CNVs) using high resolution whole genome analysis: A strategy for confirmation of genomic changes. *C. Harvard[1,5], C. Tyson[1], Y. Qiao[1,2,5], C. Fawcett[1], J. Hurlburt[2], X. Liu[3,5], JJA. Holden[3,4,5], MES. Lewis[2,5], E. Rajcan-Separovic[1,5].* 1) Departments of Pathology and; 2) Medical Genetics UBC, Vancouver, BC, Canada; 3) Departments of Psychiatry; 4) and Physiology, Kingston, Ontario; Canada; 5) ASD-Canadian American Research Consortium (www.autismresearch.com).

Array CGH using BAC arrays has been shown to successfully detect sub-microscopic chromosomal gains and losses. High resolution oligo arrays, now commercially available, can detect smaller CNVs and better characterize their breakpoints. However, the number of CNVs can be overwhelming for confirmation in a routine cytogenetic setting. We analyzed results from Nimlegen's 325K oligo array for 15 karyotypically normal subjects with idiopathic intellectual disability (ID) to provide the rationale for selecting CNVs most likely to be clinically relevant. A total of 103 CNVs were detected in our study group (2-10/subject). The criteria we used to select CNVs for further follow-up included: 1) size of the deletion (>100kb) and duplication (>200kb) respectively as these changes have a high probability of being real (Hehir-Kwa et al, 2007); 2) overlap with normal CNVs (http://projects.tcag.ca/variation/), duplicons and gaps (overlap with variants from at least 2 studies and across the entire length was considered a normal variation) and; 3) gene content. After eliminating the small CNVs (44) and those likely to be variants (55) as described above, only 4 changes remained for follow-up involving gains of: a) 9.78Mb at 5q14.1-q14.3, 0.72 Mb at 1q21.1, 0.42 Mb at 7q31,1 and 2.46 Mb at 9q21.13-31. Because some CNVs show changes in both normal and affected individuals (eg 1q21.1, Klopocki et al, 2007) and the majority of normal CNVs in the genomic variants database have not been confirmed using an independent method, we also selected two CNVs having complete overlap with normal variants and/or duplicons that contain brain function related genes (gains of 1.2Mb at 9p31. and 0.36Mb at 16p13.11). The results of FISH/qPCR confirmation and family studies of the selected CNVs resulting from our analysis will be presented.

1632/T

Advances in single copy hybridization technology for FISH and Quantitative Microsphere Hybridization (QMH). *J.H.M. Knoll[1], J.M. Cowan[1], P.K. Rogan[1,2].* 1) Pediatrics, Tufts-New England Medical Center, Boston, MA; 2) Phylogenetix Laboratories Inc., Overland Park, KS.

Single copy (sc) genomic probes, which are prepared from short, unique genomic sequences with precisely-defined chromosomal locations, have been used for high-resolution, genetic hybridization analysis of congenital abnormalities and cancer. We have previously described the development of sc probes for FISH (to detect chromosome rearrangement, deletion and duplication) and in QMH flow cytometry (to detect copy number differences). For these probes to be used clinically, hybridization efficiencies and signal intensities need to be comparable to or better than other commonly used reagents. We introduce new approaches for sc probe derivation and corresponding technical improvements in FISH and QMH methodologies using specimens previously characterized by cytogenetics and conventional FISH.

Sc probes can be developed either from repeat-masked genomic sequences or with other bioinformatic methods. We find that these different bioinformatic approaches identify the same single copy intervals with similar computational overhead, and that probes designed from these methods exhibit comparable laboratory results. Modifications of FISH protocols for cell processing, hybridization and post-hybridization wash conditions have resulted in simplified, accelerated experiments and increased hybridization efficiencies with brighter signals. Multiple probe labeling methods were also compared. For QMH, we have optimized procedures to expedite data collection and improve the statistical analysis of genomic hybridization of microsphere-conjugated sc probes. This increases the accuracy of copy number genotypes determined from mean fluorescence intensities. We demonstrate that optimization of multiple parameters in scFISH and QMH will significantly advance our goal of producing clinical assays based on sc technology.

1633/T

Dissection of apparently balanced translocations using high density SNP arrays. *J.M. Kogan, T.A. Smolarek, R.J. Hopkin, G.A. Grabowski.* Division of Human Genetics, Cincinnati Children's Hospital, Cincinnati, OH.

Some individuals with de novo apparently balanced translocations and abnormal phenotypes have cryptic genomic imbalances that likely contribute to the phenotype. Methods such as fluorescence in situ hybridization and array comparative genomic hybridization have facilitated dissection of such rearrangements. These techniques can be time consuming and have limited resolution. In comparison, SNP-based microarray offers advantages for efficient detailed copy number analyses with high density and high throughput. This approach may be particularly applicable for defining deletions or duplications in complex chromosomal rearrangements.

Employing a protocol developed to assess the usefulness of the very high density Illumina and Affymetrix SNP-based microarray platforms, several submicroscopic chromosomal rearrangements have been identified, including two in individuals with complex apparently balanced translocations. SNP microarray analysis revealed one deletion in each patient at a translocation breakpoint that was not detected by standard cytogenetic analyses. A patient with microcephaly, developmental delay, severe hypotonia, and karyotype 46,XX,t(2;9;4)(p23;q12;p16) was found to have a 3 Mb deletion at 4p16.1-p16.2, just proximal to the Wolf-Hirschhorn critical region. Another patient with dysmorphic features, seizures, developmental delay, and karyotype 46,XX,der(8)t(8;11)(q13.3;q22.2),der(11)t(8;11)(q13.3;q13.5),ins(13;11)(q33.2;q13.5q22.2) was found to have a 5.5 Mb deletion at 13q33.2-q34. Several other complex rearrangements will be discussed.

Very high density chromosome analysis using SNP microarray provides resolution to the level of individual genes with a single test that has potential for automation and high throughput. The high resolution allows for consideration of the potential contributions of specific genes involved as well as possible therapeutic targets. Additionally, analysis focused on specific regions, such as known breakpoints of a de novo translocation, may reduce the chance of confusing polymorphic variants with disease-causing abnormalities.

1634/T

Significance of submicroscopic genomic imbalances in mental retardation. *A.C.V. Krepischi-Santos[1], A.M. Vianna-Morgante[1], F. Kok[2], C.A. Kim[3], P.A. Otto[1], C. Rosenberg[1].* 1) Genetics and Evolutionary Biology, Institute of Biosciences - University of São Paulo, Brazil; 2) Department of Neurology, Hospital das Clínicas, University of São Paulo, Brazil; 3) Genetics Unit, Department of Pediatrics, Instituto da Criança - University of São Paulo, Brazil.

Cognitive impairment is the most common effect of a chromosome abnormality, frequently associated to dysmorphic features and malformations. Molecular cytogenetics is a powerful tool to identify chromosome abnormalities and the resolution was greatly improved by the use of genomic micro-arrays. We applied 1 Mb whole-genome array-CGH (comparative genomic hybridization to arrays) screening to the study of 100 mentally impaired syndromic subjects. Extensive clinical and genetic investigations could not determine the cause of their abnormal phenotypes. They all had normal G-banded karyotypes. The objective of the study was to disclose submicroscopic genomic imbalances (and genes) causally related to mental retardation. Imbalances detected by array-CGH were confirmed by other methods (FISH or MLPA). Our data indicate that 33% of the group present alterations below the level of resolution of standard clinical cytogenetics. The imbalances that were either de novo or inherited from carriers of balanced rearrangements have been considered causative (22%). The imbalanced chromosome regions are strong candidates to harbor genes related to the specific phenotypes. On the other hand, imbalances not previously found in normal controls have been detected both in the affected patients and their phenotypically normal parents (11%). The significance of this DNA copy number variation is unclear. Our inability to distinguished between copy number changes that represent rare variants from those that are related to the phenotype is due to the lack of knowledge about the copy-number variability at the population level. Our results clearly indicate that array-CGH analysis greatly contributes to elucidate the causes of mental retardation of unknown etiology. The identification of imbalances in such families can lead to detection of carriers and has clear implications for genetic counseling.

1635/T

Efficacy of whole genome array analysis for detection of chromosome abnormalities: Toward validation of a quantitative SNP array. *A. Murmann, D. Conrad, H. Ho, R. Nicolae, C. Ober, S. Schwartz.* Dept Human Genetics, Univ Chicago, Chicago, IL.

Technologies to detect cytogenetic abnormalities have changed over the past three decades and continue to evolve. As new technologies are developed it is important to determine how effectively these technologies will delineate abnormalities. In this study we tested the efficacy of a quantitative SNP array for whole genome analysis of chromosomes by studying 85 patients with confirmed cytogenetic abnormalities (either balanced or unbalanced) and 15 chromosomally normal patients. A new algorithm for the quantitation of the SNP array data and individual patient results were compared with findings of 181 normal individuals. We have been able to confirm all chromosome imbalance previously know for the cases studied and have been able to better define the precise size of the deletion and/or duplication. Twenty four deletions and duplications have been detected with the array analyses that were not seen with high resolution chromosome analysis but could be confirmed by FISH. These abnormalities were as small as 100 kb, although most were between 300 kb and 5 Mb. Results from this study reveal important information including that: (1) the newly developed algorithm allows quantitative analysis of SNP array data that can be utilized to detect unbalanced chromosomal abnormalities not seen in routine chromosome studies; (2) Our new methodology has been introduced to help both in the detection of the abnormalities and in the controlling the background noise associated with these analyses; (3) The majority of these abnormalities would not have been detected by the currently available clinical arrays; (4) These findings are important not only for clinical studies, but for research studies involving both phenotype-karyotype correlations and mechanisms underlying the etiology of structural chromosomal abnormalities; (5) We have been able to identify many new copy number variations based on our studies; (6) While copy number variation is problematic in all array studies, we believe that these studies will allow the routine detection of pathogenic deletions and duplications greater the 500kb.

1636/T

Molecular cytogenetic characterization of cryptic chromosomal abnormalities in infants and children with Congenital anomalies. *S.K. Murthy[1], A.K. Malhotra[1], P.S. Jacob[1], S. Naveed[1], E.E. Al Rowaished[1], S. Mani[1], S. Padariyakam[1], S.A.H. Al Banna[1], A. Ridha[2], M.T. Al Ali[1].* 1) Molecular Cytogenetics unit, Genetics Department, Al Wasl Hospital, DOHMS, Dubai, U.A.E; 2) Department of Pathology, Dubai Hospital, DOHMS, Dubai, U.A.E.

Congenital anomalies contribute to a significant proportion of infant morbidity and mortality. It affects 3-7% of all live born (new born to five years of age). 10-15% of them are due to chromosomal reasons, which is one of the major cause of congenital anomalies. In Dubai, of the total 1246 referrals for cytogenetic testing during 2005 and 2006, 345 (27.68%) new born and pediatric children were referred for having multiple congenital anomalies(MCA) and/or dysmorphic features. Chromosome abnormalities were detected in 110/345 (31.88%) cases. Of all the abnormal cases, 82.73% were aneuploidies (trisomy 21, 13,18, Turner, Klinefelter) and the remaining 17.27% had unbalanced structural abnormalies . Majority of them were cryptic, which were identified and characterized by molecular cytogenetic techniques - fluorescence in situ hybridization (FISH) and microarray (oligo array-CGH). Such cryptic abnormalities are normally missed by the routine cytogenetic methods. Down syndrome and cryptic unbalanced chromosomal abnormalities are the major genetic causes of congenital anomalies in this referred population of Dubai. Significance of efficient genetic diagnosis, prenatal diagnostic services and genetic counseling is discussed.

1637/T

Parental mosaicism detected in a MCA/MR family using molecular cytogenetic analysis. *M.R. Nelen, M. Eleveld, P.A. Terhal, W.A. Harts, I. de Valk, M. Poot, P.F.R. Hochstenbach, J.K. Ploos van Amstel.* Dept Medical Genetics, UMC Utrecht, Utrecht, Netherlands.

Mosaicism is a major albeit uncertain determinant of recurrence risks in sporadic/isolated genomic diseases. Therefore, the genetic counselor is left with a difficult question when patients ask about the recurrence risk in case of a de novo variation. Here we describe a MCA/MR (multiple congenital anomalies associated with mental retardation) proband of which the mother shows mosaicism. A 6 year old MCA/MR patient was presented to us for Array-CGH analysis. Routine karyotyping did not reveal chromosomal abnormalities. Array-CGH analysis showed loss of signal intensity for BAC-clone RP11-190A12. The region involved, chromosome 1q23.2, is not known to contain neutral copy number variations. FISH analysis using an adjacent clone, RP11-10P13, confirmed the deletion in all cells analyzed. To determine whether the deletion had occurred de novo, interphase FISH on cells of both parents has been performed. The mother showed segmental aneuploidy for 1q23.2 in 29 out of 100 cells analyzed. Using Array-CGH the mother revealed a reduced signal intensity for clone RP11-190A12 but within the normal range. This reduction corresponds to the degree of somatic mosaicism as identified by FISH. Upon clinical examination the mother did not shown any MCA/MR manifestations. Molecular cytogenetic analysis has revealed that microdeletions / duplications in the human genome are a major cause of MCA/MR. Segmental aneuploidy in a sporadic patient with MCA/MR can be considered most probably causal for the phenotype when it has occurred de novo. However, the frequency of parental mosaicism for the de novo segmental aneuploidies has still to be elucidated. Follow-up studies such as interphase FISH as shown will however unambiguously identify parental mosaicism. Finally, mosaicism as cause of phenotypic variability and even MCA/MR needs further investigation. Using the current normal range in Array-CGH analysis, mosaics will however largely go unnoticed as possible cause of MCA/MR.

1638/T

Application of HR-CGH and Chromosomal Microarray Analysis (CMA) in the cohort of 117 patients with mental retardation. *B. Nowakowska[1,2], E. Bocian[1], P. Stankiewicz[1,2], M. Smyk[1], E. Obersztyn[1], Z. Ou[2], J. Li[2], K. Borg[1], S.W. Cheung[2], T. Mazurczak[1].* 1) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 2) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston TX, USA.

Advances in molecular cytogenetics enable detection of small chromosomal aberrations, undetectable by routine chromosome banding, in 5-20% of patients with mental retardation (MR). The aim of this study was to compare two genome-wide screening techniques, HR-CGH and targeted array CGH, termed Chromosomal Microarray Analysis (CMA). In contrast to conventional CGH, HR-CGH enables genome-wide screening for DNA copy-number changes with a 3-5 Mb resolution. CMA version 5.0 developed at Baylor College of Medicine (853 BAC/PAC clones) enables detection of DNA copy-number changes in more than 60 chromosomal regions of known diagnostic significance and in all subtelomeric regions in a single test. In this study, we analyzed 117 patients with unexplained MR and other features suggestive of chromosomal abnormality, with apparently normal or balanced karyotypes using HR-CGH (44 patients) and/or CMA (92 patients). HR-CGH detected seven interstitial deletions and one interstitial duplication (18,2%), among which two deletions, 16p11.2p12.2 and 8q21.11q21.2 were previously described in the literature only once and a 2q23 duplication is demonstrated herein for the first time. CMA revealed 20,7% (19/92) abnormalities, among which 11 (11.8%) were clinically relevant, 6 (6.5%) cases were interpreted as polymorphic variants and two (2.1%) were of uncertain significance. HR-CGH and CMA findings varied in size from 0.5 Mb to 12.9 Mb and were all validated by FISH. In summary, our results show that HR-CGH and array CGH techniques have high detection rates of genomic imbalances in the tested groups. Both methods have become important components in cytogenetic diagnostics, particularly for detecting cryptic constitutional chromosome imbalances in patients with MR, in whom the underlying genetic defect is unknown.

1639/T

Comparative genomic hybridization in clinical evaluation of stillbirth. *G. Raca[1,2], J.S. Lafin[1], P. Modaff[3], K.D. Montgomery[1,2], R.M. Pauli[3].* 1) UW Cytogenetics Service, State Laboratory of Hygiene, Madison, WI; 2) Department of Pathology, University of Wisconsin, Madison; 3) Wisconsin Stillbirth Service Program (WiSSP), Clinical Genetics Center, UW, Madison.

Even after careful clinical evaluation and autopsy, the etiology of late gestation pregnancy loss (stillbirth) remains unexplained in up to 60% of cases. Stillborn fetuses represent a diagnostic challenge, since dysmorphic features and even major anomalies can be concealed by maceration. Additionally, cytogenetic results are not obtained in more than 50% of cases due to failure of in vitro tissue growth. To ascertain the feasibility of using array based comparative genomic hybridization (aCGH) in diagnostic evaluation of stillbirth, we tested five frozen tissue samples from cytogenetically characterized stillborn infants, obtained through the Wisconsin Stillbirth Service Program (WiSSP). Samples were tested in a blinded fashion using commercially available Constitutional Chip 3.0 BAC Arrays (PerkinElmer). In four cases findings were completely consistent with chromosome analysis and included 2 normal cases, trisomy 13 and trisomy 21. In the fifth case aCGH provided greater precision in defining the cytogenetic aberration by showing gain of ~20Mb region from 10p and loss of ~6Mb region from 18p. While initial cytogenetic evaluation detected presence of the derivative chromosome 18 with 10p material translocated to 18p, loss of 18p material was not identified by karyotyping. Clinical features in this infant, miscarried at 19 week gestation, included intrauterine growth retardation, microcephaly, encephalocele and cleft palate, which are consistent with but more severe than what might be anticipated from the combination of the two cytogenetic aberrations. We suggest that aCGH analysis should be considered instead of or in addition to karyotyping for routine diagnostic evaluation of intrauterine death. aCGH could also be a valuable research tool to examine the role of subtle, submicroscopic deletions and duplications in the etiology of intrauterine death, and to identify candidate chromosomal regions that are critically involved in survival through late gestation.

1640/T

Identification of abnormalities in subtelomeric regions by microarray analysis: A study of 5380 cases. *L. Shao, C.A. Shaw, X. Lu, A. Patel, T. Sahoo, C.A. Bacino, S. Lalani, P. Stankiewicz, A.C. Chinault, A.L. Beaudet, J.R. Lupski, S.W. Cheung.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Subtelomeric imbalances are a major cause of congenital disorders. Screening for these abnormalities has utilized GTG-banding analysis and subtelomeric FISH assays. Array CGH is a relatively new technology that can identify microscopic and submicroscopic chromosomal imbalances. Chromosome Microarray Verison 5 (CMA V5) has 853 BAC clones (www.bcm.edu/cma/table.htm, Chip Map V5.0), of which subtelomeric clones constitute a significant fraction (481/853) with an average coverage of 12 clones /10 Mb /region. CMA Version 6 (CMA V6) has 1475 BAC clones and has similar coverage at subtelomeric regions as V5 (www.bcm.edu/cma/table.htm, Chip Map V6.0). We screened 4493 consecutive clinical cases using CMA V5 and 887 cases using CMA V6 and found copy number changes in 591 patients (detection rate of 10.99%), among which pathogenic rearrangements were observed in 238 patients (4.4% of total). Among these patients, 94 had a deletion and 55 had a duplication with a size ≤ 9 Mb, 11 had a deletion and 5 had a duplication > 9 Mb, 44 had unbalanced translocations, and 19 had complex rearrangements. Almost half of the deletions (45/94) and the majority of the duplications (48/55) were ≤ 3 Mb in size. Interstitial rearrangements that would be missed by subtelomeric FISH occurred in 37/94 deletions and 44/55 duplications. In patients with known karyotype or FISH results, 37 deletions out of 70 and 31 duplications out of 35 evaded detection by karyotype and/or FISH. Deletions of 1p36.3, 22q13.3, 4p16.3, and duplications of Xq28 were the most common submicroscopic alterations. In conclusion, subtelomeric genomic imbalances contribute significantly to congenital disorders and array CGH enables greater detection rates when compared to subtelomeric FISH (4.4% versus 2.6% in Ravnan's study of 11,688 patients). Targeted array CGH with dense coverage on subtelomeric region is a vital diagnostic tool for identifying subtelomeric imbalances, especially for submicroscopic and interstitial imbalances.

1641/T

Detection of constitutional genomic imbalances with the Affymetrix GeneChip Human Mapping 250K Nsp array: Data analysis using three software packages. *I. Simonic, L. Willatt.* Medical Genetics Department, Cambridge University Teaching Hospital NHS Trust, Hills Road, Cambridge CB2 2QQ, United Kingdom.

Genotyping SNP arrays are widely used in genome-wide association studies, homozygosity mapping of rare autosomal recessive disorders, and for loss of heterozygosity (LOH) studies in malignancies. Several recent studies have shown that whole-genome SNP arrays can also be used to identify submicroscopic DNA copy number changes. We studied 50 patients with developmental delay and/or facial dysmorpism/congenital malformations using the Affymetrix GeneChip Human Mapping 250K_Nsp array and used three different software packages to analyse the results for copy number changes. Firstly we analysed all the array data with CNAG software. CNAG uses SNP genotypes generated by a DM algorithm for detection of copy number changes and allows multiple patient displays in a single window. Rapid visualisation of abnormalities, exclusion of common copy number variants and batch related array artefacts was possible. We then re-analysed all the array data with the Affymetrix CNAT and the IdeogramBrowser software packages using SNP genotypes generated by the BRLMM algorithm for copy number analysis. We concluded that CNAT was not suitable for data analysis in our diagnostic laboratory as the analysis was extremely time consuming, with a large number of false positive calls that were strongly correlated with the test sample genotype call rates. IdeogramBrowser detected all the abnormalities identified by CNAG, however the displays, particularly for some of the large abnormalities (>3Mb in size), differed significantly between CNAG and IdeogramBrowser. Furthermore, IdeogramBrowser using the analysis parameter of a minimum of 3 consecutive SNPs, identified several additional copy number variants of <0.5Mb not detected using the other software packages. All copy number changes were followed up by FISH and the potential clinical significance of these abnormalities will be presented.

1642/T

EXPANDED VERSION OF TARGETED CHROMOSOMAL MICROARRAY ANALYSIS IMPROVES DIAGNOSTIC POTENTIAL. *C. Soler-Alfonso, C.A. Shaw, A. Patel, T. Sahoo, S. Lalani, C.A. Bacino, S.H.L. Kang, A.C. Chinault, A.L. Beaudet, J.R. Lupski, S. Neill, A.N. Pursley, P.A. Ward, S.W. Cheung.* Deparment Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Chromosomal Microarray Analysis (CMA) using array comparative genomic hybridization (array-CGH) increases diagnostic capability and efficiency in detection of genomic imbalances. Previously, we reported our experience in 2513 postnatal cases using two consecutive versions, CMA V4 and CMA V5 which showed improved detection rates of 7.6% and 8.9%, respectively. Here, we report our experience with the clinical use of an expanded array, CMA V6, containing 1475 BAC clones covering greater than 150 known genomic disorders and all clinically relevant pericentromeric and subtelomeric regions. CMA V6 studies were performed in 1923 postnatal samples with a range of indications including mental retardation, autism, seizures, multiple congenital anomalies and dysmorphic features. CMA V6 identified clinically relevant genomic imbalances in 12.7% (245/1923) of cases. In cases with previous normal chromosome analysis, 11% (53/479) had abnormalities detected by CMA V6, highlighting the improved resolution of this technology. Copy number variations (CNVs) of unknown clinical significance were identified in 13.3% (256/1923) of the cases. To date, parental studies have been performed for 126 families; contributing to define 73.8% (93/126) as familial variants, and 26.2% (33/126) as de novo events. The clinical significance of these de novo changes awaits further investigation. Our findings demonstrate that array-CGH using CMA V6 has a higher sensitivity and detection rate for genomic imbalances in comparison to routine cytogenetic techniques as well as our previous clinical arrays. We have recently transitioned from using the BAC array platform to a custom oligonucleotide array referred to as CMA V6 OLIGO. This array contains an average of 30 oligonucleotides spanning each of the BAC clones on CMA V6 providing comparable genomic coverage at a higher resolution level. Our clinical experience with these two versions (CMA V6 and CMA V6 OLIGO) will be presented.

1643/T

Use of targeted array-based CGH for the diagnosis of chromosomal imbalance in polymalformed syndrome patients with apparently balanced karyotype. *A.C. Tabet[1], E. Pipiras[2], A. Delahaye[2], S. Kanafani[2], A. Aboura[1], C. Dupont[1,2], M. Uzan[4], J.F. Oury[3], B. Benzacken[1,2].* 1) UF de Cytogenetique, Hopital Robert Debre , Paris, France; 2) UF de Cytogenetique, Hopital Jean Verdier , Bondy, France; 3) Service d'Obstetrique, Hopital Robert Debre , Paris, France; 4) Service d'Obstetrique, Hopital Jean Verdier , Bondy, France.

In prenatal diagnosis as in pediatrics, some polymalformed patients with suspicion of chromosomal abnormalities have normal standard karyotype. High-resolution comparative genomic hybridization (CGH) based microarrays (array CGH) were developed to increase the resolution of chromosomal studies and to provide a comprehensive assay by using large-insert clones as the target for analysis.We propose to use this technology to better understand the pathology in few patients and fetuses with polymalformed syndrome and normal karyotype.We used a DNA microarrays (Integragen) with 3172 clones providing an average of 1 Mb resolution.

In 3 cases, this technology allowed us to correlate the abnormal phenotype with an imbalance chromosomal abnormality.In the first case, a newborn was referred for facial dysmorphy, cardiopathy and short arm segments. Her lymphocytes karyotype was normal since cultured fibroblasts failed. We performed CGH array on DNA extracted from a post-mortem pulmonary biopsy and identified a tetrasomy 12p corresponding to a Pallister Killian syndrome. In the second case, the fetus presented a complex cardiopathy with cerebral malformations. By CGH array, we report an unexpected additional deletion of 7qter in an inherited apparently balanced reciprocal translocation t(7;10)(q11.23;p14)mat. In the third case, the prenatal karyotype of a fetus with holoprosencephaly showed a *de novo* apparently balanced reciprocal translocation t(7;8)(q31.3;q12) and the CGH array identified an additional deletion in the region of the Sonic Hedgehog gene .

As a conclusion, we confirmed the feasibility and the usefulness of CGH array in constitutional cytogenetic. Use of array CGH should increase the detection of chromosomal abnormalities in polymalformed fetuses with apparently balanced chromosomal anomalies.

1644/T

Validation of single nucleotide polymorphism (SNP) array technique in 31 probands with chromosomal anomalies. *G.H. Thomas[1,2], E. Squibb[1], E. Lisi[2], A. Hamosh[2], K. Doheny[2], K. Hetrick[2], D. Valle[2], J. Pevsner[1], J.E. Hoover-Fong[2].* 1) Kennedy Krieger Institute, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Baltimore, MD.

Objective: Our objectives were to 1: evaluate whole genome SNP genotyping to detect and define chromosomal abnormalities diagnosed by G-band karyotype or fluorescence in situ hybridization (FISH), and 2: determine the frequency and significance of previously undetected copy number variants (CNVs) in these subjects. Methods: Amplified gDNA from 60 individuals was hybridized to the Illumina HumanHap550 SNP genotyping array platform (~1 SNP/5kb). 31 probands with known chromosomal anomalies (i.e. deletion=20, duplication=4, complex deletion/duplication=2, unbalanced translocation=3, ring=1, aneuploidy=1) were analyzed. 29 relatives (26 parents, 2 children, and 1 sibling) of 17 probands were also studied. Results: We identified all previously detected cytogenetic anomalies in the 31 probands. The SNP analysis more precisely defined the location and nature (i.e. presence/absence of low copy repeats) of the breakpoints involved in these alterations. Compared to their familial proband, 23 of 29 relatives did not have the same SNP anomaly, while 6 of 29 (3 parents, 2 children, 1 sibling) did. A large number of previously undetected CNVs were also identified in all 60 subjects. Conclusion: The Illumina HumanHap550 SNP array platform had 100% sensitivity to detect all previously recognized chromosomal anomalies in these 31 probands. Many additional CNVs of uncertain significance were also detected in all subjects, including phenotypically normal relatives. Consideration of parental SNP array results and phenotype with that of their dysmorphic/mentally retarded child is essential to determine which de novo CNVs are potentially related to the proband's phenotype. As SNP array resolution continues to increase and this technology becomes standard of care in the clinical setting, we must be cautious and diligent in our interpretation of the meaning of CNVs and their potential role in the continuum between disease and normal variation.

1645/T

Discrepancies of the results between MLPA and FISH, and between MLPA kits, observed in a case of subtelomeric imbalances of chromosome 12. *K. Wakui[1,2], Y. Kinishita[1], Y. Furui[3], K. Shinogi[3], T. Fukui[3], R. Kawamura[1], N. Gondo[3], S. Yokoyama[3], H. Higashi[3], Y. Fukushima[1,2].* 1) Dept Med Genet, Shinshu Univ Sch Med, Matsumoto, Japan; 2) Div Clinical and Mol Genet, Shinshu Univ Hosp, Matsumoto, Japan; 3) Biomedical Business Div. FALCO biosystems Ltd., Kyoto, Japan.

Multiplex Ligation-dependent Probe Amplification (MLPA) has come into wide use for subtelomeric screening as a new molecular cytogenetic technique. We analyzed subjects with known subtelomeric imbalances using 2 kinds of MLPA® Kits (SALSA MLPA KIT HUMAN TELOMER, P036B and P070, MRC-Holland), and evaluated the usefulness and limitations of this method compared with subtelomeric metaphase FISH analyses. Of these, some of the results showed discrepancies between MLPA and FISH, and/or between 2 kinds of MLPA kits. We report here one of such cases; a subtelomeric imbalances of chromosome 12. A case was recognized having add(12p) by initial G-banding. The 24 color FISH analysis showed that the entire der(12) was derived from chromosome 12. Subtelomeric FISH analysis performed in 2000 showed that the signals of 12qter were detected at the both chromosome ends of the der(12), and the signals of 12pter were retained at the breakpoint of the der(12). However, we detected not only gain of 12q but also loss of 12pter by MLPA analysis using P036B kit, although only gain of 12q was detected by P070 kit. We re-analyzed this case by FISH using TelVysion 12p/12q FISH Probe (Vysis), and found that the signals of 12qter was detected at the both chromosome ends of the der(12) as well, but the signals of 12pter was not detected around the breakpoint of the der(12). The target gene of the 12pter probe is *SLC6A12* (170kb distance from the 12p telomere) in P036B kit, and *RBBP2* (290kb distance) in P070 kit. The FISH probe for 12pter which we used in 2000 was 90I5/PAC (estimated about 700kb distance), and sAVH27 (90kb distance) was used as TelVysion 12pter probe. Thus, this case was re-diagnosed as having a 12q partial trisomy with 12p subtle deletion (170-290kb in size). Advance in cytogenetic testing is continuous. We always need to consider the resolution of each technique at the cytogenetic diagnosis of the structual chromosomal abnormality.

1646/T

High resolution long oligo based array CGH for clinical diagnostics: whole genome array versus targeted array. *B. Wu[1,3], Y. Shen[1,2], D. Miller[1,3], V. Lip[1], X. Sheng[1], K. Tomaszewicz[1], H. Shao[1], H. Fang[1], H. Tang[1], M. Irons[1,3], C. Walsh[1,3,4], O. Platt[1,3], J. Gusella[2,3].* 1) Lab Med & Path, Children's Hosp, Harvard Medical Sch, Boston, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, 02114; 3) Harvard Medical School, Boston, MA; 4) Howard Hughes Medical Institute.

Array CGH is now an indispensable clinical diagnostic tool for patients with genetic disorders associated with genomic imbalances. The clinical utility varies depending on the chip platform used for testing. We adopted a commercially available high resolution oligonucleotide array (Agilent 244K aCGH array) for detection of genomic imbalance events in both targeted regions that correspond to commercially available targeted arrays and across the whole genome. Clinical interpretation of arrays with whole genome coverage is complicated by detection of copy number variants (CNVs). We addressed this concern through a combined approach of dataset filtering, size cutoffs, examination of gene content, annotated database search, and general literature search. We offered parental testing free of charge to evaluate previously unreported CNVs. We tested 323 samples on with this comprehensive approach. We found 37 (11.5%) clinically relevant imbalance events in 323 samples. Seventeen of 37 samples are located outside the stated coverage of commercially available targeted arrays (equivalent to Signature v4 and Baylor v6). We detected many imbalance events below 500kb, including novel copy number variants and clinically relevant microdeletions/duplications, that would not be identified by an array based on large insert clones. Due to the redundancy of probe coverage, dye-swap confirmation was often not necessary for large regions of imbalance (>500Kb). Within these large regions, subsequent FISH was 100% concordant with array CGH data. We believe the high resolution nucleotide array transcends the traditional concept of array CGH as an alternative to multiplex FISH. With careful interpretation, the whole genome array is highly valuable in the clinical detection of unsuspected genomic imbalance events among patients with unexplained developmental defect.

1647/T

Duplication of 16q22.3qter/13.6 Mb DNA characterized by G-banding, FISH, SKY and array CGH. *J. Xu[1], B. Hamilton[1], V.M. Siu[2].* 1) Cytogenetics; 2) Medical Genetics, London Health Sciences Centre and University of Western Ontario, Canada.

A 6-year-old girl presented with developmental delay, mild dysmorphism and extreme anxiety. G-banding showed additional material of unknown origin attached to a distal 3q. Telomere FISH and SKY identified the addition as being a translocation of 16q22.3qter onto 3q. The patient's karyotype is 46,XX,der(3)t(3;16)(q29;q22.3).ish der(3)(wcp16+,3qter+,16qter+). The mother had a normal female karyotype and the father was not available for study. Genomic DNA was extracted from 11-month old fixed cell pellet leftover from the original cytogenetic investigations. Array CGH analysis using CytoChip (BlueGnome) at 870 kb resolution with 3550 BACs showed duplication of 14 clones covering 13.6 Mb (genomic position: 74852437.50-88532307.50) in the 16q. In addition, the array identified deletion of 2 clones of 71.6 kb (genomic position: 2638138-2719693) at 6p22.1. The presence of 3qter probe in the der(3) and the results of CGH analysis indicate that there is very little deletion of distal 3q material, making this a "pure" dup(16)(q22.3qter). Six cases of dup 16q22qter have been reported in literature; all had another chromosomal rearrangement. This is probably the first case of "pure" dup 16q22.3qter. Similar findings to a dup 16q case with der(Y)t(Y;16)(q12;q22) (Clin Dysmorphol 2005,14:177-81) include developmental delay, speech delay and dysmorphism. More case reports are needed to establish a phenotype of dup 16q22.3qter. This study further demonstrates that CGH array can assist in the molecular definition (size and genomic position) of cytogenetic rearrangements and detect submicroscopic aberrations. As well, our data suggest that fixed cytogenetics cell pellets (up to 11 months in our lab) may be used for array CGH analysis.

1648/T

Custom design and validation of an oligonucleotide microarray combining whole genome and targeted strategies for clinical cytogenetics. *E.L. Baldwin, J. Lee, D. Blake, B. Bunke, C. Alexander, A. Kogan, J. Hauenstein, D.H. Ledbetter, C.L. Martin.* Dept Human Genetics, Emory Univ, Atlanta, GA.

Array Comparative Genomic Hybridization (aCGH) has rapidly become an integral part of cytogenetic diagnostics. We report the design, validation, and clinical utility of a custom oligonucleotide array which combines whole-genome coverage at a high-resolution (equivalent to a 6,000 band karyotype) with enhanced coverage at clinically relevant regions, including telomeres, centromeres and the common microdeletion/duplication regions. Individual probes were placed every 75 kb across the entire euchromatic genome to establish a chromosomal backbone. Although the 75 kb spacing allows detection of imbalances of ~300 kb, we have chosen a limit of 500 kb to decrease the identification of benign copy number variants, ~95% of which are <500 kb in size. Thirty patient samples were tested on the array as part of the validation study. These cases included normal samples and cases with trisomy 21, sex chromosome abnormalities, telomere imbalances, unbalanced translocations, microdeletions and duplications. For all 30 samples, the array results were consistent with previous FISH and/or karyotype findings, validating the array for clinical detection of copy number imbalances greater than 500 kb. In addition, we have carried out prospective clinical analysis of 75 samples using this custom array. Of these 75 cases, 61 were found to have normal array results, while 14 contained various abnormalities. Nine of the abnormal cases were submitted for array studies to further define an abnormality originally detected by G-banding. Five of the abnormal samples, however, had previously been reported as normal by G-banding. Three of these cases (two deletions of 15q11-13 and a 4.4 Mb 21q telomere deletion) would have also been detected with targeted arrays which have limited coverage for certain regions of the genome. However, two imbalances, a ~3 Mb 2p interstitial deletion and a ~9 Mb 2q interstitial duplication, would only be identified with whole-genome coverage. Our early results highlight the diagnostic utility of this custom array design for detecting clinically relevant cytogenetic imbalances.

1649/T

A novel 3.4 Mb deletion at Xq22.2-Xq22.3 including PLP1 detected by oligonucleotide based array-CGH. *S.E. Im[1], E.J. Seo[1,2,3], J.O. Lee[3], K.J. Kim[3], T.S. Ko[4], J.K. Ko[4], H.W. Yoo[3,4], I.S. Park[3,4].* 1) Medical Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center; 3) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center; 4) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Duplication of the proteolipid protein gene (PLP1) located on Xq22.2 is a major mutation for Pelizaeus-Merzbacher disease (PMD). Whereas deletions of less than 0.5 Mb containing PLP1 have been observed infrequently in patients with dysmyelination and spasticity. An Xq22.3 duplication has been reported as a FGS5 locus for FG syndrome. We present a male infant with a de novo 3.4 Mb interstitial deletion of the long arm of chromosome X. The patient, a 5-month-old Korean boy, showed ventricular septal defect, pulmonary stenosis, dextrocardia, agenesis of the corpus callosum, blindness, micropenis, undescended testis and dysmorphism. Karyotype was found to be normal. The 244k oligonucleotide based array-CGH (Agilent) analysis could define a novel 3.4 Mb deletion at Xq22.2-Xq22.3 including RAB40A, TCEAL4, TCEAL1, MORF4L2, TMEM31, PLP1, RAB9B, MCART6, ESX1, IL1RAPL2, NRK, SERPINA7, MUM1L1, RNF128, CLDN2, and several open reading frames. Some genes located in the deleted region may be possible candidates for patient's phenotype. ESX1 is involved in the embryonic development of head, thorax and abdomen. RAB40A, TCEAL1, PLP1, and RNF128 are highly expressed in brain, and NRK in heart predominantly. We now propose a new contiguous gene deletion syndrome in Xq22.2-Xq22.3 characterized by PMD, congenital heart defect, agenesis of corpus callosum and other anomalies.

1650/T

Use of High Density Oligo Array CGH to Characterize Chromosomal Aberrations Associated with Unexplained Clinical Presentations. *M.M. Li[1, 2, 3], X. Hu[3], T. Narumanchi[1,2], C. Dvorak[1,2], D. Mercer[1], G. Pridjian[1,2], H. Andersson[1,2].* 1) Hayward Genetics Ctr;; 2) Dept. of Pediatrics, Tulane Univ. Sch. Med; 3) Louisiana Cancer Research Consortium, New Orleans, LA.

Many chromosomal aberrations identified through conventional cytogenetic studies do not completely explain patients' phenotype. Cryptic copy number variations (CNVs) have been suggested to be responsible for the discrepancies. We have recently used high density oligo array CGH (aCGH) to characterize a series of cytogenetic aberrations associated with dissonant phenotypic presentations. A patient with a balanced t(15;22) showed developmental delay and growth defects. aCGH identified a 3.3 Mb deletion adjacent to the chromosome 15 breakpoint. A young girl with Williams syndrome exhibited severe developmental delay, absence of speech, and an inability to crawl or walk. aCGH revealed a 4 Mb deletion including the Williams critical region. A newborn diagnosed prenatally to have a 4q- displayed Pierre Robin sequence, facial and digital anomalies, and undescended testes. aCGH uncovered that the "4q-" was in fact an der(4)t(3;4)(q27.2;q32.2). Two twin brothers were identified as carrying a r(9)(p22.3q34.3). However, the phenotype of the twins resembled 9p deletion syndrome: craniosynostosis, thick eyebrows and synophrys, cleft palate, and gastroesophageal reflux. aCGH revealed a 14.5 Mb deletion on the short arm of the ring and no deletion of the long arm. We also studied a der(20)t(16;20)(q22.3;p13) in a patient with midface hypoplasia, gastroesphageal reflux, and hearing and vision impairments. Using aCGH we demonstrated a 16 Mb duplication of 16q and no copy number change on the 20p, showing that the phenotype of this patient was actually the result of partial trisomy 16q. Our experiences have demonstrated that high density aCGH is far superior to conventional cytogenetics and FISH in the diagnosis and phenotype/genotype correlations of multiple congenital anomalies, developmental delay, and mental retardation. With continuously increasing array density and decreasing array cost, high density whole genome aCGH will soon become the primary and preferred method for the diagnosis of genomic CNVs.

1651/T

Short Telomeres in a case of acrogeria. *R. Drouin, O. Samassekou.* Service of Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, Universite de Sherbrooke, Sherbrooke, PQ, Canada.

Telomeres play a major role in the biology of cancer and aging. Changes in telomere homeostasis during premature aging syndromes, specifically the reduction of their length, have been reported. We report the case of 4 years old girl, first child of her family (she has a younger brother), who presented the following clinical manifestations: The facial features include micrognathia, alopecia, and prominent scalp veins; a short stature; growth delay; and thin and wrinkled skin. Besides these morphological abnormalities, she had an atrium septal defect. A diagnosis of acrogeria was proposed. Blood samples and skin biopsies were obtained from each member of the family. Regular blood and fibroblast cultures were set up to perform cytogenetic studies. Conventional cytogenetic studies and FISH (fluorescence in situ hybridization) using subtelomeric probes were performed on the lymphocyte culture and no chromosomal abnormalities were found. The same results were obtained for her parents and brother. The quantitative FISH technique was carried out on the metaphases of each member of her family. Ten metaphases of each individual were analyzed by measuring the signal intensity on each arm of the chromosomes. This signal intensity is proportional to the telomere length. The Q-FISH analysis showed a dramatically reduced telomere length of the patient when compared to her brother or even to her parents. Compared to all other members of her family, when analyzing each telomere length individually, the pattern of telomere length distribution was different. The Telomere Restriction Fragment (TRF) analysis, which assessed the average telomere length of the whole genome, confirmed the short telomere length observed by the Q-FISH. The sequencing of LMNA (lamin A) gene did not revealed any mutations, excluding the progeria diagnosis. Fibroblasts of all members of the family are cultured and the telomere length is assessed at every two or three passages. Further molecular studies will be needed to identify the gene responsible for this disease.

1652/T

Exploring cryptic genomic aberrations related to multiple congenital anomaly with mental retardation using in-house CGH-arrays. *S. Hayashi[1,2], S. Honda[1,2], I. Issei[1,2], J. Inazawa[1,2].* 1) Dept Molecular Cytogenetics, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan.

We have constructed several types of bacterial artificial chromosome (BAC)-based CGH-arrays and screened genomic aberrations in patients with multiple congenital anomalies with mental retardation (MCA/MR) including congenital disorders. Our purpose is not only to explore copy-number aberrations (CNAs) responsible for such disorders but also to establish a system for diagnosis of MCA/MR using array-CGH. For primary screening of unknown MCA/MR cases without cytogenetic abnormalities in conventional karyotyping, we used MCG Genome Disorder (GD) Array containing BAC clones covering loci associated with known genomic disorders and subtelomeric regions of all chromosomes except short arms of acrocentric chromosomes, and detected CNAs in 24 of 244 cases (9.8%). In cases without any CNAs by GD Array, we next employ MCG Whole Genome (WG) Array, which harbors 4523 BACs throughout human genome, and detected CNA in 10 of 35 cases (28.6%). In addition to this cohort, we have performed array-CGH in MCA/MR cases and other congenital disorders without known causative genes, and identified CNA in 43 of 118 cases (36.4%). In a patient with Norrie disease with atypical characteristic, for example, cryptic deletion involving genes which would explain their phenotypes was detected. In MCA/MR patient with congenital sclerocornea glaucoma and syndactyly cryptic deletion including a candidate causative gene was detected. During these analyses, we also created a database accumulating phenotypes of congenital disorders we have analyzed to identify new disease entries with common features and their correlation with specific genomic abnormalities. In conclusion, array-CGH provides a useful strategy to investigate cryptic genomic aberrations responsible for unknown MCA/MR, and/or to establish a new syndrome.

1653/T

Oligo Fluorescence in situ hybridization (Oligo-FISH), a new strategy for enumerating chromosomes in interphase nuclei. *J. Aurich-Costa, P. Keenan, L. Zamechek, S. Bradley.* Research and Development, One Cell Systems/Cellay, Cambridge, MA.

Objectives: Development of FISH probes using labeled oligonucleotides (ODNs) for 5 color chromosome enumeration in interphase nuclei. 10-20 ODN for chromosomes X, 15, 17 and 20 α-satellite repeats and for chromosome Y alpha 3 repeat were designed in regions where the satellite 3 pentamer or the α-satellite consensus sequence was underrepresented, 5' end labeled, and tested individually on human metaphases from 5 chromosomally normal individuals. Only ODNs exhibiting signal specific for the target region were selected for each chromosome cocktail. For each chromosome, ODNs were mixed together and hybridized on human cells and signal to noise ratio (S/N), sensitivity and specificity were assessed according to the Standards and Guidelines for Clinical Genetic Laboratories of the American College of Medical Genetics. Only probes exhibiting S/N >2 calculated on 30 interphase nuclei, and sensitivity and specificity ≥98% were included in each chromosome specific cocktail. Next, we selected 5 fluors that could be simultaneously assessed using epifluorescence. To rank fluor intensity, chromosome Y probe was labeled with all 5 fluors, and S/N was assessed. S/N obtained for each probe labeled with A568, was inversely matched with the ranking of the fluors. Finally, all the probes were mixed together, and varying hybridization times were tested until the shortest time giving the same S/N was found.

Results: We designed specific ODNs for 5 chromosomes. After probes were labeled with the 5 fluors and combined, all probes exhibited S/N >2. Hybridization time was determined at < 1hr.

Conclusions: FISH ODN probes' short length permits rapid hybridization, a significant advantage for time critical procedures such as enumeration of chromosome in interphase nuclei for preimplantation genetic diagnostics. Due to the oligo size, hybridization time was < 1hr, a significant advantage for FISH.

Support: NIH grant: 1R43 HD052597-01.

1654/T

FISH confirmation of array-detected microduplications: an assessment of discrepancies with real-time qPCR findings. *N. Riendeau[1], C. Harvard[2], Y. Qiao[2], X. Liu[3], J.J.A. Holden[3], S. Lewis[1], E. Rajcan-Separovic[2].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; 3) Department of Psychiatry and Physiology, Queen's University, Kingston, ON, Canada.

Microarray comparative genomic hybridization, or array-CGH, can identify microdeletions and microduplications at the whole genome level in individuals affected with a variety of disorders. In addition to detecting clinically relevant changes, array-CGH reveals a large number of apparently benign copy number variants (CNVs), which are then catalogued in a public database (http://projects.tcag.ca/variation/). Interpretation of CNVs can be challenging as very few of the >6000 normal CNVs have been confirmed using independent methods. In our study of subjects with Autism Spectrum Disorders (ASD), we aim to confirm all new CNVs by real-time quantitative PCR (RT-qPCR) and/or FISH. Here we describe the assessment of 7 microduplications using both methods and discuss the reasons for discrepant findings. Briefly, 3/7 array-detected microduplications had been confirmed by RT-qPCR (clones RP11-808H17, RP11-105C15 and RP11-91A13) and 4 had not (clones RP11-2L22, RP11-212I21, RP11-278A4 and RP11-366M24). FISH results were concordant with RT-qPCR results for all clones. Using FISH we could explain the discrepancy for 2/4 discrepant clones (RP11-2L22 and RP11-212I21) as they hybridized to two pairs of homologous chromosomes. For the remaining 2/4 discrepant clones, FISH was not suggestive of a microduplication and we will provide possible explanations for the array vs RT-qPCR and FISH discrepancy. Our findings suggest that all novel CNVs need to be confirmed, sometimes using two independent methods, to select those that are potentially clinically relevant. Ideally, all CNVs already listed as normal variants should also be confirmed, to minimize the possibility that clinically relevant changes are discarded as previously reported normal copy number variants, when some may be artifacts.

1655/T

FISH in Suspension Applied to Interphase Aneuploidy and Metaphase Quantification. *X. Wu[1,2], L.M. Whelchel[1], ZH. Chen[3], J.N. Lucas[1].* 1) ChromoTrax, Inc, Frederick, MD; 2) Union Hospital, Huazhong University of Science and Technology, Wuhan, Hubei, China; 3) University of Utah, Salt Lake City, UT.

The detection sensitivity of classical fluorescence in situ hybridization (FISH) performed on slides is 1 in 100-1000 cells. PCR is very sensitive, but lack of very accurate quantification still is a problem. By contrast, our innovative approach will greatly facilitate accurate and quantitative detection of chromosomal aberrations. It will significantly increase the speed of analysis and number of samples that can be analyzed. Our techniques include a novel approach of hybridizing chromosomes in suspension with fluorescently-labeled DNA probes in combination with flow cytometric analysis, in order to sensitively, precisely and rapidly quantify chromosomal abnormalities. Firstly, we demonstrated the efficacy of our approach for interphase cell analysis by detecting aneuploidy in trisomy 21 human cells with a DNA probe specific for chromosome 21q. For direct comparison, FISH in suspension and FISH on slides were performed simultaneously on cells from four normal controls and four patients with Down syndrome. In the normal group, the average percentages of cells with two signals between FISH in suspension and on slides were 75.4% vs. 74.5%, and in the trisomy 21 group the average percentages of cells with three signals between FISH in suspension and on slides were 73.1% vs. 73.6%. These findings strongly indicate that our interphase FISH in suspension approach is able to generate similar efficacy as standard FISH in detecting numerical chromosome abnormalities. Secondly, we demonstrated that flow cytometry could be used to detect and analyze FISH signals on chromosomes hybridized in suspension. We measured the frequency of chromosome 1 using flow after FISH in suspension and determined the detection sensitivity using serial dilution. The sensitivity was measured to be within one in 10,000. These experiments demonstrate: (1) The hybridized signals are sufficiently bright for flow detection; (2) Chromosome breakage during flow is not a problem. We therefore expect our method will be valuable to flow cytometry in medical genetic.

1656/T

CGH array analysis of a cohort of patients with mental retardation reveals imbalances in at least 20% of the cases and suggests new candidate genes. *S. Jaillard[1,2], C. Dubourg[1,3], L. Pasquier[4], C. Bendavid[1], C. de La Rochebrochard[4], D. Bonneau[5], A. Guichet[6], H. Journel[7], B. Gilbert-Dussardier[8], A. Toutain[9], A. David[10], D. Martin[11], P. Parent[12], J. Mosser[1], V. David[1,3], S. Odent[1,4].* 1) UMR 6061, IFR 140 GFAS, Faculty of Medicine, Rennes, France; 2) Cytogenetics, CHU, Rennes, France; 3) Molecular Genetics, CHU, Rennes, France; 4) Medical Genetics, CHU, Rennes, France; 5) Medical Genetics, CHU, Angers, France; 6) Cytogenetics, CHU, Angers, France; 7) Medical Genetics, CHBA, Vannes, France; 8) Medical Genetics, CHU, Poitiers, France; 9) Medical Genetics, CHU, Tours, France; 10) Medical Genetics, CHU, Nantes, France; 11) Medical Genetics, CH, Le Mans, France; 12) Medical Genetics, CHU, Brest, France.

Mental retardation (MR) affects 1 to 3% of the general population. It is described by impaired intelligence and function in adaptative skills, before age 18. Its etiology is very heterogeneous: numerous genetic and environmental causes have been reported, but the molecular bases remain undetermined in half of MR patients. We initiated an array CGH study using high performance Agilent arrays 4x44K, to detect microrearrangements that could not be observed by standard karyotyping and subtelomeric FISH. Clinically relevant copy number abnormalities (from 210 kb to 12.5 Mb) were identified in at least 20% of the 65 studied patients: 11 deletions and 6 duplications. Novel microdeletions in 2q and in 17q were detected, and fit with new microdeletional syndromes characterized by dysmorphic features, developmental delay, hypotonia. These regions include candidate genes for RM. In the same way, a microdeletion in 17p11 was observed in a RM patient with a muscular hypertrophy. This CGH array technology also allowed us to detect a rearrangement of the chromosome 18 and to suspect a mosaicism, which was then confirmed by FISH showing an isodicentric chromosome 18 in 5% of the cells. Our results indicate that the diagnostic yield of CGH array approach in the general population of patients with MR is at least twice higher than standard karyotyping. Therefore this powerful molecular tool allowed us to give accurate genetic counselling in mental retardation.

1657/T

Development and validation of array CGH for detection of copy number mutations in the Duchenne muscular dystrophy (DMD) gene. *B.A. Boggs, D. del Gaudio, Z. Ou, Y. Yang, J. Wiszniewska, J.R. Lupski, A.L. Beaudet, A.C. Chinault, C.M. Eng.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Duchenne/Becker muscular dystrophy (DMD/BMD) is caused by mutations in the DMD gene located at Xp21. This is one of the largest genes identified in the human genome with 79 exons spanning ~2.2 megabases that are expressed as multiple isoforms in different tissues. A large percentage of mutations (~70%) in the DMD gene are due to deletions or duplications affecting one or more exons. Current diagnostic evaluation for such mutations involves several different approaches in male and female patients. These include dosage-sensitive Southern hybridization analysis, multiplex PCR amplification of DMD exons, and/or multiplex ligation-dependent probe amplification (MLPA). Given recent progress with array based CGH for high-resolution detection of genomic copy number gains and losses, we designed an Agilent oligonucleotide-based array with high-density coverage of the DMD gene to test the ability of this platform to detect and precisely map the end points of deletions and duplications in both male DMD/BMD patients and heterozygous females (which are more difficult to detect by current methodology). Initially, 6 samples (3 male, 3 female) were analyzed in a blinded study using an array that included 1125 oligos at DMD. Four deletions of 35-728 kb and a duplication of 113 kb were detected; in addition a non-contiguous duplication of 535 kb and 325 kb separated by a non-affected region of ~71 kb was found. All these findings were in complete concordance with the known abnormalities from previous studies. We subsequently obtained results for additional clinical samples, which included a male patient with a 5-kb duplication and a female with an apparent whole gene deletion that was actually shown to be due to loss of an entire X chromosome in ~30% of the cells. These studies provide initial experience with the use of the oligoarray platform to enhance detection of gene rearrangements in this and other disease genes as well as potentially replace existing methodologies in the clinical diagnostic laboratory.

1658/F

Stable Transfection of BACs Containing Fragile Site Sequence Recapitulate Fragile Site Instability at the Site of Integration. *R.L. Ragland, M.W. Glynn, T.W. Glover.* Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

Common fragile sites (CFSs) are loci that preferentially form gaps and breaks on metaphase chromosomes under conditions of replication stress. While much has been learned about the methods of induction and checkpoint and repair pathways that act in response to gaps and breaks at CFSs, little is understood about what makes a CFS "fragile". Sequences at CFSs are highly conserved through evolution and are exceptionally AT-rich, containing a high number of AT-rich flexibility peaks. In addition, partial deletions of CFS regions lead to reduction of associated metaphase gaps and breaks following replication stress. Given these and other data, we hypothesized that the sequence of CFSs is central to the fragility of these sites. In order to examine this possibility, we stably transfected into HCT116 cells two BACs containing FRA3B sequence and two control BACs containing non-fragile site sequence with similar sequence content. The transfections resulted in six clones containing sequence from the CFS FRA3B and six clones containing control sequence, each at unique chromosomal loci. Integrated BAC sequences were present at several hundred to just a few contiguous copies, arising either from concatamer formation or BAC amplification following integration. Clones containing sequence from FRA3B showed a significant, three to seven fold, increase in gaps and breaks over controls after treatment with aphidicolin, and most control BACs showed no or few breaks. Many FRA3B integration sites showed multiple breaks and other chromosome aberrations (circular chromosomes, amplified signal, etc.) indicative of instability. Furthermore, these sites were at least as prone to forming gaps and breaks as the endogenous FRA3B site. Loci with a greater copy number of inserted FRA3B BAC were more fragile than those with only a few copies. This is the first direct evidence in human cells, that introduction of CFS sequences into endogenous non-fragile loci, can recapitulate the instability found at CFSs. These data support the hypothesis that the sequences at CFSs are inherently instable, and contribute to the gaps and breaks seen at these sites.

1659/F

Presence of Yq microdeletions in men with Cryptorchidism. *Z. Arora[1], R. Kumar[2], R.K. Sharma[4], R. Kumar[3], R. Dada[1].* 1) 2ND YEAR, AIIMS, New Delhi, Delhi, India; 2) anatomy,AIIMS,New Delhi,India; 3) UROLGY,AIIMS,New Delhi,India; 4) Army R and R Hospital.

The incidence of cryptorchidism in full-term infants is approximately 3%, whereas it is 33% in males born prematurely.However, spontaneous testicular descent may occur in the newborn, and by 1 yr of age. In recent years, a growing body of evidence has demonstrated the existence of a genetic basis for primary testiculopathies related to microdeletions in the euchromatic region of the Y chromosome long arm (Yq11), where an azoospermia factor (AZF) has been suggested to exist. Analysis of the microdeletions resulted in the identification of three loci in Yq11 involved in the control of spermatogenesis, corresponding to three nonoverlapping regions: AZFa, AZFb, AZFc. The AZFa locus is located on proximal Yq11 (Yq11.21), while AZFb and AZFc are located on distal Yq11 (Yq11.23). The aim of this study was to study Yq microdeletion in those cases with Cryptorchidism which do not show any improvement in semen parameters post surgery. A total of 21 normal patients with confirmed Cryptorchidism and 20 fertile cases (controls) were included in the study. Cytogenetic analysis was done in all the cases and controls to rule out any chromosomal abnormality.PCR for the microdeletion analysis was done in each case using the primers for AZF region on peripheral blood. The sequence tagged site primers tested in each case were sY86 (AZFa); sY127 (AZFb); sY254 (AZFc). The PCR products were analyzed on a 1.8% agarose gel. PCR amplifications found to be negative were repeated at least three times to confirm the deletion of a given marker. All the 21 cases band of appropriate size was observed in cases for AZFa (320bp) and out of the 21 patients 19 cases had a AZFc(350bp) loci intact. In 20 out of the 21 patients a band of appropriate size was also observed for AZFb locus (274bp).1 case showed a microdeletion in the AZFb region.2 case showed AZFc deletion. None of the controls had any of such deletions.All the bilateral cryptorchoid cases were azoospermic with FSH levels.

1660/F

The molecular analysis of apparently balanced chromosome translocations in two unrelated patients with hypogonadotropic hypogonadism. *H.G. Kim[1], K. Norris[2], A.S. Kulharya[2], L.C. Layman[1].* 1) Section of Reprod Endocrinol & Genet, Dept OB/GYN, Inst Molec Med Genet, Medical College of Georgia, Augusta, GA; 2) Depts. Pathology and Pediatrics, Medical College of Georgia, Augusta, GA.

Patients with idiopathic hypogonadotropic hypogonadism (IHH) present with absent puberty due to a hypothalamic-pituitary defect, and may be either normosmic (nIHH) or anosmic (Kallmann syndrome). Although mutations in genes such as FGFR1, KAL1, and GNRHR constitute the most commonly encountered etiology in IHH, the molecular basis for most patients remains unknown. Apparently balanced chromosomal rearrangements found in some patients may actually disrupt a gene at the breakpoint, thereby aiding in identification of the causative gene. We have characterized translocations in two unrelated IHH patientsone a 46,XY,t(10;12)(q26.3;q13.1) in a male with Kallmann syndrome, and the other a mos46,-XY,t(3;12)(p13;p13)[18]/46,XY[3] in a male with normosmic IHH and cerebellar ataxia. The 10q26 breakpoint has been reported previously in a Kallmann syndrome patient with monosomy 10q26 and in several cases of translocations involving urogenital anomalies and hypogenitalism. In our patient, the 10q26 translocation breakpoint maps proximal to BAC clone RP11-95I16 and that of 12q13.1 was narrowed to 4.3 Mb between RP11-88L2 and RP11-204C20. In the second patient with the mosaic 3;12 translocation, homogeneous lymphoblastoid cell lines with the balanced translocation were successfully transformed from peripheral white blood cells. IHH and cerebellar ataxia often occur together and they are seen in Gordon Holms syndrome and Boucher-Neuhauser syndrome. We hypothesize that one of the breakpoints of this translocation case is likely to harbor a gene responsible for this phenotype. A positional cloning technique was applied to clone each of the breakpoints. FISH mapping for a breakpoint at 12q13 in this patient has led to the isolation of a BAC RP11-4N23, which crosses this breakpoint. Currently FISH with fosmid clones overlapping this clone is underway to refine the breakpoint region. These chromosome translocations afford the potential to define additional genes involved in IHH/Kallmann syndrome.

1661/F

***De Novo* Interstitial Deletion 1p31.1: A Distinct Syndrome?** *A. Battaglia[1,2], J. Palumbos[2], N. Pihl[2], A. Brothman[2], E. Ashton[2], J.C. Carey[2].* 1) Inst Child Neurology & Psych, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Dept of Peds/Div of Med Genetics, University of Utah Med Ctr, Salt Lake City, UT.

The 1p36 deletion syndrome has become well established as a recognizable entity over recent years. Less well-characterized and delineated are the phenotypes due to more proximal interstitial 1p deletions. An informative patient with an MCA/MR syndrome consisting of global developmental delay/mild mental retardation, microcephaly, short stature, distinctive facial features (telecanthus, epicanthal folds, synophyrs, overfolded small ears, narrowed ear canals), short neck, and accessory left nipple was referred for diagnostic evaluation to the Division of Medical Genetics. On examination, height was far below the 2nd centile; OFC fell below the 2nd centile; weight was just above the 5th centile. He also had severe genu valgum, requiring surgery; and early cryptorchidism with vanishing testes (not found at surgery). HRB chromosomes were apparently normal. Array-CGH was then carried out and showed a deletion at 1p31.1 of 73kb-5Mb in size; 1 BAC deleted RP11-80G24 (not deleted in his parents). Therefore, the patient's karyotype shows: 46,XY, arr cgh del1p31.1 (RP11-80G24). To date, 4 cases of visible interstitial deletions 1p31 have been published. All have short stature, microcephaly, developmental delay, and the male individuals have cryptorchidism. However, compared to the patients reported in the literature, our propositus shows more distinctive facial features. Based on the literature and on our patient, there is evidence for a critical region within 1p31.1 for the distinctive facial features and the vanishing testes. We suggest that this chromosome disorder may constitute yet another recognizable microdeletion syndrome.

1662/F

17q duplication: a rare chromosomal abnormality associated with brachyrhizomelia. *D.F. Garcia[1], C.M. Lourenço[1], A.C. Laus[2], S.A. Santos[2], L. Martelli[1,2].* 1) Medical Genetics Division, School of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil; 2) Department of Genetics, School of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil.

Abnormalities of chromosome 17 are relatively rare , apart from those found in hematologic malignancies such as the isochromosome 17q. Duplication 17q has been associated with a clinically recognizable syndrome, characterized by psychomotor retardation, short stature, microcephaly, narrow palpebral fissures, flat nasal bridge, long philtrum, cleft palate, large mouth, thin upper lip, low-set and malformed ears, brachyrhizomelia and hyperlaxity of limb joints. In addition, cardiac and cerebral anomalies are described. We report a female with 17q duplication and severe rhizomelic shortening of the limbs and brachydactyly. The patient is a 18 month-old female, the first child of healthy, young and non-consanguineous parents. She was born at term and by cesarean delivery. Her birth weight was 1900g, length 64.5cm and OFC41.5cm (all bellow 3rd percentile). She had a patent ductus arteriosus surgically corrected soon after birth. She developed seizures with one month-old and had delayed milestones. Physical exam showed microcephaly, curly hair with alopecia areas, midface hypoplasia, bulging eyes with convergent strabismus, short neck, short nose with depressed nasal bridge, low-set and posteriorly rotated ears, umbilical hernia, bilateral clinodactyly of 5th fingers, brachydactyly and rhizomelic limb shortness. Her x-rays showed no metaphyseal anomaly or calcifications. Abdominal and cranial ultrasonography were normal.Blood karyotype showed duplication of the distal region of 17q in all metaphases. Parental karyotypes were normal. Until now, few liveborn cases of partial trisomy for the distal region of 17q were reported. Cases of 17q duplication show marked variability in clinical expression, possibly related to the extent of the duplicated segment. A study of more patients is needed to refine the phenotypic mapping of chromosome 17 and to correlate different clinical syndromes with the extent of the 17q duplication.

1663/F

A genotype-phenotype correlation of small supernumerary marker chromosomes (sSMC) in human. *T. Liehr[1], K. Mrasek[1], N. Kosyakova[1,2], J. Vermeesch[3], S.W. Cheung[4], A. Weise[1].* 1) Institute of Human Genetics and Anthropology, Friedrich Schiller University, Jena; Germany; 2) Research Centre for Medical Genetics, Russian Academy of Medical Sciences Moscow, Russia; 3) Center for Human Genetics, University Hospital Leuven, Herestraat 49, Leuven, Belgium; 4) Baylor College of Medicine, Houston, Texas, USA.

Small supernumerary marker chromosomes (sSMC) are present in 0.044% of newborn and 0.075% of prenatal cases. In infertile or mentally retarded 0.125% or 0.288% are sSMC-carriers, respectively (Liehr & Weise 2007, Int J Mol Med 19:719-31). Overall, in about 30% of sSMC carriers an abnormal phenotype is observed. However, individual clinical outcome of sSMC presence is difficult to predict. Phenotypic consequences can appear due to differences in euchromatic DNA-content, uniparental disomy of the sSMC's homologous chromosomes, and/or different degrees of mosaicism. We did own studies on ~400 cases with sSMC using (sub)centromer-specific probe-sets and/or array-CGH. Moreover, we performed a review of the literature and thus, we suggested a first chromosome specific genotype/phenotype correlation for sSMC (Liehr et al., 2006 Cytogenet Genome Res 112:23-34) based on ~1650 cases. At present (04/2007) the sSMC-homepage (http://www.med.uni-jena.de/fish/sSMC/00START.htm) comprises ~2400 cases with sSMC. Thus, we are now able to present upgraded data on centromere-near chromosomal imbalances and their clinical consequences. Interestingly, clinically normal cases are reported, which have - due to sSMC presence - partial trisomies of several MB in size. The possible mechanisms involved have to be determined in future studies. In summary, ~50 year after first description of an sSMC, molecular cytogenetics provides now approaches for the comprehensive characterization of these 'marker chromosomes'. This will lead to an improved genetic counseling of cases especially with de novo sSMC and it will be possible to define clear syndromes within the clinically and genetically group of 'patients with sSMC'. Supported by the DFG (436 WER 17/5/05, 436 RUS 17/22/06, WE 3617/2-1, LI820/11-1), Boehringer Ingelheim Fonds, Ev. Studienwerk Villigst.

1664/F

Clinical and molecular-cytogenetic studies in a family with features of Jacobsen syndrome caused by an ~5 Mb deletion del(11)(q24.3). *J. Pietrzak[1], K. Szczaluba[1], E. Bocian[1], M.M. Sasiadek[2], I. Makowska[2], P. Stankiewicz[1], R. Smigiel[2].* 1) Dept of Medical Genetics , Institute of Mother and Child, Warsaw, Poland; 2) Dept of Genetics, Wroclaw Medical University , Wroclaw, Poland.

To date, over 100 cases with terminal deletion of 11q have been described and the resulting Jacobsen syndrome (JBS; MIM147791) has been well characterized. Clinical expression in JBS depends on the deletion size that varied between ~7-15 Mb. Typical features include developmental delay/mental retardation, short stature, congenital heart defects, thrombocytopenia, and a characteristic facial dysmorphism. In most JBS cases, *de novo* deletions have been found. In the remainder, the monosomy was the result of a product of a balanced chromosome translocation present in one of the parents. We present a family, in which a 4-year-old girl, her mother and mother's brother have features of JBS, including square asymmetric face, broad forehead, epicanthal folds, thick eyebrows, short nose with long philtrum, downturned corners of the mouth and low-set posteriorly rotated ears. In addition, the proband has psychomotor and speech delay while her uncle is mentally retarded and has psychiatric disturbances such as dementia, oligophrenic symptoms, and psychoorganic delusions. Notably, neither thrombocytopenia nor congenital defects were detected in this family. The initial G-banding analyses in this family were normal. Using FISH, we have identified a deletion of the terminal part of chromosome 11q in all three family members. The breakpoint was subsequently mapped to 11q24.3 between BAC clones RP11-507F16 and RP11-678L3, thus defining the deletion size ~ 5 Mb. This is the smallest terminal deletion associated with features of JBS. Interestingly, the *ETS* (*v-ets erythroblastosis virus E26 oncogene*) and *FLI1* (*friend leukemia virus integration 1*) hematopoiesis factor genes located ~6.5 Mb from 11qter and usually deleted in patients with JBS, are intact. We propose that one of these genes can be responsible for thrombocytopenia in JBS.

1665/F

Clinical Presentation of Newly or Rarely Described Chromosomal Rearrangements. *J. Alfardan, G. Scharer, J. Thomas, R. Gallagher, D. Manchester, A. Tsai.* UCHSC, TCH, Department of Pediatrics, Division of Genetics, Denver, CO.

Introduction: We describe 16 patients referred to our genetic service for developmental delays and/or congenital anomalies over a 6 month period and diagnosed with new or rare chromosomal rearrangements. Most of the rearrangements have not been described. Testing included HRC, FISH and/or DNA array and parental testing. **Patients 1 and 2:** 8 yo girl presented at birth with ASD, dysmorphy and global delays. HRC/FISH showed a small dup 7p22.1-22.2. Her mother has the same rearrangement but showed a milder phenotype. Presentation resembles larger dup 7p. We suggest that 7p22.1-22.2, specifically 7p22.1, is a dup 7p critical region. **Patient 3:** 6 yo boy with global delays and dysmorphy. DNA array showed del 13q32.3-33.1 and included ZIC2 gene. **Patients 4, 5 and 6:** 3 and 6 yo sisters with global delays and dysmorphy. Their mother had early developmental delays. The three have del 16q24.2-24.2 detected by DNA array. **Patients 7 and 8:** 6 yo boy with global delays and seizures. DNA array showed dup 5p13.2. His mother, who had normal development, has the same duplication. **Patients 9, 10 and 11:**(Pt 9 and 10) are unrelated 15 yo girl and 5 yo boy with dysmorphy and global delays. Mother (pt 11) of the 5 yo had multiple miscarriages, dysmorphy and cognitive impairment. HRC for those patients showed del 8q21.12-21.3. **Patient 12:** 1 week old boy with TOF, dysmorphy, and club foot. HRC showed del 9q34.2. **Patients 13 and 14:** 1 yo boy with developmental delays whose DNA array showed dup 8p23.3. His father also tested positive but had normal early development. **Patients 15 and 16:** 1 day old girl with dilated aortic arch, dysmorphy and hypotonia. Her mother had two miscarriages and reported early learning disabilities. Both have del 4q35.2-35.2. **Results:** We documented several newly/rarely reported chromosomal rearrangements. While some are de novo, others familial. Of the latter, some parents showed similar phenotype while others reportedly normal. **Conclusion:** We recommend DNA array for patients with developmental delays and/or congenital malformations as a first or second tier genetic testing. If a parent is also positive, this does not automatically indicate a polymorphism.

1666/F

Apparently balanced translocations are molecularly distinct in clinically affected patients by comparison with unaffected controls. *J. Baptista[1], S. Gribble[2], E. Prigmore[2], N. Carter[2], P. Jacobs[1], J. Crolla[1].* 1) Wessex Regional Genetics Laboratory, Salisbury, UK; 2) The Wellcome Trust Sanger Institute, Cambridge, UK.

De novo apparently balanced translocations (ABTs) can be present in both clinically affected and unaffected individuals. Molecular studies of ABTs have shown that an abnormal phenotype might be due to cryptic imbalances and/or gene disruption. To test the hypothesis that these features would be found in ABTs of affected patients, but not in those of unaffected individuals, we have analysed 14 affected and 18 unaffected cases all of whom have been examined by a Clinical Geneticist. For affected patients, breakpoint mapping was done by array painting and FISH, and a whole genome scan was conducted using array CGH on the Sanger 30K WGTP array. Molecular characterisations in unaffected individuals were done by FISH and array CGH on the Sanger 1 Mb array.

Array CGH imbalances were detected in 6/14 affected patients, but in none of the unaffected individuals. All of the imbalances were deletions from 200 Kb to 2.5 Mb in size and these were present at/near the breakpoints in 3 cases and in chromosomes unrelated to the translocations in 3 other cases. In 5 affected patients, one breakpoint disrupted a specific gene and in a further 7 gene disruption is likely. Surprisingly, gene disruption by a breakpoint was seen in 8/18 unaffected individuals and in a further 7 gene disruption was likely. As hypothesized, an abnormal phenotype might be explained by genomic imbalances in 40% of patients and by breakpoint-mediated pathogenic gene disruption in a minimum of 35%. The analysis of unaffected individuals showed none to have an imbalance, but a minimum of 44% to have a breakpoint disrupting a gene. In view of the fact that only about 5% of the genome is comprised of genes, this finding in the unaffected individuals suggests that some feature of the chromatin of genes makes them susceptible to breakage. The genes disrupted in this group are presumably not dosage sensitive and thus provide a platform of comparison that might help to establish genotype-phenotype correlations.

1667/F

The direct characterisation of breakpoints : a new approach for the segregation analysis of paracentric inversions in human sperm. *S. Bhatt[1, 5], K. Moradkhani[3, 5], K. Mrasek[4], J. Puechberty[1, 3], G. Lefort[3], J. Lespinasse[6], P. Sarda[3, 5], T. Liehr[4], S. Hamamah[1,2,5], F. Pellestor[1,2,5].* 1) INSERM U847, Montpellier, France; 2) Departement of Reproduction Biology, CHU Montpellier, Montpellier, France; 3) Department of Medical Genetics, CHU Montpellier, Montpellier, France; 4) Institute of Human Genetics and Anthropology, Jena, Germany; 5) University of Montpellier I, Montpellier, France; 6) Laboratory of Cytogenetics, CHR Chambery, Chambery, France.

We report the sperm FISH analysis of two paracentric inversions of chromosome 14 and chromosome 5, based on the direct breakpoint identification by the use of BACs spanning the inversion breakpoints. Sperm analysis was performed by multicolor FISH. Total of 7670 and 4807 spermatozoa were scored for inv(14) and inv(5) respectively. The breakpoints for inv(14) case were found to be in 14q23.2 and 14q32.13. The breakpoints for inv(5) case were found to be in 5q13.3 and 5q33.1.The breakpoints for both inversions were found to be in G light region. The inverted segment length for inv(14) was 31 Mb and 29% of the total length of the chromosome was involved in the inverted segment. The frequency of sperm harbouring normal, inverted, deleted, duplicated chromosome 14 was found to be 49.62%, 46.66%, 3.43% and 0.29% respectively. The inverted segment length for inv(5) was 75 Mb and 42% of the total length of the chromosome was involved in the inverted segment. The frequency of sperm harbouring normal, inverted, deleted, duplicated chromosome 5 was found to be 45.64%, 44.67%, 8.73%, 0.96% respectively.This study shows that the breakpoint characterisation could be an efficient approach for segregation analysis of paracentric inversion. Using the BACs spanning the breakpoints, the percentage of normal, inverted, duplicated deficiency and deleted segregation product could be accurately estimated.*Supported by a INTAS research grant (03-51-4060)* .

1668/F

A case of pure, non-mosaic trisomy 8q with multiple cardiac defects and congenital anomalies. *N. Cohen, S. Pardo, N.B. Kardon, L. Edelmann.* Dept Genetics and Genomic Sciences Mt Sinai Sch Med, New York, NY.

We report on an infant born at 38 weeks of gestational age with multiple cardiac and congenital anomalies, resembling a severe form of mosaic trisomy 8 syndrome. Conventional cytogenetic analysis by high resolution GTG-banding revealed an abnormal male karyotype with a de-novo, non-mosaic, partial trisomy of chromosome 8q designated 46,XY,add(8)(p23). Upon physical examination, the infant was noted to be severely dysmorphic with facial features that included frontal bossing, downslanting long palpebral fissures, low set dysplastic ears, wide/broad nasal bridge, short smooth philtrum and micrognathia. Additional findings included a webbed neck, hypoplastic and wide spaced nipples, bilateral cryptorchidism, anteriorly placed anus and deep sacral crease. Multiple hand and foot anomalies were noted, including overlapping digits, 2-3rd toe syndactyly and deep sole creases. Neurological exam showed diffuse hypertonicity with limited range of motion of all joints and bilateral wrist contractures. Echocardiogram revealed multiple cardiac defects, including patent ductus arteriosis, critical coarctation of the aorta and ventricular septal defect. Fluorescence In Situ Hybridization (FISH) analysis with a whole chromosome 8 paint (WCP 8, Vysis) verified that the additional material at 8p was derived from chromosome 8. Telvysion FISH with probes for 8p and 8q (Vysis) indicated that the 8p subtelomere was intact and that the duplicated segment extended to include the 8q subtelomere. The revised karyotype was designated as 46,XY,add(8)(p23).ish der(8)dup(8)(qter>q13::pter)(8ptel+,8qtel++). Oligonucleotide array CGH analysis (Agilent) confirmed that the proximal breakpoint of the duplicated segment was within 8q13 and that the whole p-arm of chromosome 8 was intact. To date, all cases of partial trisomy 8q reported in the literature involved the segments from 8q22-8q24 to 8qter. To our knowledge, this is the first report of a pure partial trisomy of 8q involving 8q13 to 8qter in a patient. In summary, the non-mosaic nature and size of the duplicated segment of 8q contributed to the severe phenotypic presentation of trisomy 8 syndrome in this patient.

1669/F

X-linked gene expression in fibroblasts and brain tissue from patients with supernumerary X chromosomes. *T. Helling[1], C. Jie[2], L. Zhang[1], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Microarray Core, Johns Hopkins University, Baltimore, MD.

Sex chromosome number abnormalities in humans occur 1 in 400 males and 1 in 650 females. Patients with supernumerary X chromosomes (SNX) have significant defects in cognitive and language development. With each extra X chromosome, there is ~15 points of IQ reduction with increasing severity of language defect. The apparent dependency of the phenotype on copy number of the X chromosome strongly suggests a dosage effect of certain X-linked genes. We hypothesize that steady-state transcript levels of certain X-linked genes at given stages of development may have an effect on the clinical phenotype of SNX patients. Using a custom human X chromosome cDNA microarray, we examined the relative abundance of X-linked transcripts in fibroblasts from SNX patients. We used 1.5-fold change as an arbitrary cut-off for significance and identified 57 genes that showed altered transcript abundance in fibroblasts with 49XXXXY. We observed the expected increase of transcripts from XIST (2.76 fold) and 8 out of 9 genes in the pseudoautosomal regions (PAR) in 49XXXXY fibroblasts. The folds of increase for the 34 non-PAR genes ranged from 1.55 to 3.48. Among these, 16 (47%) were mapped to Xp22; 5 (14.7%) to Xp11, 3 (8.8%) to Xq13, 6 (17.6%) to Xq21-22, 1 (2.9%) to Xq26, and 3 (8.8%) to Xq28. This observation is consistent with the published report (Nature 434:400) that ~50% of the genes that escape X-inactivation reside in the distal Xp near PAR1. Among 22 genes that have comparable data with the published report, 16 (72.7%) showed consistent (n=12) or variable (n=4) escape of X-inactivation. Additionally, we identified 11 genes that showed transcript reduction (1.8 to 2.5 fold) in fibroblasts with 49 XXXXY as compared to that with 46 XY. We performed qRT-PCR or northern blot to validate microarray data from selected genes using RNA from fibroblasts with 49XXXXY and brain tissue from a patient with 49XXXXY. Results of the study will help to understand the mechanism of copy number-dependent dosage effects of X-linked genes on human cognitive and language function and diseases.

1670/F

Verification of CNVs identified with high density oligo aCGH using an optimized Q-RT PCR assay. *X. Hu[1], M.M. Li[1,2,3].* 1) Louisiana Cancer Research Consortium; 2) Hayward Genetics Center; 3) Dept. of Pediatrics; Tulane Univ. Sch. Med, New Orleans, LA.

High density oligo microarray CGH (aCGH) has been revolutionizing the diagnosis of patients with developmental delay and mental retardation. However, higher density oligo arrays also brought with it a higher rate of copy number variations (CNVs). How to distinguish true CNVs from artifacts, especially those duplications/deletions smaller than 0.5 Mb, has proved to be a significant challenge. In this study, we attempted to develop a SYBR Green-based quantitative Real Time PCR (Q-RT PCR) method to verify small deletions and duplications identified by CGH. Five genes, UTS2D and CLND from 3q, ANXA29 and AMAD10 from 4q, and DSCR1 from 21q were selected for this study. In addition, two housekeeping genes, ABL and GUSD were utilized as internal controls. The sensitivity and linearity of the Q-RT PCR assay were established using serial dilutions of reference DNA (Promega). The standard curves showed a correlation coefficient between 0.99 and 0.997 for starting templates ranging from 101 to 106 copies. The specificity of the test was validated by a single peak melting curve with an identical melting temperature for all samples. The intra-assay coefficient of variation (CV) was < 2%, and the inter-assay, < 5%. Using this method we studied DNA samples from a patient with an unbalanced translocation der(4)t(3;4)(q27.2;q32.2) and a patient with Down syndrome. The Q-RT PCR data were analyzed using the standard curve method and three relative quantification methods. The results confirmed a duplication of the UTS2D gene (1.63 fold, $p<0.05$) and the CLND gene (1.67 fold, $p<0.05$) and a deletion of the ANXA29 gene (0.5 fold, $p<0.05$) and the AMAD10 gene (0.49 fold, $p<0.05$) in the patient with partial trisomy 3q and partial monosomy 4q and a duplication of the DSCR1 gene (1.67 fold, $p<0.05$) in the patient with Down syndrome. In conclusion, we have developed and optimized a Q-RT PCR assay that can quantify copy number changes in genomic DNA with high sensitivity and specificity. This assay can be used to confirm small deletions or duplications identified with high density oligo aCGH.

1671/F

Neocentromere marker chromosome of distal 3q mimicking dup(3q) syndrome phenotype. *K. Kosaki[1], K. Izumi[1], M. Aramaki[1], R. Kosaki[2].* 1) Department of Pediatrics, Division of Medical Genetics, Keio University Tokyo, Japan; 2) Department of Clinical Genetics and Molecular Medicine, National Ctr for Child Health and Development, Tokyo, Japan.

Supernumerary marker chromosomes lacking alpha-satellite sequences and possessing a newly derived functional centromere are referred to as neocentromere marker chromosomes. Although the delineation of the chromosome content of these neocentromere marker chromosomes would be helpful for genetic counseling, such fine mapping has been difficult because of the limited sizes of the involved segments. We report a female patient with mosaic neocentromere marker chromosome involving 3q26.3-3qter, the content of which was determined using an array CGH analysis. Our results support the validity of an array CGH-based approach to investigating the origins of SMCs. Further FISH analyses revealed that the neocentromere marker chromosomes represented de novo inverted duplications of the distal segments of chromosome 3, 3q26.3-3qter. The present case had many manifestations of dup(3q) syndrome, the critical interval of which is considered to be 3q26.3-q27. Common features included mental and growth retardation, hirsutism, synophrys, a broad nasal root, anteverted nares, downturned corners of the mouth, and malformed ears. The observation gives further credence to the concept that the critical region responsible for the dup(3q) phenotype to 3q26.3-q27.

1672/F

CHROMOSOMAL DELETIONS WITHOUT PHENOTYPIC EFFECT: GENE DENSITY IN THE HUMAN GENOME. *S. Li, R. Hyde, P. Blackett, J.J. Mulvihill.* Dept Ped/Gen, BSEB 224, Univ Oklahoma Hlth Sci Ctr, Oklahoma City, OK.

The infrequent reports of constitutional chromosomal deletions with no phenotypic abnormality beg an explanation. One speculation was that such abnormalities occur in genomic regions of low gene density. With Borgaonkar's Chromosomal Variation in Man and PubMed searching, most reports of such deletions in clinically normal individuals were assembled. The distribution of genes on all chromosomes was retrieved from online databases for the human genome and averaged 8.64 genes/Mbp. For all 52 normal individuals reported with cytogenetic deletions, the calculated gene density of each deleted region averaged 4.14 ± 3.20 genes/Mbp. For comparison, in individuals with mild clinical abnormalities, the deleted regions had a calculated average gene density of 7.03 ± 2.14 genes/Mbp (n=41). We therefore conclude that there is a significantly ($p=0.002$) lower gene density in the deleted regions associated with a normal phenotype. It appears that the severity of the phenotypic outcome is at least partly controlled by the gene density of the deleted region; or, conversely, only regions of sparse gene densities can deleted without clinical phenotypic abnormalities.

1673/F

The relationship between meiotic recombination in human spermatocytes and aneuploidy in sperm. *R. Martin[1], F. Sun[1], M. Oliver-Bonet[1], E. Ko[1], A. Rademaker[2], P. Turek[3].* 1) Department of Medical Genetics, University of Calgary, Calgary, AB; 2) Department of Preventive Medicine, Northwest University Medical School, Chicago, IL; 3) Departments of Urology and of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, San Francisco, CA.

In humans, abnormalities in the number or position of crossovers have been associated with nondisjunction in both males and females. We previously determined, by single sperm PCR, that a decreased frequency of recombination between the X and Y chromosomes is associated with nondisjunction and the production of aneuploid human 24,XY sperm. The aim of this study was to determine if there is an association between the frequency of recombination observed in human pachytene spermatocytes and the frequency of aneuploidy in sperm from the same men. Six patients who were undergoing vasectomy reversals donated a small sample of testicular tissue for meiotic analysis. Six to twelve months after the vasectomy reversals, a second sperm sample was obtained from each patient to test for aneuploidy frequencies using fluorescence in situ hybridization (FISH) analysis. The meiotic analysis of pachytene spermatocytes was performed using new immunocytogenetic techniques which allow the visualization of the synaptonemal complex (SCP1, SCP3), the centromere (CREST), and the sites of recombination (MLH1, a DNA mismatch repair protein that marks the location of recombination). A minimum of 100 pachytene spermatocytes were analyzed for each male. Individual meiotic bivalents were identified with centromere-specific multicolour FISH and the number of MLH1 signals recorded for individual chromosomes. The frequency of sperm aneuploidy was analyzed for chromosomes 1,9,13,21,X and Y. A Pearson correlation coefficient was used to determine the relationship between the frequency of meiotic recombination for an individual chromosome (eg 21) with the frequency of sperm aneuploidy for that chromosome. No correlation was observed for any of the chromosomes. This first direct test of an association between recombination and sperm aneuploidy for autosomes suggests that there is no direct association, at least for normal men.

1674/F

An i(21) case caused by paternal low level mosaicism. *H. Numabe[1], H. Uchio[2], H. Doi[2], S. Adachi[2], T. Yorifuji[2], T. Nakahata[2], K. Tomiwa[1], S. Kosugi[1].* 1) Department of Clinical Genetics, Kyoto University Hospital, Kyoto, Kyoto, Japan; 2) Department of Pediatrics, Kyoto University Hospital, Kyoto, Kyoto, Japan.

Case: The case is a 7-day-old boy with Down syndrome who is the first child by ICSI (intracytoplasmic sperm injection). He was transferred to our hospital because of heart murmur. At his birth the mother was 33 and the father was 31 years old. Labor and delivery were uneventful. The gestational age was 37 weeks and 1 day and the birth weight 2,764g. A cardiac echogram demonstrated ASD (atrial septal defect), VSD (ventricular septal defect), and PDA (patent ductus arteriosus). His karyotype was 46,XY,i(21)(q10). To reveal the origin of i(21), we performed the chromosome analyses of his parents who have normal phenotypes. The mother's karyotype was 46,XX,inv(9)(p12q13) which is known as a normal variant. The father's karyotype was 45,X,dic(Y;21)(p11.3;p11.2) in total count of 20 cells, and 45,X,dic(Y;21)(p11.3;p11.2)[48]/46,XY,r(21)(p13q22.3)[2] in 50 cells. To reveal low level mosaicism of i(21), we examined 100 cells employing the FISH technique. As the final result, the fathers karyotype was ish dic(Y;21)(SRY+,D21S259/D21S341/D21S342+,Tel 21q+),21(D21S259/D21S341/D21S342x1, Tel 21qx1)[91]/Yp11.3(SRYx1),21(D21S259/D21S341/D21S342x1,Tel 21qx1),r(21)(D21S259/D21S341/D21S342+,Tel21q+)[8]/Yp11.3(SRYx1),21(D21S259/D21S341/D21S342x1, Tel 21qx1),?i(21)(Tel 21q++, D21S259/D21S341/D21S342++)[1]. The father has a low level mosaicism of i(21) in blood cells, and may have a similar or higher level mosaicism in gonad.

1675/F

A study on the chromosome abnormalities in women with premature ovarian failure. *F. Pouresmaeili[1], M. Fallahian[2], F. Azizi[3], E. Azargashb[4], N. Arian[5], S. Karimi[6], A. Shirafkan[2].* 1) Shaheed Beheshti University of Medical Sciences, Faulty of medicine, Genetics & Biochemistry Dept., Evin square, Chamran highway, Tehran 19395-4719, Iran & Fertility-Infertility Health Research Center of Taleghani Hospital, 3rd floor, Evin square, Chamran; 2) Taleghani Hospital, Gynecology & Obstetrics Dept., Evin square, Chamran highway, Tehran, Iran; 3) Taleghani Hospital, Endocrinology Research center, Evin square, Chamran high way, Tehran 19395-4763, Iran; 4) Shaheed Beheshti University of Medical Sciences, Faulty of medicine, Social Medicine Dept., Evin square, Chamran highway, Tehran 19395-4719, Iran; 5) Shaheed Beheshti University of Medical Sciences, Faulty of medicine, Genetics & Biochemistry Dept., Evin square, Chamran highway, Tehran 19395-4719, Iran; 6) Shaheed Beheshti University of Medical Sciences, Faulty of medicine, Genetics & Biochemistry Dept., Evin square, Chamran highway, Tehran 19395-4719, Iran.

Failure of germ cell development is associated with complete ovarian failure,while their decreased number more likely is associated with partial ovarian failure,which takes the form of secondary amenorrhea.Both are highly heterogeneous conditions whose aetiology in most cases is still unknown but they may be considered different manifestations of the same underlying pathogenetic and etiologic processes.A genetic basis has long been suggested even if the frequency and types of chromosome aberrations and gene mutations have still to be determined.We performed a systematic genetic study on 32 women affected by secondary amenorrhea. The inclusion criteria were menopause before the age of 40yrs and FSH levels>40IU/l.The overall percentage of chromosome anomalies was 18.7%with identifable abnormal X chromosome, which were 1 XX/XXX ;2 XX/XO; 1 XX/XY musaics and 2 pure autosomal anomalies,XO.We suggest chromosome study to be performed for all patients with POF. The obtained information will be usefull for patient management, genetic counselling, and future family planning.

1676/F

Prenatal Diagnosis of Mosaic Variegated Aneuploidy with Premature Chromatid Separation (MVA with PCS). *P.L. Sinanaj, S. Whitmer, J.B. Ravnan.* Genzyme Genetics, Santa Fe, NM.

Amniotic fluid was received for chromosome analysis from a 35-year-old Asian female. Clinical indications included advanced maternal age, positive maternal serum screen for trisomy 18, and multiple abnormalities noted on ultrasound including a possible heart problem, nuchal thickness, microcephaly, and abnormal cisterna magna. Interphase FISH analysis showed no abnormalities of chromosomes 13, 18, 21, X, and Y. Cytogenetic analysis of 15 colonies from six independent cultures showed varying aneuploidies in every cell examined with no normal cells seen. Two colonies were 47,XX,+7 with the remainder of the colonies showing different abnormalities. Trisomy 7 and trisomy 17 were the most common trisomies, usually seen in conjunction with other aneuploidies. In addition to the aneuploidies, many metaphase spreads showed a distinct morphology with the chromatids completely separated and lying side by side. A cytogenetic diagnosis of mosaic variegated aneuploidy with premature chromatid separation (MVA with PCS) was reported.

Characteristic features of MVA with PCS include microcephaly, growth deficiency, CNS anomalies, mental retardation, flat and broad nasal bridge, low-set ears, eye and skin abnormalities, and ambiguous genitalia in male patients. Diagnosis is usually made in infancy, but some adults have been diagnosed as well. Almost 1/3 of individuals with MVA with PCS have been reported to develop leukemia, rhabdomyosarcoma, or Wilms tumor in childhood. Approximately twenty-five probable or definite cases have been reported world-wide. Inheritance appears to be autosomal recessive with affected sibs in a few families. Parental chromosome studies showed increased levels of premature chromatid separation in both, suggesting they are carriers of the PCS trait.

Mutations in the BUB1B gene encoding BUBR1, a key protein in the mitotic spindle checkpoint have been found in some families with MVA with PCS. Blood from the parents was sent to a research laboratory for mutation analysis of the BUB1B gene.

1677/F

Sperm aneuploidy frequencies analyzed before and after chemotherapy in testicular cancer and Hodgkin's lymphoma patients. *H.G. Tempest[1], E. Ko[1], P. Chan[2], B. Robaire[2], A. Rademaker[3], R.H. Martin[1].* 1) Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada; 2) Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada; 3) Department of Preventative Medicine, Northwestern University, Chicago, Illinois, USA.

In this study, multi-color fluorescent in-situ hybridization (FISH) was utilized to detect sperm aneuploidy for chromosomes 13, 21, X and Y in individuals before and after chemotherapy for testicular cancer (n=12) and Hodgkin's lymphoma (n=11). Aneuploidy was assessed before, 6 months and 1-2 years after the initiation of treatment and compared to age matched controls (n=19). Slides were coded and analyzed "blindly" with a minimum of 5,000 sperm cells scored per patient, per chromosome at each time point where sperm was available (635,396 sperm scored). At 6 months, testicular cancer and Hodgkin's lymphoma patients showed significant increases in XY disomy and nullisomy 13 frequencies, and significantly higher frequencies of sex chromosome disomy and nullisomy 21 were found in testicular cancer patients. Aneuploidy frequencies, for the most part, declined to pre-treatment levels at 12 months. However, there were elevated aneuploidy frequencies for some chromosomes up to 24 months after the intiation of treatment. When Hodgkin's lymphoma and testicular cancer patients were compared to each other and with controls, cancer specific differences were identified. Hodgkin's lymphoma patients, in particular, exhibited a significant increase in aneuploidy frequencies for all chromosomes at all time points compared to controls and testicular cancer patients. Because of elevated aneuploidy frequencies prior to and up to 24 months from the start of chemotherapy, and to facilitate informed decisions regarding their future reproductive choices, patients should receive genetic counseling about the potentially increased risk of an aneuploid conceptus from sperm cryopreserved prior to chemotherapy, and for conceptions up to two years from the initiation of treatment.

1678/F

Skewed X-Inactivation in Women with Karyotyped Spontaneous Abortiions. *D. Warburton[1], J. Kline[1], S. Brown[2], A. Kinney[1], C-Y. Yu[1], B. Levy[1], V. Jobanputra[1], B. Levin[1].* 1) Columbia Univ, New York; 2) Univ. Vermont.

Several previous reports suggest that highly skewed X inactivation (HSXI) is associated with recurrent spontaneous abortion. A possible explanation is that HSXI is associated with trisomy. X-chromosome genetic changes could lead both to HSXI and to increased oocyte atresia, with a resulting increase in trisomic conception. Alternatively, an anomalous X chromosome could cause embryonic death in male conceptions. To test these hypotheses we measured XCI skewing in women ascertained through a karyotyped spontaneous abortion and in age-matched controls with births at the same hospital. We used the HUMARA assay with control Rsa1 and MIC2 digestion. Because ratios >=90% were infrequent (~2%), we defined HSXI as >=85%. A ratio >=85% occurred in 5.4% of controls (n=427), 5.5% of women with trisomic losses (n=163) and 2.2% of women with normal male losses (n=46). In comparison with controls, the age-adjusted odds ratio for XCI >=85% vs. XCI 50-<60% was 1.2 (95% CI 0.5-2.8) for trisomic losses and 0.3 (95% CI 0.04-2.4) for chromosomally normal male losses. Thus, our study does not support an association between HSXI and either trisomic or normal male loss. In secondary analyses, the skewing distribution was significantly different for non-trisomic abnormal losses (p=0.02), due mostly to increased HSXI (21.1%) among monosomy X losses (n=19). When trisomies were classified by type, the skewing distribution was significantly different for non-acrocentric trisomies other than 16 (n=38; p=0.03); 13.2% had HSXI. Because of small samples and the multiple tests performed, the latter results require confirmation. HSXI was unrelated to recurrent loss in our sample. All 45 women with HSXI had normal X chromosomes by G-banding; high-resolution oligonucleotide microarray analysis on 15 revealed no additional changes. Among 90 women for whom we measured skewing in left and right buccal smears, the correlation between the average ratio in buccal smears and blood was 0.53. Only 3 women showed skewing >=85% in all samples. This low correlation indicates that the XCI skewing ratio in a blood sample is not a good indicator of X-chromosome anomalies leading to skewing in all tissues.

1679/F

Phenotypic characterization of patients with interstitial duplications of 15q11-q13 detected by array based comparative genome hybridization (array CGH). *H. Yonath, C. Bacino, S.R. Lalani, A. Patel, A.L. Beaudet, S.W. Cheung, T. Sahoo.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Interstitial duplications and isodicentric chromosomes involving the 15q11q13 region are a relatively common cytogenetic abnormality associated with altered behavior, developmental delay/mental retardation (DD/MR), autism, and seizures. This chromosomal region has considerable genomic instability and is subject to imprinting in a parent-of-origin manner. Deletions in this region give rise to Prader-Willi/Angelman syndromes. There is a lack of exhaustive phenotypic data from cases with interstitial duplications of 15q11q13, that involve the Prader-Willi/Angelman syndrome critical region (PWACR). There is evidence that duplications of maternal origin cause autism and ones of paternal origin are generally benign. In order to identify patients with this duplication, we screened 6000 cases that were referred for targeted array CGH. The array CGH was found to provide a more accurate detection and identification of these submicroscopic duplications, most of which could not be identified cytogenetically. We report here the cytogenetic, molecular and phenotypic characterization of 12 patients harboring a duplication of the PWACR. A combination of array CGH, fluorescence in situ hybridization, methylation analysis, and a custom 44K array CGH focused on 15q11-q13 allowed delineation of the size and nature of the duplicated segment; in 6 cases (5 interstitial and 1 inv dup 15) the duplication extended from breakpoints 1 to 3 (class I), in 5 cases from breakpoints 2 to 3 (class II), and one case of inv dup 15 included a more distal breakpoint (breakpoint 4). Detailed patient information was obtained form the referring physician after obtaining informed consent from the patients or their parents. The indications for the array CGH were DD/MR in 6 patients, DD and absent speech in 1, autistic spectrum in 2, seizures in 2 and behavioral problems in one patient. In one case the duplication is de novo and the rest of the cases are awaiting the parental results. A genotype/phenotype correlation including parent of origin effect and size of the duplication will be presented.

1680/F

Contiguous gene deletion of 16q24.3 in an individual with venous malformations, distichiasis and other anomalies. *M.G. Butler[1], S.L. Dagenais[1], J.L. Garcia-Perez[1], J.W. Innis[1,2], T.W. Glover[1,2].* 1) Human Genetics, University of Michigan, Ann Arbor, MI; 2) Pediatrics, University of Michigan, Ann Arbor, MI.

Varicose veins and distichiasis, or growth of accessory eyelashes from the Meibomian glands, occur together in lymphedema-distichiasis syndrome (LD). LD is an autosomal dominant syndrome also characterized by pubertal onset of bilateral lower extremity edema. A 12 year-old female presented with a subset of LD features including distichiasis and severe varicose veins, but without lymphedema. She also had microcephaly and mental retardation, features not typically associated with LD. Additional members of the proband's family had distachiasis and less severe varicose veins. Our lab demonstrated that mutations in the forkhead transcription factor FOXC2 cause LD, but cases without FOXC2 mutations have been reported. Mutational screening of FOXC2 failed to identify mutations in the proband, but chromosomal microarray identified a deletion of BAC clone RP11-106D4 located ~ 750 kb telomeric to the FOXC2 locus on chromosome 16. The deletion was also identified in 3 affected family members but was not present in 1 unaffected relative. The RP11-106D4 clone rarely varied in over 800 unrelated samples tested previously. The presence of the deletion was confirmed by FISH using an overlapping BAC clone RP11-178L8. Using a combination of techniques, we cloned the breakpoints of the deletion. It spans 260 kb on 16q24.3 including three genes: FBXO31 (F-box Protein 31), MAP1LC3B (microtubule-associated protein 1 light chain 3 beta), and ZCCHC14 (zinc finger, CCHC domain containing 14) and extends into a conserved gene desert distal to FOXC2. We studied the expression pattern of each deleted gene in mouse embryos using in situ hybridization. Fbxo31 is expressed strongly in the eyelid, surrounding blood vessels, and kidney, which are also sites of Foxc2 expression. Map1lc3B is expressed in the stomach, and Zcchc14 is expressed in the ear and skull. Based on expression analysis, deletion of FBXO31 could be the cause of varicose veins and distichiasis in this family, although deletion of a long range FOXC2 enhancer element cannot be ruled out. *FOXC2FBXO31MAP1LC3BZCCHC14.*

1681/F

Molecular cytogenetic characterization of an interstitial de novo 13q deletion in a 3-month-old with severe pediatric gastroesophageal reflux. *N.L. Champaigne[1], N. Laird[1], J.K. Northup[2], G. Velagaleti[1,2].* 1) Dept Pediatrics, Univ Texas Medical Branch, Galveston, TX; 2) Dept Pathology, Univ Texas Medical Branch, Galveston, TX.

Gastroesophageal reflux (GER) occurs when gastric contents travel back into the esophagus through the esophageal sphincter. GER is very common in infants with most growing out of it, but some continue to have chronic symptoms throughout childhood and adulthood. Previous linkage analysis identified a gene, GERD1, responsible for severe pediatric GER. GERD1 has been mapped to 13q14. We report here a de novo interstitial deletion of chromosome 13 in a 3-month-old biracial male who presented with severe GER and failure to thrive. His height, weight, and OFC were all below the 3rd percentile. On physical examination, he was noted to have hypotonia and multiple dysmorphic features including large eyes with downslanted palpebral fissures, a very short frenulum, absent uvula, small mandible, a short neck with extra nuchal folds and coronal hypospadias. Chromosome analysis from cultured peripheral blood lymphocytes showed an interstitial deletion of chromosome 13, with the karyotype 46, XY, del(13)(q12.3q14.1). FISH analysis with several BAC probes localized to the 13q12.2-q14.3 region showed the proximal breakpoint to be between RP11-203D17 (13q12.3) and RP11-90M5 (13q12.3). Similarly, the distal breakpoint is between RP11-13I8 (13q14.11) and RP11-160G19 (13q14.11). Based on the BAC-FISH analysis, the deletion appears to encompass at least 12.3 Mb and does involve the GERD1 locus. The GERD1 locus has been mapped to a 9-cM interval located between the markers CAGR1 and D13S263, both of which we have shown to be deleted in our patient. Further FISH analysis with overlapping BAC clones localized to the breakpoint regions is in progress to more precisely determine the extent of the deletion. We propose that the GER phenotype in our patient is due to a haploinsufficiency for GERD1.

1682/F

Atypical 22q11.2 Deletions - What Have We Been Missing? *D. McDonald-McGinn[1], S. Saitta[1], C. Catania[1], P. Kaplan[1], J. Coppinger[2], L. Shaffer[2], B. Emanuel[1], E. Zackai[1].* 1) Div Human Gen, Children's Hosp Philadelphia, Philadelphia, PA; 2) Signature Genomics, Spokane, WA.

The majority of patients with a 22q11.2 deletion have the same large >3Mb/A-D deletion identified using N25/TUPLE probes. Nonetheless, there is significant inter & intra familial variability with minimal evidence of genotype-phenotype correlations. In addition, many patients are referred with clinical symptoms of the deletion whose FISH studies are negative. We identified 7 patients with atypical deletions using research probes or clinical CGH. These include one with pulmonary valve stenosis, cleft palate, scoliosis, feeding difficulties, developmental delay & dysmorphia with a 2.6 Mb/midA-D deletion including UFD1L & TBX1 & another with recurrent URI's, VPI, developmental delay, & dysmorphia with a 1.4 Mb/midA-C deletion including TBX1. Both were studied using research probes as the patients clearly had clinical features of the deletion. In addition, 3 others, including one with cleft palate, developmental delay & dysmorphia; another with FTT, microcephaly, aplasia cutis congenita, feeding difficulties & developmental delay; & another with microcephaly, a preauricular tag, micrognathia, dysphagia, & polymicrogyria all had a B-D deletion distal to TBX1 using CGH. Lastly, one patient with scoliosis, speech delay/hypernasality, OCD, and dysmorphia had a C-D deletion and an infant with preauricular tags, VSD's, and dysmorphia had a midE-midF deletion just beyond the classic 22q11.2 deletion region using CGH. So, in summary, we have identified 7 patients with atypical 22q11.2 deletions whose diagnoses would have been missed using conventional FISH. Thus, other individuals with findings ordinarily associated with the deletion may in fact have atypical deletions requiring alternative methods of identification such as CGH. Conversely, a new subset of patients is being elucidated via CGH & although the clinical findings in this latter cohort are variable, not unlike the patients with the standard deletion, these individuals will likely be key in advancing the search for genotype-phenotype correlations & in providing answers to the question "What have we been missing?".

1683/F

A 46,XX,del(20)(q11.23q13.11) with normal adenosine deaminase (ADA) activity. *T. Sonoda, M. Tomimori, S. Iwashiro, N. Ikewaki, S. Iwamoto.* Occup Therapy, Sch Hlth Scu, Kyushu Univ Health & Welfare, Nobeoka, Japan.

We present a patient with 46,XX,del(20)(q11.23q13.11). The proband, a female infant, was born after 41-weeks gestation. The birth weight was 3,082 g, length 51.0 cm and head circumference 34.2 cm. Physical findings included: deep-set eyes, anteverted nostrils, a portwine stain on the forehead, low-set ears, bilateral high axial triradius, right hip dislocation, and general muscular hypotonia. There was no history of recurrent infection. Development quotient (DQ) at 7 months after birth was about 60. The patient's G-banded karyotype analyzed on cultured lymphocytes revealed she had an interstitial deletion of 20q (q11.23→q13.11). The level of the patient's adenosine deaminase (ADA), for which the gene locus is mapped on 20q13.11 was 14.6 IU/L (normal range: 6.8-18.2).

Structural abnormalities of chromosome 20 are rare. A few cases have been reported with ring 20, a few with complete or partial trisomy (mostly of the short arm), and a very few with partial deletion of the short or the long arm. Deletion of the long arm in particular is extremely rare. To our knowledge, only 2 cases have been reported. We present an additional case with interstitial deletion of 20q. We also discuss clinical features and the gene locus of adenosine deaminase (ADA), which is mapped on 20q.

1684/F

Investigation of 300 patients with delayed psicomotor development, obesity, hyperphagia, learning disabilities and behavioral problems. *M.C. Varela[1], C.S. D'Angelo[1], I. Kohl[1], C.I.E. Castro[1], F. Kok[1,2], C.A. Kim[3], C.P. Koiffmann[1].* 1) CEGH, Dept Gen & Evol Biol, Univ São Paulo; 2) Child Neurol Service, Dept Neurol, Univ São Paulo School of Medicine; 3) Children's Institute, Hospital das Clínicas, School of Medicine, Univ São Paulo, São Paulo, Brazil. E-mail: mcvarela@ib.usp.br.

Obesity in association with phenotypic abnormalities and mental retardation characterizes syndromic obesity. Herein we present molecular studies in 300 patients with syndromic obesity. On an ongoing research we diagnosed 142 patients with Prader-Willi syndrome (PWS), the most common form of syndromic obesity: 86 cases had a paternal deletion of 15q11-q13, 54 maternal uniparental disomy of chr15, and 2 had a defect in the imprinting center. These patients were diagnosed by methylation pattern analysis of the SNRPN-SNURF gene and by microsatellite profiling of loci within and outside the 15q11-q13 region. Two patients with unbalanced translocations were detected. Amongst the remaining 158 patients one had 6q16.2 deletion (including SIM gene) diagnosed by GTG-banding. Multiplex ligation-dependent probe amplification (MLPA) studies (SALSA P147, P036/B, P064) were performed on the remaing patients. These analyses disclosed 4 patients with <3Mb 1p36 terminal deletion, 1 patient with an atypical 17p11.2 deletion (including RAI1 gene) and 1 patient with an inherited atypical 22q11.2 deletion further characterized by SALSA P023; P204, FISH and microsatellites analyses. Besides, subtelomeric MLPA detected a deletion of chromosome 3p probe in 1 patient, subsequentely confirmed with SALSA P208 which revealed a <2.5Mb terminal deletion. The clinical features of patients with 1p36, 17p11.2 and 22q11.2 deletions were either not typical or specific enough to allow diagnosis before MLPA analyses. Human disorders such as PWS and other syndromes associated with obesity and hyperphagia as those described here could play an important role in the understanding of feeding behavior. Investigation of patients with syndromic obesity in spite of the overlap of their phenotypes can lead to the recognition of new syndromes. Supported by: FAPESP, CEPID/FAPESP, CNPq.

1685/F

Characterization by microarray CGH of a de novo partial monosomy 10q26.3 and trisomy 17q25.3 in a patient with dysmorphic features and developmental delay. *Y. Wang, J.E Martinez, J. Chaplin, C.M Tuck-Muller, W. Wertelecki, T.J Chen.* Dept Medical Genetics, Univ South Alabama, Mobile, AL.

Patients with terminal deletion of 10q or partial duplication of terminal 17q have been suggested to have a characteristic phenotype (Irving, 2003; Brisset, 2006) and previously reported cases of partial trisomy 17q were associated with partial monosomies of other chromosomes (e.g. 5p15, 12p13.3, 9p21 and 12q24). We report a patient with dysmorphic features and global developmental delay with a de novo monosomy 10q26.3 and trisomy 17q25.3, karoytype that has not been previously reported. The patient is a 7 year old white male with history of early FTT and global developmental delay. He is the product of the only pregnancy to young, non-consanguineous parents. The child was born at term with a birth weight of 2.8 kg from a pregnancy that was uneventful and at 16 months of age, his measurements were: OFC 46 cm (5th%); Height 74 cm (<5th%) and Weight 9.6 kg (10th). He was short and disproportioned with a short arm span, craniofacial dysmorphism including prominent forehead, high nasal bridge, hypertelorism, long palpebral fissures, dysplastic ears, joint laxity, broad distal phalanges and brittle finger nails. Cytogenetic studies revealed 46, XY, add (10)(q26.3). Parental chromosomes were normal and analysis of subtelomeric regions by multiple ligation-dependent probe amplification (MLPA) revealed a deletion in 10q26.3 and duplication of 17q25.3. To precisely determine the deleted and duplicated regions, we performed microarray analysis using high resolution oligo CGH array. The deletion in 10q26.3 was about 1.7 Mb in size (from 133.6 MB to 135.3 MB of chromosome 10). The duplication in 17q25.3 was about 5.4 Mb in size (from 73.4 MB to 78.8 MB of chromosome 17). Both 10q26.3 and 17q25.3 are gene rich regions. Monosomy 10q and trisomy 17q are both characterized by postnatal growth retardation, hypertelorism, and microcephaly. Our patient also had severe, universal and progressive hypotonia, which has not been delineated in previous reports on either monosomy 10q or trisomy 17q .

1686/F

Two, non-identical, *de novo* markers derived from chromosome 1 associated with cardiac abnormalities and cleft lip and palate. *C. Astbury[1], L. Christ[2], C.A. Curtis[2], R.L. Hassan[1], M. Jamehdor[1], G.E. Tiller[1].* 1) Genetic Testing Laboratory and Department of Genetics, S Calif Permanente Med Group, Los Angeles, CA; 2) Center for Human Genetics Laboratory, University Hospital Case Medical Center, Cleveland, OH.

Marker chromosomes are seen in approximately 1 in 1,000 prenatal studies, generally in a mosaic form with a normal cell line, and, when de novo, require rapid and precise characterization. Amniocentesis performed at our institution on a 37 year old G5P3sAb1 woman referred for AMA revealed two abnormal cell lines. Thirteen of 20 colonies revealed 48,XY with two small, non-identical marker chromosomes; the remaining colonies showed 47,XY with one marker chromosome. The markers were de novo, non-satellited, and not derived from the X or Y chromosomes or chromosomes 15 or 22 (excluding the centromeric region). Prenatal ultrasound at 16 weeks only revealed cleft lip. A 3,230g infant was born at term with unilateral cleft lip and palate, hypoplastic aortic arch, ventricular septal defect, and patent ductus arteriosus. He underwent successful cardiac surgery in the newborn period. In peripheral blood leukocytes, three cell lines were seen {mos 48,XY,+2mar dn[20]/47,XY,+mar dn[7]/46,XY[3]}; following FISH with alpha satellite-specific probes, the non-identical markers were determined to be derived from chromosome 1. Phenotypes associated with chromosome 1 markers thus far reported in the literature vary from normal to mild facial dysmorphism with developmental delay. We propose that trisomy/tetrasomy for pericentric loci from chromosome 1 is responsible for the infant's structural defects. Breakpoint mapping studies in this case are in progress.

1687/F

Pure partial trissomy 4q32q34: a familial report with the associated phenotype. *A. Guichet[1], E. Colin[1], O. Ingster[1], D. Bonneau[1,2].* 1) Department of Biochemistry and Genetics, CHU, Angers, France; 2) INSERM U694, Angers, France.

Duplication of the long arm of chromosome 4 has been described in more that 60 patients. In most cases, the duplication resulting from an unbalanced segregation of a balanced translocation in one of the parents is associated with partial monosomy for other chromosomal material. This leads to wide phenotypic variability complicated by the number and size of the breakpoints reported. However, partial duplication of chromosome 4q has been very rarely reported. We present a case of familial pure partial duplication of 4q32q34, affecting a mother and her two sons, characterized by distinctly dysmorphic features and mental retardation. The index case was a boy referred for developmental delay at 18 months of age. The pregnancy had been uneventful. The child had dysmorphic features but showed no growth retardation. Bilateral convergent strabismus had appeared at the age of 12 months. At the time of consultation, the psychomotor delay was such that the child could neither walk nor talk. Cytogenetic analysis performed on a peripheral blood sample revealed a 46,XY, der(6)ins(6;4)(q26;q32.1q35) abnormality. FISH analysis (using commercial, BAC, and MultiFish probes) confirmed the pure trisomy 4q32q34. When the parental karyotyping was done, the mother's karyotype showed the same 46,XX, der(6)ins(6;4)(q26;q32.1q35) abnormality. She also had distinctively recognizable dysmorphic features, and suffered from moderate mental retardation and strabismus. The couple had a second child for which they refused a prenatal cytogenetic diagnosis. Eventually, at birth they allowed the child to be karyotyped. He was then discovered to have the same karyotype as the mother and his older brother. He too had dysmorphic features but no strabismus as yet. As simple duplications are more useful for defining the trisomy 4q phenotype, this case of familial pure partial trisomy 4q32q34 should contribute to the karyotype/phenotype correlation for this critical region.

1688/F

Unique interstitial 3p duplication in a patient with multiple congenital anomalies. *E.M. James[1], K.R. Schmidt[1], U. Surti[2], L.A. Gole[2].* 1) Medical Genetics, Children's Hospital of Pittsburgh, Pittsburgh, PA; 2) Pittsburgh Cytogenetics Laboratory, Magee-Womens Hospital, Pittsburgh, PA.

Here we report on a ten-year-old female patient with a duplication of 3(p23p25) identified by karyotype. Although duplication of the entire p-arm of chromosome 3 and terminal duplications of 3p have been well-characterized, literature review has not identified any other cases of this specific interstitial duplication. Our patient's clinical features include tetralogy of Fallot, cortical dysplasia with pachygyria/polymicrogyria, mental retardation, and minor dysmorphic features including a square face, full cheeks, and hypertelorism. She developed seizures at two years. At the age of ten years, she uses only a few words and signs but does communicate using a picture board. Several other patients have been described in the past with overlapping duplications, including two related patients with partial trisomy 3p and congenital heart disease, psychomotor retardation, and similar facial features. She is similar to another patient with an overlapping 3p duplication in her speech delay, mental retardation, and psychomotor retardation. A fourth patient with a terminal deletion of 3p with a slight overlap at 3p25 had mental retardation, hypotonia, and mild dysmorphic features. Fifteen other patients have been reported with terminal duplications of 3p. Ten of fifteen had congenital heart defects. Several had cleft lip with or without cleft palate. Our patient differs from those previously reported in that she has the smallest duplicated area, and she is the first reported with documented structural abnormalities of the brain. Narrowing the duplicated area should help to delineate genes within this area important in cardiac and brain malformations.

1689/F

A 46,XX male with bilateral ovotestes and no detectable SRY in peripheral blood or gonadal material. *JJD. Morrissette[1], D. Paduch[2], JP. de Chadarévian[1], F. DeLuca[3], T. Shaikh[4], R. Steckler[5].* 1) Dept Pathology & Lab Medicine, St Christopher's Hospital for Children, Philadelphia, PA; 2) Dept Urology, Weill Medical College of Cornell University, Population Council Center for Biomedical Research, New York, NY; 3) Section Endocrinology, St Christopher's Hospital for Children, Philadelphia, PA; 4) Dept Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Section Urology, St Christopher's Hospital for Children, Philadelphia, PA.

We present a 4 year-old boy with normal male external genitalia, a 46,XX karyotype in peripheral blood, as well as left and right gonad, and left and right scrotal skin. The patient came to medical attention with a history of right testicular pain, intermittent over the past year. Physical examination revealed a red, swollen right scrotum. Ultrasound evaluation seemed consistent with right testicular torsion, with the right scrotum appearing atrophic and fixed. Surgical exploration revealed multiple "matted masses" adjacent to the right testicle, that grew Staphylococcus aureus. Gross examination identified a small hydrocele, an abnormal appearing right testicle, and an epididymis that looked abnormal and enlarged, which prompted genetic evaluation. Cytogenetic studies revealed a 46,XX karyotype in peripheral blood, which was SRY-negative by fluorescence in situ hybridization (FISH). Biopsy material from both gonads revealed ovotestes bilaterally, with ovarian follicles adjacent to testicular structures consisting of seminiferous tubules with no conspicuous Leydig cells. No primordial germ cells or spermatogonia were identified. Molecular genetic and cytogenetic studies confirmed that both gonads and scrotal skin were 46,XX and SRY negative in over 500 cells studied from each tissue. Nested PCR failed to identify SRY or Y sequences in peripheral blood or tissue from either gonad. Array CGH from gonadal tissue demonstrated an apparently normal female karyotype. This represents a rare case of a true gonadal hermaphrodite with normal male external genitalia, apparently normal female karyotype, and no demonstrable SRY.

1690/F

Branchiootic syndrome-3 and Oculoauriculovertebral spectrum features in a family with a duplication including *SIX1* and *SIX6*. *Z. Ou[1], D.M. Martin[2], M.L. Cooper[1], A.C. Chinault[1], P. Stankiewicz[1], S.W. Cheung[1].* 1) Dept Molecular & Human Genetics, Baylor Colloge Medicine, Houston, TX; 2) Dept Pediatrics and Human Genetics, University Michigan Medical School, Ann Arbor, MI.

Chromosomal insertions are rare structural aberrations with an estimated frequency of 1 in 5,000 infants. We present a 7-month-old boy with developmental delay and multiple congenital anomalies, including multiple preauricular and facial skin tags, right optic nerve hypoplasia, occipital encephalocele with large fontanels, poorly ossified and irregular skull shape, posteriorly sloping prominent forehead, prominence of the maxilla, severe hypoplasia of the mandible, low set ears with abnormally formed antihelix and ear lobe, highly arched palate, broad nasal bridge, small kidneys, small genitalia with cryptorchidism, and bilateral talipes equinovarus. These features suggested branchiootic syndrome-3 (MIM 608389, BOS3) and oculoauriculovertebral spectrum (MIM 164210, OAVS). His father had mental retardation, short stature, hypernasal speech, minor craniofacial dysmorphisms, including tall forehead and crowded dentition. Chromosomal microarray analysis revealed a copy number gain detected by a single BAC clone RP11-79M1 at band 14q23.1. FISH analysis using this clone as a probe showed the third copy of this sequence inserted into the mid long arm of one chromosome 13. The same insertion duplication was also present in the father. To delineate the chromosome breakpoints, array CGH with 244K oligonucleotide probes was used. It revealed an ~11.69 Mb gain of chromosome 14q22.3-q23.3 and a loss of an ~4.38 Mb chromosome fragment in 13q21.31-q21.32 in both the proband and his father. The deleted region on 13q is extremely gene poor and harbors only one gene, *PCDH9*, which encodes protocadherin-9 and has not been related to any disease. Chromosome region 14q22.3-q23.3 contains 51 genes, including *SIX1* and *SIX6*. Interestingly, mutations in *SIX1* have been reported in patients with BOS3 and mutations in *SIX6* cause microphthalmia, cataract, and nystagmus (MIM 212550). We propose that duplication of *SIX1* and *SIX6* causes BOS3 and OAVS-like features in our patients, respectively.

1691/F

Identification of mosaic partial trisomy 12p (Pallister-Killian Syndrome) by FISH analysis. *C.W. Yu[1], O.B. Evans[2], H. Huang[1], C. Thompson[1], O.A. Abdul-Rahman[1].* 1) Dept Preventive Medicine; 2) Dept Pediatrics, Univ of Mississippi Med Ctr, Jackson, MS.

Pallister-Killian Syndrome (PKS) was first reported in 1977 with mosaicism in fibroblasts for an extra chromosome composed of 12p. PKS was characterized by profound psychomotor retardation, inability to sit or speak, seizures, and joint contractures. We report a case of partial duplication of 12p in an infant with unusual chromosome rearrangements identified from blood lymphocytes. A Caucasian female infant was born at 39 weeks gestation to a 22 year-old primigravida mother and 22 year-old father. The mother experienced tonsillitis during the pregnancy, but was otherwise uncomplicated. The infant could not sit or crawl at nine months and was hospitalized for seizures at ten months. On physical examination at 11 months, the infant had a head circumference 45.3 cm, height of 74.6 cm, and weight of 12.6 kg. The dysmorphic features included a board forehead with high anterior hairline, hypertelorism, flat nasal bridge, short upturned nose, down turned corners of the mouth, short fingers, single transverse crease in the right hand, and hypotonia. Chromosomes from PHA stimulated blood cultures were analyzed. Five of the eighty G-banded metaphases examined had extra material on the terminal short arm of chromosome 12. G-banding suggested that it might be a translocation from chromosome 21 or a duplication of 12p. Both parental chromosomes were normal. Tissue chromosome study was not available. FISH studies were done using locus specific probes for loci TEL (12p13.1) and AML1 (21q22), telomere probes for 12pter and 12qter, and whole chromosome panting probes for 12. FISH and cytogenetic studies suggest that the derivative 12 possibly has double interstitial duplications from 12p12.2 to 12p13.31, which might have resulted from repeated DNA replications within a crossing-over loop in a somatic cell. The infant has mosaic tetrasomy of segment 12p12.2 to 12p13.31 (three on the derivative chromosome 12 and one on the normal chromosome 12) and has clinical features of PKS, further demonstrating the critical genomic regions and the phenotypic expressions.

1692/F

True hermaphroditism with a 46,XX/46,XY karyotype. *A.L. Zaslav[1], L. Mehta[2], J. Jacob[3], T. Mercado[1], L. Palmer[4].* 1) Cytogenetics, State University of New York at Stony Brook, Stony Brook, N.Y; 2) Schneider Children's Hospital, LIJ, New Hyde Park, N.Y; 3) Division of Cytogenetics, Dept. of Lab Medicine, LIJ, New Hyde Park, N.Y; 4) Department of Urology, Schneider Children's Hospital, LIJ, New Hyde Park, N.Y.

True hermaphroditism (TH) is a rare condition in which the external genitalia are ambiguous and the gonads have both ovarian and testicular elements. We present a newborn infant with TH and a 46,XX/46,XY karyotype. At birth the infant presented with ambiguous genitalia, no palpable testes and clitoromegaly. Two different surgical procedures (S1,S2) were performed. Endoscopic evaluation revealed a 1cm urogenital sinus and a vagina with a single cervical os posteriorly. Laparoscopy (S1) showed a single uterus with two gonads. Gonadal biopsy revealed predominantly testicular tissue with normal appearing seminiferous tubules (ST). Close to the surface ST appeared smaller and poorly formed indicating gonadal dysgenesis. On the left was a fallopian tube. The biopsy of this structure revealed ovarian tissue, with a mixture of germ cells consistent with gonadoblastoma. At the second surgery the child underwent bilateral gonadectomy and awaits vaginoplasty and clitoroplasty. Chromosome analysis and FISH analysis performed on peripheral blood (BL) and skin (S), left (LG) and right (RG) gonadal tissue from the two surgical procedures showed 46,XX/46,XY in BL, S, LG, RG (S2). Only 46,XX cells were observed from the RG from S1. The LG from S1 failed to grow. FISH on 200 nuclei, S and G using the CEP X/CEP Y probes and SRY/CEP X probe (Vysis, Downers Grove, IL) on BL was performed. All tissues except RG (all XX) from S1 revealed a XX/XY signal pattern in varying proportions. Metaphase FISH on BL revealed that 7/10 cells were positive for one X centromere and the SRY critical region of the Y. TH is rare with only 10% having a 46,XX/46,XY karyotype. Patients present with significant diagnostic and management challenges involving various disciplines. This case study will assist in increasing knowledge of long-term genetic, physical and psychosocial care for patients with TH.

1693/F

Micronuclei in diabetes: folate supplementation diminishes micronuclei in diabetic patients but not in an animal model. *M.L. Ramos-Ibarra[1], C.M. Batista-González[1], B.C. Gómez-Meda[1], A.L. Zamora-Perez[2], T. Muñoz-Magallanes[3], C. Ramos-Valdés[3], M.P. Gallegos-Arreola[4], G.M. Zúñiga-González[1].* 1) Laboratorio de Mutagénesis, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Laboratorio de Farmacogenómica y Biomedicina Molecular, CIIDIR, IPN, Durango, Dgo., México; 3) Servicio de Endocrinología, Unidad Médica de Alta Especialidad, Hospital de Especialidades, Centro Médico Nacional de Occidente "Lic. Ignacio García Téllez", IMSS, Guadalajara, Jal., México; 4) Laboratorio de Genética Molecular, CIBO, IMSS, Guadalajara, Jal., México.

Diabetes mellitus (DM) is associated with a high risk of health complications, mainly due to excessive free radical (FRs) production that could result in an increased frequency of micronuclei. The consumption of antioxidants, like folic acid (FA), may mitigate the effects of the FRs. Micronucleated polychromatic erythrocyte (MNPCE) frequencies were determined in blood sampled weekly from the tails of pregnant female Wistar rats and pregnant Wistar rats with experimental diabetes that were given unsupplemented diets and diets supplemented with FA. At birth, the pups were sampled to analyze micronucleated erythrocyte (MNE) and MNPCE frequencies. Micronucleated cells (MNCs) were evaluated in buccal mucosa samples taken from 81 healthy adult subjects, 48 patients with DM, and 30 DM patients who were sampled before and after FA treatment. Increases in MNPCE frequencies were significant only at the first sampling ($P<0.01$ and $P<0.03$) in pregnant rats with experimental diabetes. Pups from the diabetic group and from diabetic group treated with FA had higher frequencies of MNEs ($P<0.03$ and $P<0.001$, respectively) and MNPCEs ($P<0.009$ and $P<0.05$, respectively) than controls. No differences were found in diabetic rats and newborn rats born to diabetic mothers treated with FA compared with untreated animals. Patients with DM had a higher frequency of MNCs compared with healthy subjects ($P<0.001$). FA reduced the frequency of MNCs in DM patients ($P<0.001$). These results indicate that diabetes results in elevated frequencies of micronuclei, and that, at least in humans, FA can protect against the elevation.

1694/F

Non-disjunction of chromosome 13. *A. Collins[1], M. Bugge[2], M.B. Petersen[3].* 1) Dept Human Genetics, Univ Southampton, Southampton, United Kingdom; 2) Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark; 3) Department of Genetics, Institute of Child Health, Athens, Greece.

We performed a molecular study with 21 microsatellites on a sample of 82 trisomy 13 conceptuses, the largest number of cases studied to date. The parental origin was determined in every case and in 89% the extra chromosome 13 was of maternal origin with an almost equal number of maternal MI and MII errors. The latter finding is unique among human autosomal trisomies, where maternal MI (trisomies 15, 16, 21, 22) or MII (trisomy 18) errors dominate. Of the 9 paternally derived cases 5 were of MII origin but none arose from MI errors. There was some evidence for elevated maternal age in cases with maternal meiotic origin for liveborn infants. We find clear evidence for reduced recombination in both maternal MI and MII errors and the former is associated with a significant number of tetrads (33%) that are nullichiasmate, which do not appear to be a feature of normal chromosome 13 meiosis. This study supports the evidence for subtle chromosome-specific influences on the mechanisms that determine non-disjunction of human chromosomes, consistent with the diversity of findings for other trisomies.

1695/F

Comparison of X Chromosome Inactivation Patterns in Multiple Tissues from Human Females. *D.C. Bittel, N. Kibiryeva, Z. Talebizadeh, M.G. Butler.* Section of Medical Genetics, Children's Mercy Hospital and Clinics and University of Missouri-Kansas City, MO.

X-chromosome inactivation (XCI) is the mechanism by which gene dosage uniformity is achieved between female mammals with two X chromosomes and male mammals with a single X chromosome. XCI occurs early in embryonic development of somatic cells in human females and is subsequently stable in all daughter cells. Therefore, each tissue of a female is composed of cells which actively transcribe genes from either the maternal or the paternal X chromosome. X chromosome silencing is thought to occur at random in the general female population; therefore the ratio of the maternal to paternal XCI should have a normal distribution with an average ratio of maternal to paternal XCI of approximately 50:50. However, females have been reported with X-linked disorders (e.g., Rett syndrome) and XCI skewness (e.g., > 80:20). For genetic testing, tissues of convenience (e.g., blood) are commonly used; however, the relationship with inaccessible tissues (e.g., brain) is poorly understood. For accessible tissues to be informative for genetic analysis, a high degree of concordance of genetic findings among tissue types would be required. We analyzed XCI patterns in multiple tissues from human females to determine the relationship of XCI among several tissues within individuals at different ages. We analyzed 278 autopsy tissues from 26 females grouped by age as follows: fetus (N=4); 0 - 2 yrs (N=5); 5 - 8 yrs (N=3); 15 - 20 yrs (N=4); 21 - 40 yrs (N=4); 41 - 60 yrs (N=3) and > 60 yrs (N=3). Thirty six different tissues were collected from the three embryonic germ layers with an average of 4 tissues from endoderm (e.g., liver, pancreas, lung, colon), 6 tissues from mesoderm (e.g., blood, spleen, heart, kidney, psoas, adrenal) and 2 from ectoderm (e.g., skin, cerebrum). There was a trend for increasing XCI skewness in blood DNA with age. However, the variation in XCI pattern was reasonably consistent within a subject (< 17% variation) particularly in younger females suggesting it may be reasonable to extrapolate between accessible and inaccessible tissues of interest for study.

1696/F

GENETIC ANALYSIS IN RECURRENT IVF FAILURE. *M. Bilal Shamsi[1], R. Dada[1], R. Kumar[1], N.P. Gupta[2], R.K. Sharma[3], R. Kumar[2].* 1) Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) Department of Urology, All India Institute of Medical Sciences, New Delhi, India; 3) Research and Referral Hospital, Delhi Cantt.

Infertility is the lack of pregnancy after one year of regular unprotected sexual intercourse. About 15-20% couples harbour genetic abnormalities. These Chromosomal abnormalities in infertile couples results in spermatogenic arrest, premature ovarian failure, implantation failure and consequently failure of InVitro fertilization (IVF). The aim of the study was to determine genetic basis for recurrent ART/IVF failure. Fiftyfour infertile couples with IVF failure having poor blastocyst development and implantation were analyzed cytogenetically and for molecular analysis of AZF loci in the men. Two females with recurrent IVF failure showed partial deletion of Xq, three had deletion in the Xp22.3-24, and the other female had 10% cell line showing deletion of pericenteromeric region of long arm of chromosome number 1. Four men had chromosomal abnormality. Out of this two had a translocation in the D-D group chromosomes and two had balanced reciprocal translocations on autosomes. Of these couples microdeletion analysis of 50 cytogenetically normal infertile men, only two cases showed deletion; one with AZFc loci and the other case had deletion of AZFb loci. The couples where female partner had deletion of long arm of X chromosome (Xq-) resulted in repeated failure of blastocyt development, in 4 IVF cycles. The case with AZFb microdeletion had maturation arrest and case with AZFc deletion had hypospermatogenesis. In these cases sperms could be retrieved from the testis and to be used for IVF or Intracytoplasmic sperm injection. (ICSI). In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and consequently poor implantation, which may be a result of high segregation abnormalities and may negatively affect the outcome of assisted reproductive techniques. ART is a very expensive technique and recurrent ART/IVF failure results in severe financial stress coupled with emotional stress, thus all couples opting for ART must undergo genetic analysis.

1697/F

Shorter Telomeres in Older Male Individuals with the Fragile X Premutation, FXTAS, and FXTAS with Dementia. *E.C. Jenkins[1], F. Tassone[2,3], L. Ye[1], H. Gu[1], W.T. Brown[1], R.J. Hagerman[2,4], P.J. Hagerman[2,3].* 1) Dept Hum Genetics, NYS Inst Basic Res Dev Disab, Staten Island, NY; 2) MIND Institute, UC Davis Health System, Sacramento, CA; 3) Dept Biochem Mol Med, UC Davis School of Medicine, Sacramento, CA; 4) Dept Ped, UC Davis Health System, Sacramento, CA.

We have recently reported shorter telomeres, chromosomal termini with highly conserved TTAGGG repeats, in T lymphocytes of people with trisomy 21 Down syndrome (DS) and dementia compared to people with DS only. We have since hypothesized that similar shortening may occur in people with fragile X-associated tremor/ataxia syndrome (FXTAS) and dementia. Shorter telomeres also have been associated with cell senescence, Alzheimer Disease, neoplastic transformation, and increased psychological stress. To address the question of telomere shortening in FXTAS, approximately 300,000 mononuclear cells/ml were PHA-cultured from 10% dimethylsulfoxide(DMSO)-cryoprotected buffy coats that had been obtained from male individuals who carried the premutation with or without FXTAS and/or dementia. Telomeres were detected with an FITC-labeled peptide nucleic acid (PNA) probe [(CCCTAA)3-Cy3]. Telomere fluorescent light intensity measurements were obtained as previously described (Jenkins et al., 2006). Twenty metaphases were analyzed for each individual specimen type and compared to an age-matched control. Five control specimens were studied pairwise with age-matched premutation specimens. Shorter telomeres were observed in 4/4 individuals with FXTAS and dementia, in 4/4 individuals with FXTAS, and in 2/2 individuals with the fragile X premutation only (p values ranged from <.000001 to <.05). We were surprised to observe shorter telomeres in people with the premutation only. Additional studies to test younger individuals with the premutation are being carried out to see at what age significant telomere shortening begins compared to controls. It is possible that increased telomere shortening may serve as a biomarker before the emergence of both tremor/ataxia and dementia thus facilitating future prevention, intervention or treatment strategies. This work was supported in part by the NYS Office of Mental Retard. and Develop. Disabil.[553x105].

1698/W

Joint Effects of Interleukin 6 Pathway Genes and the Risk of Cardiovascular Disease. *M. Alanne[1], K. Auro[1], K. Kristiansson[1], K. Silander[1], K. Kuulasmaa[1], J. Saarela[1], L. Peltonen[1,2,3], V. Salomaa[1], M. Perola[1,2].* 1) Molec Med, National Public Health Inst, Helsinki, Finland; 2) Med genet, Faculty of Med, Univ of Helsinki, Finland; 3) The Broad Institute, MIT, MA.

Interleukin 6 (IL-6) is a cytokine regulating a wide range of inflammatory responses. Our aim was to identify cardiovascular disease (CVD) risk-modifying variations and their joint effects in the IL-6 pathway in two independent case-cohort samples from prospectively followed population-based cohorts, FINRISK 92 and 97 (n=999 and 1223, respectively). We selected 33 common (>5%) SNPs in angiotensin receptor 1, angiotensin I converting enzyme, IL-6, C-reactive protein, and fibrinogen alpha (FGA), beta and gamma genes based on haplotype information and previous reports. To identify joint effects of these genes, we first analyzed them by using recursive partitioning techiniques and random sampling. All SNPs (n=15) present in >10% of the classification trees and their pairwise combinations were then analyzed with Cox's model, using false discovery rate (FDR) and replication to control for the number of tests. The results indicate that men carrying the minor alleles of both a SNP in IL-6 and in FGA gene have decreased risk of CVD compared to men that carry a minor allele of either of the SNPs: a hazard ratio 0.27 (95% CI: 0.12-0.61) was observed in the FINRISK 97 cohort, and the trend was similar in the FINRISK 92 cohort (0.52 (0.22-1.19). The association was significant after FDR when both male cohorts were combined (0.35 (0.20-0.62)) and when both sexes from both cohorts were combined (0.40 (0.25-0.63)). Meanwhile the comparison between individuals with no copies of the minor alleles and carriers of minor allele from either of the SNPs was nonsignificant. The IL-6 SNP minor allele associated risk was 0.80 (0.60-1.09) and for the FGA SNP 0.66 (0.46-0.93) in combined male cohorts. The results were nonsignificant in females. Our two-stage strategy revealed a new combination of genetic polymorphisms in a biological pathway influencing the risk of CVD consistently in two separate samples from Finland. The method can be applied to larger datasets to identify interacting SNPs in other pathways as well.

1699/W

***OSBPL11* Gene Polymorphisms are Associated with Obesity-Related Metabolic Complications and Diabetes in Obese Individuals.** *L. Bouchard[1,2,3], G. Faucher[1,2,3], A. Tchernof[2,3,4], Y. Deshaies[5], S. Marceau[6], O. Lescelleur[6], S. Biron[6], M.-C. Vohl[1,2,3].* 1) Lipid Research Center; 2) Nutraceuticals and Functional Foods Institute; 3) Department of Food Science and Nutrition; 4) Molecular Endocrinology and Oncology Research Center; 5) Department of Anatomy and Physiology and Laval Hospital Research Center; 6) Department of Surgery, Faculty of Medicine and Laval Hospital, Laval University, Quebec, Canada.

Hyperglycemia, dyslipidemia and hypertension are commonly observed in obese individuals and define the metabolic syndrome (SM). Although heritability studies provided evidence for a significant contribution of genetic factors in MS, only a limited number of genes have been consistently associated with this condition. Recently, an inventory of MS candidate genes has been generated by comparing the omental adipose tissue gene expression profile of non-diabetic obese men with and without MS. Of the genes found to be differentially expressed, the oxysterol-binding protein-like protein 11 (*OSBPL11*) was found to be slightly but significantly overexpressed in the MS group (1.34-fold, P<0.05). **Objective**: To determine whether *OSBPL11* gene polymorphisms are associated with MS and its individual components. Methods: *OSBPL11* gene promoter and coding regions were sequenced in 25 obese men and common tagging SNPs (r^2<0.75 between the SNPs; rs7625936, rs1055419, rs2979382, rs12496976, rs12487030 and IVS12+95) were genotyped in a sample of 958 obese individuals. Chi-square tests were applied to compare genotype frequencies between low and high-risk groups according to MS as defined by the NCEP-ATPIII guidelines. **Results**: rs7625936, rs2979382 and IVS2+95 were associated with diabetes (p=0.039, p=0.008 and p<0.0001), whereas significant associations were observed between diastolic blood pressure and rs1055419, rs12496976 and rs12487030 (p=0.014; p=0.042 and p=0.006). Moreover, IVS2+95 was associated with fasting plasma glucose (p=0.001), HDL- (p=0.011), LDL- (p=0.001) and total-cholesterol (p=0.011) levels as well as with MS (p=0.006). **Conclusion**: These results suggest that *OSBPL11* gene polymorphisms are associated with obesity-related metabolic complications and diabetes.

1700/W

Evidence Of Common Genetic Determinants For Insulin Resistance And Thrombosis. *J. Cui[1], X. Guo[1], K.D. Taylor[1], S. Cheng[2], R. Hughes[2], J. Li[2], W. Hsueh[3], J.I. Rotter[1].* 1) Cedars-Sinai Med Ctr, Los Angeles, CA; 2) Roche Molecular Systems, Inc., Alameda, CA;; 3) UCLA, Los Angeles, CA.

The metabolic syndrome (MS) is characterized by the clustering of a number of metabolic abnormalities in the presence of underlying insulin resistance, with a strong association with diabetes and cardiovascular disease morbidity and mortality. It has been suggested that MS may contribute to the development of venous thromboembolism and may act as a link between venous thrombosis and atherosclerosis. MS and thrombosis have been shown to have underlying genetic determinants individually. However, no data is available regarding the possibility of a common genetic basis for the association between MS and thrombosis. In this study, we evaluated the association between insulin resistance and 7 variations in 5 genes (F7, FGB, ITGA2, ITGB3, and SERPINE1) that were previously implicated in thrombosis. Families were ascertained via a coronary artery disease proband in the Mexican-American Coronary Artery Disease Project. Mexican Americans have been shown to be the population with the highest risk for MS. 656 individuals from 100 Mexican-American families were genotyped for 7 literature-associated thrombosis SNPs in the 5 genes (2 SNPs in F7 and SERPINE1, and 1 in all others). 449 adult offspring and offspring spouses were phenotyped for insulin sensitivity by the hyperinsulinemic euglycemic clamp and the insulin sensitivity index (SI) was derived. The generalized estimating equation method was utilized to evaluate the associations. Both SNPs (I/D and R353Q) in F7 were found to be associated with SI (p=0.038, 0.048), as was SNP L33P in gene ITGB3 (p=0.027). SNP G(-455)A in gene FGB was not significantly associated with SI, however a significant association (p=0.016) with homeostasis model assessment (HOMA) was identified. Our results suggest that genes in the thrombosis pathway, including F7, FGB, and ITGB3 are associated with insulin resistance/sensitivity. This supports the hypothesis that there might be genes that link insulin resistance and thrombosis in Mexican Americans, a group at high risk for the metabolic syndrome.

1701/W

Myeloperoxidase gene variations are associated with low-density-lipoprotein characteristics. *G. Dolley[1,2,3], B. Lamarche[3], J.P. Després[4,5], C. Bouchard[6], L. Pérusse[1,5], M.C. Vohl[1,2,3].* 1) CRML, CHUL Research Centre, Quebec, Canada; 2) Department of Food Science and Nutrition, Laval University, Quebec, Canada; 3) Nutraceuticals and Functional Foods Institute (INAF), Quebec, Canada; 4) Quebec Heart Institute, Quebec, Canada; 5) Department of Social and Preventive Medicine, Laval University, Quebec, Canada; 6) Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA.

Background: The small, dense LDL phenotype is associated with an increased cardiovascular disease risk. A genome-wide scan performed on 236 nuclear families of the Quebec Family Study (QFS) revealed a QTL for LDL peak particle size (LDL-PPD) on the 17q21 region. This region contains the myeloperoxidase gene (MPO). MPO is thought to be part of an important pathway for LDL oxidation and atherosclerosis progression. MPO is able to oxidize LDL, and MPO-modified LDL particles have been detected in atherosclerotic plaques. **Objectives**: To test the association between MPO gene polymorphisms and LDL-PPD as well as plasma lipid levels. **Methods**: Analyses were performed on 680 subjects of QFS. LDL-PPD was measured by gradient gel electrophoresis on non-denaturating 2-16% polyacrylamide gradient gels. Direct sequencing of the coding regions, exon-intron splicing boundaries and the regulatory regions was performed on 25 subjects. Genotyping was performed either by taqman or direct sequencing. **Results**: MPO gene sequencing revealed 16 polymorphisms. Three SNPs not in LD (r2 ≥0.73) were retained for genotyping on the whole cohort (c.-653G>A, c.157G>T and c.2149A>G). No significant association was found with LDL-PPD. However, the c.-653G>A MPO polymorphism was associated with lower plasma total cholesterol, LDL-cholesterol and LDL-apolipoprotein B (apoB) levels (p=0.036, p=0.049 and p=0.016, respectively). These associations were observed when the phenotypes were adjusted for the effects of age and sex, and remained significant with further adjustment for body mass index. **Conclusion**: The MPO gene variants are not associated with LDL-PPD. However, the c.-653G>A is associated with significant variation in LDL-cholesterol and LDL-apoB concentrations.

1702/W

Mutations of *FOG2* gene in patients with septal and conotruncal defects. *D.Y. Gibbs[1], P.C. Paluru[1], S. Woyciechowski[1], E. Goldmuntz[1, 2].* 1) Division of Cardiology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA.

Friend of *GATA2* is a zinc finger protein (*ZFPM2/FOG2*) expressed during early heart development which acts as a co-regulator of the transcription factor *GATA4*. Disease related mutations of *GATA4* and its molecular partner *NKX2.5* have been identified in patients with congenital heart defects. Studies in the mouse suggest an important role for *FOG2* in cardiac development. We hypothesized that patients with septal and conotruncal defects might have mutations in *FOG2*. The *ZFPM2/FOG2* gene maps to 8q23 and contains 9 exons encoding a protein of 1151 residues. We sequenced the coding region of *FOG2* for sequence variants in 494 patients with septal and conotruncal heart defects (97-double outlet right ventricle, 100-D-transposition of great arteries, 98-tetralogy of Fallot, 12-interrupted aortic arch, 29-truncus arteriosus, 63-ventricular septal defect, and 95-atrial septal defect). A total of twelve novel missense mutations were identified in 20 unrelated subjects, which were absent in 200 ethnically matched control samples and public databases. One mutation maps into the third zinc finger domain. Though the other mutations map outside of known conserved domains, seven of them alter amino acids conserved across all species including zebrafish, and four alter aminoacids conserved across all species other than zebrafish. The etiologic role of the novel mutations remains unclear. Functional analysis is needed in order to characterize these mutations and establish their role in altering the structure and function of the protein.

1703/W

Inflammatory genes confer risks of subclinical carotid atherosclerosis. *Y.C. Guo[1], H.F. Lin[1], E. Hsi[2], T. Rundek[3], R.L. Sacco[4], S.H.H. Juo[2,3].* 1) Hsiao-Kang Hospital, Kaohsiung City , Taiwan; 2) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, USA; 4) School of Medicine, University of Miami, FL, USA.

Background and Purpose Carotid intima-media thickness (IMT) is a reliable surrogate marker for subclinical atherosclerosis and cardiovascular risks. Family studies have provided significant evidence for the heritability of IMT, but its genetic determinants remained unsolved.

Methods A total of 96 single nucleotide polymorphisms (SNPs) at 25 inflammatory genes were genotyped in 414 Caribbean Hispanics from the Northern Manhattan Study(NOMAS). Genotyping was performed by the Illumina technology and carotid IMT was assessed by high-resolution B mode ultrasound. The relationships between SNPs and the maximal difference between intima and media (max-IMT) at common carotid artery (CCA) and carotid bifurcation (BIF) were evaluated by multivariate regression analyses. Haplotype analyses were performed by Hap-Clustering program.

Results In the Hispanic population, the CCA max-IMT was significantly associated with age and hypertension (Pearson correlation p <0.01) and the BIF max-IMT was associated with sex and diabetes (p < 0.05). Although the two phenotypes were highly correlated (r^2 = 0.46, p < 0.001), we found different genes in relation to the max-IMT values at these two segments (p < 0.005). The interleukin 6 receptor (IL6R) and the transforming growth factor beta 2 (TGFB2) genes were related to BIF max-IMT; while CXC motif chemokine ligand 12 gene (CXCL12) and the interleukin 6 (IL6) genes were related to CCA max-IMT. Haplotype analysis yielded similar results as single SNP analysis. Since the hemodynamic factors have different influences on CCA and BIF, the genetic determinants of the max-IMT values at different segments of carotid artery may vary substantially.

Conclusion The present study identified four inflammatory genes that confer risks to the subclinical carotid atherosclerosis.

1704/W

Phosducin, a novel candidate gene for human essential hypertension. *M.D. Harrison[1], M. Stoll[2], K. Maresso[1], R. Lorier[1], E. Virlee[1], L. Hein[3], P. Hamet[4,5], D. Gaudet[4], O. Seda[5], J. Tremblay[4,5], M. Kaldunski[1], T. Kotchen[1], A.W. Cowley[1], U. Broeckel[1].* 1) Medical College of Wisconsin, Milwaukee, WI; 2) University of Münster, Germany; 3) University of Freiburg, Germany; 4) Université de Montréal, Canada; 5) Centre Hospitalier de l'Université de Montréal, Canada.

Hypertension and its complications represent leading causes of morbidity and mortality and evidence suggests that a significant portion of risk is determined by genetic factors. Recently, phosducin (PDC) emerged as a novel candidate, since a knockout mouse model develops profound hypertension particularly during stress. The aim of this study was to test whether SNPs in phosducin influence blood pressure (BP) phenotypes in humans. We studied French-Canadians (FC) in a unique founder population, and African-Americans (AA), for 849 and 341 individuals, respectively. To dissect BP as a complex phenotype, we measured resting as well as various stress induced and other related BP measurements as intermediate phenotypes. To describe the haplotype structure for the PDC region, we resequenced PDC and genotyped 21 SNPs covering a region of 500,000 base-pairs centered on PDC. Several new SNPs were found that could be functionally important. Significant associations ($p<0.01$) for BP phenotypes were found in both populations at markers in the PDC, and SNPs in strong linkage disequilibrium (LD) extending into the neighboring prostaglandin G/H synthase 2 (PTGS2), the gene encoding cyclooxygenase 2. High levels of LD in the FC population cover the entire region, but LD is broken down between the 2 genes in the AA population. By focusing on 6 traits, we found PDC to be associated with resting BP in FC ($p=0.0002$) and BP response to stress in AA ($p=0.00005$). PTGS2 is associated with stress BP in FC ($p=0.0003$) and overall BP regulation in AA ($p=0.001$). To conclude, PDC is associated with BP phenotypes in both human populations with an emphasis on resting BP in FC and stress BP in AA. The presence of candidate genes in close proximity, PDC and PTGS2, supports the notion of functional gene units. Our findings provide further evidence that PDC is involved in the control of BP, and is therefore an important new candidate gene for hypertension.

1705/W

Association of TCF7L2 variants with high serum triglycerides in Mexican dyslipidemic families. *A. Huertas-Vazquez[1], T. Tusie-Luna[2], E. Nikkola[1], C. Aguilar-Salinas[2], P. Pajukanta[1].* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Instituto de Investigaciones Biomédicas de la UNAM, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City, Mexico.

Transcription factor 7-like 2 (TCF7L2) has been shown to be strongly associated with an increased risk of type 2 diabetes in different populations. Familial combined hyperlipidemia (FCHL), characterized by elevated levels of serum total cholesterol (TC), triglycerides (TGs) or both, is the most common mixed dyslipidemia in Mexicans. However, the Mexican population has been underinvestigated for the genetic factors conferring the susceptibility to dyslipidemias. Considering the clear phenotypic overlap between type 2 diabetes and FCHL, both predisposing to high serum triglycerides, glucose intolerance and coronary artery disease, we hypothesized that TCF7L2 may contribute to the genetic susceptibility to FCHL. We investigated the effect of the TCF7L2 variants, rs7903146 and rs12255372, previously associated with T2DM on the most clinically relevant lipid traits in FCHL (TGs and TC) in 759 individuals from 55 extended Mexican FCHL families. For the SNPs rs7903146 and rs12255372, the frequencies of the T allele (0.15 and 0.16 respectively), and linkage disequilibrium between these SNPs ($D'=0.86$, $r2=0.7$ in spouses) were lower than the corresponding values reported in European and African American populations. Family-based association analyses using the FBAT software showed significant evidence for association with both the qualitative and quantitative TG traits for the SNP rs7903146 ($P= 0.002$ and $P= 0.005$). The SNP rs12255372 showed similar results ($P= 0.03$). These results suggest for the first time that variants in TCF7L2 are significantly associated with high TG levels in Mexican families with FCHL.

1706/W

Association of polymorphism of the liver X receptor gene with angina pectoris in the Japanese population. *M. Inoue[1], R. Uemura[1], T. Ikezaki[1], S. Kobayashi[1], S. Ikeda[2], S. Kohno[2], K. Tsukamoto[1].* 1) Dept of Pharmacotherapeutics, Nagasaki Univ Graduate Sch of Biomed Sci, Nagasaki, Japan; 2) 2nd Dept of Internal Med, Nagasaki Univ Sch of Med, Nagasaki, Japan.

Objective: Coronary artery disease (CAD) is a multifactorial disorder and consists of two major forms, angina pectoris (AP) and myocardial infarction (MI). The etiology of CAD contributes to atherosclerosis of coronary arteries. As a candidate gene susceptible to CAD, we focused on liver X receptor (LXR). LXR is a nuclear receptor that binds oxysterols. LXR has two isoforms, LXRα and LXRβ, which play a role in decreasing cholesterol accumulation through inhibiting intestinal cholesterol absorption and transfer from peripheral tissues to the liver, while through stimulating uptake into liver, catabolism into bile acids, and biliary secretion, leading to protection against atherosclerosis and hypercholesterolemia. Thus, we have examined an association of *LXR* polymorphisms with CAD in the Japanese population. Methods: We studied 146 patients with AP, 97 patients with MI, and 165 gender- and age-matched control subjects with normal coronary artery after coronary angiography. Four single nucleotide polymorphisms (SNPs) in *LXR*α were detected by PCR-restriction fragment length polymorphism and PCR-direct DNA sequencing analysis. Haplotypes composed of these 4 SNPs were estimated using SNP Alyze 6.1. The frequencies and distributions of haplotypes and diplotypes were compared between patients and controls by multivariate logistic regression analysis. Results: The frequencies of haplotype 2 ("G" at rs12221497, "T" at rs2279239, "C" at rs2279238, and "T" at rs7120118: G-T-C-T) and haplotype 3 (A-C-C-C) were significantly higher in AP patients than in controls (30.1% vs. 22.4%, odd ratio (OR) = 1.488, $P = 0.031$; and 10.8% vs. 6.4%, OR = 1.789, $P = 0.049$, respectively). Of a total of 143 patients with AP, 11 (7.7%) had a "hap 2/hap 3" diplotype, the frequency being significantly higher than that in controls (4/165, 2.4%)(OR = 3.354, $P = 0.042$). Conclusion: The present study indicates that *LXR*α may be one of the determinants of AP in the Japanese population.

1707/W

Independent genetic mechanisms downregulate genes involved in catecholamine biosynthesis, storage and secretion in the spontaneously hypertensive rat. *M.L. Jirout[1], R.S. Friese[1], N.R. Mahapatra[1], M. Mahata[1], S.K. Mahata[1], M. Pravenec[2,3], V. Kren[3,2], N. Hubner[4], T.J. Aitman[5], M.G. Ziegler[1], N.J. Schork[1,6], D.T. O'Connor[1].* 1) University of California, San Diego, La Jolla, CA, USA; 2) Czech Academy of Sciences, Prague, Czech Republic; 3) Charles University, Prague, Czech Republic; 4) Max-Delbruck-Center for Molecular Medicine, Berlin-Buch, Germany; 5) Imperial College, London, UK; 6) Scripps Health/TSRI, La Jolla, CA, USA.

We investigated the regulation of genes involved in catecholamine biosynthesis, storage and secretion in the chromaffin cell of the spontaneously hypertensive rat (SHR). Integration of transcriptional profiling, biochemical phenotyping and quantitative trait locus (QTL) mapping was pursued in the adrenal tissue of the HXB/BXH recombinant inbred (RI) strains, which segregate the SHR genome. The results suggest that several independent genetic mechanisms in the SHR lead to downregulation of genes involved in the biosynthesis, storage and secretion of catecholamines. These mechanisms converge on *Dbh*, *Pnmt* (catecholamine biosynthesis) and *Vamp1* (catecholamine secretion) whose expression levels appear to be regulated by variations in these genic regions (i.e., in *cis*). In the SHR, *Dbh* is decreased (both, mRNA and tissue enzymatic activity), coupled with increased dopamine. Expression and physiological QTLs overlap and map in *cis* to chr 3 at 6 Mbp. The dopamine QTL co-localizes with the *Dbh* QTLs. *Pnmt* is also decreased (both, mRNA and tissue enzymatic activity). Expression and physiological QTLs overlap and map in *cis* to chr 10 at 90 Mbp. *Vamp1* expression QTL maps in *cis* to chromosome 4 at 161 Mbp. In addition, expression QTLs for *Vmat1* and *Chga* (both involved in catecholamine storage) co-localize to the *Pnmt* region. *Pnmt* re-sequencing revealed several promoter polymorphisms, which result in a decreased in-vitro promoter responsiveness to dexamethasone in the SHR. Finally, the SHR allele at the QTL peak locus for all above genes was associated with lower transcript levels, underscoring the additive nature of apparently independent genetic modulators of the catecholamine biology in the SHR.

1708/W

Concept and design of a custom 50K SNP array for large-scale interrogation of vascular disease cohorts. *B.J. Keating[1], T. Bhangale[2], T.S. Price[1], S.S. Tischfield[3], J.C. Barrett[4], P.I. de Bakker[3], M. Fornage[5], D.A. Nickerson[2], M.I. McCarthy[4], S.S. Anand[6], J.C. Engert[7], S.B. Gabriel[3], D.J. Rader[1], J.N. Hirschhorn[3], G.A. FitzGerald[1].* 1) Inst Translational Medicine & Therapeutics, Uni. Pennsylvania, Philadelphia, PA; 2) Dept Genome Sciences, Uni. Washington, Seattle, WA; 3) Broad Inst of Harvard & MIT, Cambridge, MA; 4) Wellcome Trust Centre Human Genetics, Uni. Oxford, UK; 5) Candidate-gene Association Resource (CARE), SNP Selection Committee; 6) Dept Medicine, McMaster Uni., Hamilton, Ontario, Canada; 7) Dept Medicine & Human Genetics, McGill, Montréal, Québec, Canada.

The recent wave of genome wide association studies (GWAS) has identified many novel genes of unknown function linked to cardiovascular, metabolic and inflammatory phenotypes in humans. Delineation of the actual causal variants, and any context specific functionality, requires very large cohorts with carefully defined phenotypic data for robust within and across cohort meta-analyses. Analyses with large cohorts are also critical for effective assessment of gene-gene and gene-environment interactions. We have designed a 50K SNP array to extensively assess the genetic diversity relevant to a spectrum of cardiovascular and metabolic diseases. The array employs a cosmopolitan tagging approach to assess diversity in all major populations represented in HapMap and Seattle SNPs across ~2100 genes. The gene content is based on recent GWAS; expression quantitative trait loci mapping of disease related genes; pathways based approaches and a comprehensive literature search. Over 140,000 extensively phenotyped individuals will be interrogated initially. The 50K array has significantly denser coverage for most cardiovascular related candidate genes compared to WGA panels and will greatly increases power for association testing in those regions. It will also facilitate unprecedented collaborative power to perform cross cohort meta-analyses for the replication of primary CVD associations in similar and different populations as well as allowing for rigorous assessment of gene-gene and gene-environment interactions.

1709/W

De novo submicroscopic deletion of 20p12.3 involving BMP2 gene in an individual with Wolff-Parkinson-White syndromeIdentifying a new locus for WPW. *SR. Lalani[1], X. Wang[1], W. Bi[1], MS. Bray[1,3], C. Shaw[1], J. Towbin[1, 2], RA. Friedman[2], G. Zapata[1], A. Pursley[1], SW. Cheung[1], JW. Belmont[1], L. Potocki[1].* 1) Dept of Genetics, Baylor Col Medicine, Houston, TX; 2) Dept of Cardiology, Baylor Col Medicine, Houston, TX; 3) Dept of Pediatrics, Baylor Col Medicine, Houston, TX.

Wolff-Parkinson-White (WPW) is a cardiac conduction abnormality that arises from a developmental defect in the atrio-ventricular electrical insulation due to the presence of an accessory pathway. It is known that about 3.4% of the affected individuals (1-3 persons in 1000) have first-degree relatives with pre-excitation, usually inherited as a mendelian autosomal dominant trait. PRKAG2 (7q34-q36) is the only gene, unequivocally known to be associated with familial WPW syndrome. Here, we report an individual with WPW, who bears a de novo submicroscopic deletion of 1.3 Mb on 20p12.3. The deletion, initially detected by clinical BAC array-CGH and fine-mapped with an Illumina HumanHap300 array, involves a single gene, BMP2. This individual was incidentally found to have WPW during the evaluation for suspected seizures and mild respiratory difficulties in the newborn period. Echocardiogram showed an ASD. Although WPW is asymptomatic in this patient, subsequent evaluations revealed pectus deformity, failure to thrive, and mild delay in language expression and comprehension at age 19 months. It is known that Bmp2 is required for myocardial patterning and plays an important role in atrioventricular cushion morphogenesis in mice. We have performed mutation analysis of BMP2 gene in 30 individuals with either isolated, familial, or syndromic WPW and have not found mutations in the coding region of this gene. Quantitative-PCR studies for BMP2 copy number alterations in these individuals are currently being analyzed. Interestingly, another individual with WPW syndrome associated with a larger deletion of 20p12.3 has recently been identified (Thakuria et al., this meeting). We propose that genetic aberrations of BMP2 can cause pre-excitation and deletions involving this gene on 20p12.3 can cause Wolff-Parkinson-White syndrome.

1710/W

Multiple genetic determinants of plasma lipid levels in Caribbean Hispanics. *Y.C. Liao[1], H.F. Lin[2], T. Rundek[3], R. Cheng[4], E. Hsi[1], R.L. Sacco[5], S.H.H. Juo[1,2,3].* 1) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Neurology, Columbia University, New York, USA; 4) The Gertrude H. Sergievsky Center, Columbia University, New York, USA; 5) Department of Neurology, Miller School of Medicine, University of Miami, FL, USA.

Objective

To identify candidate genes in relation to plasma lipid levels in Caribbean Hispanics.

Methods and Results

A total of 114 single nucleotide polymorphisms (SNPs) at 17 lipid-related genes were genotyped in 477 Caribbean Hispanics from the Northern Manhattan Study (NOMAS). Analyses for each SNP and haplotype were performed to evaluate the associations with four lipid traits: high- and low-density lipoprotein cholesterol (HDL-C, LDL-C), triglyceride (TG) and total cholesterol (TC). We identified 19 SNPs at 10 genes that were significantly related to lipids ($p<0.01$), including nine involved in the reverse cholesterol transport pathway, and one involved in bile acid synthesis. The significant genes, in conjunction, explained 10.5%, 8.7%, 8.6% and 8.6% of variation in HDL-C, LDL-C, TG and TC levels, respectively. Three genes, namely the apolipoprotein A5, apolipoprotein B and cytochrome p450 polypeptide 7A1 genes, accounted for the largest proportion of variation in HDL-C/TG, TC and LDL-C respectively; while other single genes explained less than 2% of lipid variation.

Conclusions

The present study identified 10 candidate genes that influenced plasma lipid levels in Caribbean Hispanics. Although the genetic effect of individual genes is modest, the cumulative effects of multiple genes lead to a substantially better prediction of inter-individual variations in lipid levels.

1711/W

Conditional linkage with the UGT1A1 gene and whole-genome association studies of serum bilirubin. *J-P. Lin[1], J.P. Schwaiger[2], L.A. Cupples[3], C.J. O'Donnell[4], G. Zheng[1], V. Schoenborn[2], S.C. Hunt[5], F. Kronenberg[2].* 1) Office of Biostatistics Research/NHLBI, NIH; 2) Division of Genetic Epidemiology/Innsbruck Medical University, AUSTRIA; 3) Department of Biostatistics, Boston University School of Public Health; 4) Framingham Heart Study/NHLBI/NIH; 5) Department of Internal Medicine, University of Utah.

Many studies have shown an inverse association between serum bilirubin and cardiovascular disease (CVD). Previously, we conducted genome-wide linkage studies and identified a major locus at the 2q telomere affecting bilirubin levels with LOD=3.8 (the highest LOD in the rest of the genome was 1.3). Within this region, there is a candidate gene, UDP-glucuronosyltransferase (UGT1A1). The insertion of a TA in the promoter, allele 7 (wild type: allele 6) designated UGT1A1*28, decreases gene transcription. We conducted association studies in 1780 Framingham Heart Study (FHS) subjects followed for 24 years and found individuals with genotype 7/7 to have significantly higher bilirubin levels and 1/3 the CVD risk, RR=0.30, than those with genotypes 6/6 and 6/7. We carried out a conditional linkage study using the largest 330 families from the FHS to investigate whether the UGT1A1*28 was responsible for the linkage peak of our previous linkage study. Conditional on the UGT1A1*28 association, LODs of the linkage peak dropped from 3.8 to 0.4, suggesting that the UGT1A1 association may fully explain the linkage result. We also carried out an Affymetrix 100K SNP whole-genome association study on bilirubin using GEE and confirmed the top ranked SNPs with SOLAR. The closest SNP, rs1113193, about 15,000 bp away is in linkage disequilibrium with UGT1A1*28. This SNP always ranked within the top 10 SNPs using different analytic methods, different Framingham examinations, and different ranking methods, with p-values ranging from 1.38E-08 to 9.33E-06. Our studies suggested that the UGT1A1 may be the only gene with a large effect controlling serum bilirubin levels. Since the polymorphism may have a substantial impact on the development of CVD, and the 7/7 genotype is found in 10-16% of Caucasians, the gene may be an important target for therapeutic intervention.

1712/W

Association of a *VEGF* functional allele with cardiac atrioventricular septal defects and a possible genetic interaction with *CRELD1* mutations. *C.L. Maslen[1], D. Babcock[1], C.D. Morris[2], S. Sherman[3], L.J.H. Bean[3], K.V. Dooley[4], E. Feingold[5].* 1) Molec/Med Genetics; 2) Medical Informatics, Oregon Health Sci Univ, Portland OR; 3) Genetics; 4) Pediatrics, Emory Univ, Atlanta GA; 5) Human Genetics, Univ. Pittsburgh, Pittsburgh PA.

VEGF is a potent signaling molecule that regulates endothelial cell growth and migration. It plays a key role in heart development in the formation of endocardial cushions, the precursors of atrioventricular (AV) valves and septa. Failure of this process results in the congenital heart defect known as an atrioventricular septal defect (AVSD). Increased expression of VEGF interferes with AV endocardial cushion morphogenesis. A functional *VEGF* polymorphism, +405G/C, is associated with altered VEGF expression. To test the hypothesis that this variant influences risk for AVSD, we compared the allele frequencies between subjects with AVSD and a control population of individuals without a heart defect or family history of congenital heart disease. We analyzed 65 individuals with either complete or partial AVSD (cases) and 34 controls. Among the cases, 33 had Down syndrome (DS) and 32 were non-DS and among controls, 24 had DS and 10 were non-DS. The +405C allele was more prevalent in cases than controls overall ($p<0.025$) and when stratified by DS and non-DS case/control groups ($p=0.04$ and $p=0.04$, respectively). When all cases were stratified by complete versus partial AVSD, there was a highly significant association with the +405C allele compared to controls only among complete AVSD ($p<0.009$), but not among partial cases ($p>0.10$). Overall these results indicate a specific association of the *VEGF* +405C allele with complete AVSD in both the DS and non-DS populations. In previous studies we showed that mutations of *CRELD1* are associated with AVSD. Interestingly, 4/5 AVSD cases with a *CRELD1* mutation also carried at least one *VEGF* +405C allele, and in each case where the *CRELD1* mutation was inherited it came from one unaffected parent while the *VEGF* +405C allele was inherited from the other parent. These preliminary data suggest that there may be an interaction between the *CRELD1* and *VEGF* alleles that increases the risk of AVSD.

1713/W

Essential Hypertension is associated to several worldwide genetic factors in a Sardinian genetic isolate. *E. Mocci[1], V. Cabras[1], N. Pirastu[1], M.P. Concas[2], C. Fraumene[1], M. Adamo[1], I. Persico[1], G. Biino[1,2], M. Pirastu[1,2], A. Angius[1,2].* 1) Shardna Lifesciences, Cagliari, Italy; 2) Inst Population Genetics, Alghero, Italy.

Essential hypertension (EH) affects approximately 20% of the adult population and has a multifactorial origin. Epidemiological survey of 9 villages in the secluded area of Ogliastra revealed that in the genetic isolate of Talana there is the highest EH prevalence (26%). We performed medical examination on the whole population and identified 98 affected individuals with high diastolic blood pressure (>95 mmHg), which belong to a single 12 generation pedigree. This large family was divided in 12 three generation pedigrees comprehensive of 185 members including 71 patients. We performed a genome wide linkage analysis on these families using 1000 microsatellites. Recombination maps and allele frequencies were calculated in the same population using 800 people. Statistical analysis, with Simwalk2, allowed the identification of 6 loci on chromosome 2, 8, 17, 18, 22 ($-\log(P)>3$); all of them but the one on chr 22 have been already described in association with EH in several population even with the same most significative markers. Genome wide search was replicated on different family structure with 16k SNPs evenly distributed every 150 Kb. For statistical analysis we used Merlin because LD modelling is a concern with highly dense marker maps. With this approach we were able to confirm and refine all the loci but the one on chr18. We are currently carrying out fine mapping in all the positive loci using high density SNPs. On chr 22 locus we identified a gene involved in cardiovascular development which shows a strong association with EH. Preliminary results of GWA using 90 cases and 90 controls, genotyped with 500k SNPs, showed several genes linked to EH i.e. NEDD4L, TGFA, ADIPOQ, which have been already associated with EH in other studies. To replicate these results we collected a large cohort of 450 cases and 450 controls from 9 different villages in Ogliastra. Our data indicate that several genetic factors common to worldwide population contribute to EH in Talana.

1714/W

Novel *HEY2* mutations in patients with left sided cardiac defects. *P.C. Paluru[1], N. Navabi[2], J. Garbarini[1], E. Goldmuntz[1,2].* 1) Division of Cardiology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA.

The hairy/enhancer of split-related with YRPW motif (*HEY2*) gene plays an important role in mammalian heart development. Mice lacking *Hey2* expression exhibit several types of congenital heart defects. *HEY2* is an established downstream target gene for the Notch signaling cascade and functions as a repressor through its basic helix loop helix (bHLH) DNA binding domain. Because *NOTCH1* mutations have been found in humans with aortic valve disease, we hypothesized that mutations in *HEY2* would be found in patients with similar heart malformations. *HEY2* contains 5 exons encoding a protein of 337 residues. By direct sequencing we analyzed the coding region of the human *HEY2* gene in 289 unrelated subjects for mutations including: 66 with valvar aortic stenosis (AS), 105 with coarctation of the aorta (CoA), 18 with coarctation of the aorta and ventricular septal defect (CoA/VSD), and 100 with hypoplastic left heart syndrome (HLHS). Four nonsynonymous mutations were identified in six patients, of which three had AS and three had CoA. No mutations were found in the cohort with HLHS. These novel mutations were not observed in public SNP databases or in 200 ethnically matched control samples. Two of the unique mutations map into the bHLH domain and one in the conserved orange domain. In addition, five silent mutations and multiple known polymorphisms were seen in the patient cohort. Further studies are required to investigate the effects of these mutations on protein function. This study suggests that *HEY2* may be an important disease gene for valvar AS and CoA and further implicates the Notch signalling pathway in cardiovascular disease.

1715/W

Frequency and function of an Asian specific novel CETP variant. *J.M. Reynolds[1], D.L. Lloyd[1], S.P. Williams[2], L.S. Wood[1], J.T. Thompson[1].* 1) Pharmacogenomics, Pfizer, Inc., Groton, CT; 2) DNA Sequencing, Pfizer, Inc., Groton, CT.

Cholesteryl ester transfer protein (CETP) plays an important role in modulating lipid levels and promoting reverse cholesterol transport. Genetic variation in CETP has been clearly associated with HDL cholesterol levels but its association with cardiovascular disease and related phenotypes has been more controversial. Some of this lack of reproducibility arises from studies attempted with small population sizes but an additional portion of the variability may also arise from polymorphisms that occur at markedly different frequencies in different ethnic populations. To properly compare results across populations, there must be a good understanding of the variation unique to each population as well as which elements of variation are common across populations. To assess whether there might be undetected common variations in individuals of Asian ancestry that contribute to CETP heterogeneity, all exons were resequenced in 96 individuals. One novel SNP, S332Y, was identified and then characterized in more detail in functional assays and its frequency determined across multiple ethnic groups. This variant is secreted less well than wild type protein but retains significant transfer activity.

1716/W

Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. *S. Sookoian, C. Gemma, T. Fernández Gianotti, A. Burgueño, C.J. Pirola.* Department of Molecular Genetics and Biology of Complex Diseases.Instituto de Investigaciones Medicas A Lanari. CONICET. University of Buenos Aires,Argentina.

Altering circadian rhythmicity results in pathophysiological changes resembling metabolic syndrome and fat accumulation. Then we investigated the role of gene variants and derived haplotypes of the CLOCK transcription factor in obesity and related quantitative metabolic traits. Research Design and Methods: 715 lean and 391 overweight/obese unrelated subjects, aged 34.4±8.6, were included in a population-based cross sectional study. Six tag SNPs showing a minor allele frequency >10 % (rs1554483 C/G; rs11932595 A/G; rs4580704 C/G; rs6843722 A/C; rs6850524 C/G and rs4864548 A/G) encompassing 117 kb of chromosome 4 and representing 115 polymorphic sites (r2 >0.8) were genotyped. Association was tested by PLINK and WHAP software while multiple testing was controlled by permutation test. Results: the genotype frequencies of four tSNPs, rs1554483, rs6843722, rs6850524 and rs4864548, showed significant (empiric p= 0.009950, 0.01492, 0.01492 and 0.009950 respectively) association with overweight/obesity. Haplotype analysis showed that only paired haplotypes including rs1554483 and rs4864548 showed a significant effect on disease status. Combinations of these SNPs (haplotype block CG and GA) are responsible for the gene effect (GA frequencies cases: 0.47% vs. controls: 0.41%, empiric p=0.0102). These findings were replicated in an independent case-control hospital-based study and the combined Mantel-Haenszel's fixed effect (MH) was OR 1.82, CI: 1.31-2.54, p=0.00034, for the paired haplotype which included CG and GA for the rs1554483 and rs4864548. Conclusions: our study suggests a putative role of the CLOCK polymorphism and related haplotypes in susceptibility to obesity. Carrying the diploid haplotype of rs1554483G and rs4864548A was associated with 1.8-fold increase for being overweight/obese.

1717/W

Comprehensive Genetic Analysis of the Platelet Activating Factor Acetylhydrolase Gene and Cardiovascular Disease in Case/Control and Family Datasets. *B. Sutton[1], D. Crosslin[1], S. Shah[1,2], S. Nelson[1], A. Bassil[1], A. Hale[1], C. Haynes[1], P. Goldschmidt-Clermont[3], J. Vance[3], W. Kraus[2], S. Gregory[1], E. Hauser[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Division of Cardiovascular Medicine, Duke University Medical Center, Durham, NC; 3) Department of Medicine, University of Miami, Miami, FL.

Platelet Activating Factor Acetylhydrolase (PAFAH) is a potent pro- and anti-inflammatory molecule that has been implicated in multiple inflammatory diseases, including cardiovascular disease. The goal of this study was to investigate the genetic effects of PAFAH in two large, independent coronary artery disease (CAD) datasets to better elucidate its genetic role in CAD. Using a haplotype tagging (ht) approach, 19 htSNPs were genotyped in CATHGEN case/control samples (cases = 807 and controls = 267) and in the GENECARD Family Study (1,101 families, 2,954 individuals). Single SNP analysis using logistic regression was performed on all CATHGEN subjects, resulting in nine SNPs showing significant association (P-values 0.0004-0.02). CATHGEN cases were further stratified into subgroups based on age of CAD onset (AOO) and severity of disease; 600 young affecteds (YA, AOO<56) and 207 old affected (OA, AOO >56) to provide a consistent validation set for the early onset CAD GENECARD Family study. After AOO stratification, the OA subgroup remained the most associated, with 14 SNPs significantly associated (P-value 0.0001-0.02). Similar association to that seen in the YA subgroup was detected in the GENECARD probands (P-values 0.002-0.05). Three SNPs, I198T, A379V, and R92H, were nonsynonymous coding changes. Interestingly, A379V and R92H constituted the most significantly associated SNPs, even after Bonferroni correction and appear to represent independent associations. Haplotype analysis was performed on all 19 SNPs using a two-SNP sliding window approach, with significant association identified in 161 of the 171 haplotypic combinations. In summary, PAFAH represents an important, potentially functional candidate in the pathophysiology of CAD based on numerous associations using two independent data sets and multiple statistical approaches.

1718/W

A multi-stage evaluation of genetic association with early-onset CAD in MYLK gene. *J. Vance[1], L. Wang[2], E.R. Hauser[2], D. Crosslin[2], S. Nelson[2], A.B. Hale[2], S.G. Gregory[2], S.H. Shah[2,3], GENECARD. Investigators[3,4,5,6], W.E. Kraus[3], P.J. Goldschmidt-Clermont[7].* 1) Miami Inst Human Genomics, Univ Miami Miller Sch Medicine, Miami, FL; 2) CHG, Duke Univ Medical Ctr, Durham, NC; 3) Division of Cardiology, Duke Univ Medical Ctr, Durham, NC; 4) Vanderbilt Univ, Nashville, TN; 5) Univ of Wales College of Medicine, Cardiff, UK; 6) Univ of Sheffield, Sheffield, UK; 7) Univ Miami Miller Sch Medicine, Miami, FL.

We and others have reported linkage evidence for coronary artery disease (CAD) at chromosome 3q13-21. To fine map the region, we previously conducted a peak-wide association survey using SNPs spaced at 100 kb intervals. While strongest association was found at Kalirin gene, multiple genes were associated with early-onset CAD, including the myosin light chain kinase (MYLK) gene. MYLK is a member of the Kalirin-RhoGTPase pathway that we have proposed previously to be important in CAD. To further define association in MYLK, we examined 46 tagging and functional SNPs across the gene in the CATHGEN case-control samples with early-onset CAD (N=750), resulting in an average density of one SNP every 6 kb. Significant results were then validated in GENECARD families with early-onset CAD (N= 2954). As a third evaluation, the GENECARD probands (N=560) were compared to the CATHGEN controls (N=291). Finally, validated SNPs were examined for correlation with atherosclerosis in 145 human aortas. Multiple SNPs were significant in the initial screening (p=0.001 to 0.038). However, only three of them were validated in all datasets (p=0.0011 to 0.0014 in CATHGEN, p=0.021 to 0.046 in GENECARD, p=0.0055 to 0.0075 in the comparison of GENECARD probands with CATHGEN controls). The risk alleles of the three validated SNPs were also correlated with higher atherosclerosis burden in aortas (p=0.007 to 0.040). The three SNPs are in linkage disequilibrium with each other (r2>0.9) and span over 50 kb region at the 5' end of the gene. Our multi-stage evaluation strongly supports MYLK as a novel CAD risk gene. MYLK regulates smooth muscle cell contraction and endothelial cell permeability. The known functions of MYLK provide a plausible mechanism for its role in the development of CAD.

1719/W

Genomic convergence identified CAPG and VAMP8 as candidate genes for coronary artery disease. *L. Wang[1], E.R. Hauser[1], D. Crosslin[1], S. Nelson[1], A.B. Hale[1], S.G. Gregory[1], S.H. Shah[1,2], GENECARD. Investigators[1,3,4,5], D. Seo[6,7], W.E. Kraus[2], P.J. Goldschmidt-Clermont[6], J.M. Vance[7].* 1) CHG, Duke Univ Medical Ctr, Durham, NC; 2) Division of Cardiology, Duke Univ Medical Ctr, Durham, NC; 3) Vanderbilt Univ, Nashville, TN; 4) Univ of Wales College of Medicine,Cardiff, UK; 5) Univ of Sheffield, Sheffield, UK; 6) Miller School of Medicine, Univ of Miami, Miami, FL; 7) MIHG, Univ of Miami, Miami, FL.

By combining evidence from linkage and microarray study, we identified capping protein, gelsolin-like (CAPG) and vesicle-associated membrane protein 8 (VAMP8) as candidate genes for coronary artery disease (CAD). The two genes reside within 200 kb on chromosome 2p11.2, where consistent linkage evidence was found for myocardial infarction (MI). Both genes were upregulated in aortas with atherosclerosis. A SNP rs1010 in VAMP8 was associated with MI in a genome-wide association study. To thoroughly evaluate association in the two genes, we examined all 16 tagging and 11 exonic SNPs. To validate association, multiple datasets were used, including a family-based (N=2954), a White case-control (N=982), and an African-American case-control (N=250) dataset. Stratified analyses on the MI subphenotype and gender were performed. Allele-specific gene expression was evaluated in 92 aortas. VAMP8 SNP rs3731828 was the most significant result, with association in both Whites (p=0.008, OR=1.4) and African-Americans (p=0.007, OR=2.3), and highly significant in the joint analysis of both ethnic groups (p=0.0001, OR=1.6). The risk allele of rs3731828 was correlated with higher VAMP8 expression (p=0.05). One SNP in CAPG and the previously reported rs1010 were also significant (p<0.05). However, the association at rs1010 could be accounted for by rs3731828. No MI or gender specific associations were found. In summary, our study supports CAPG and VAMP8 as CAD risk genes. Importantly, our data suggest VAMP8 as a major CAD risk gene as its genetic effect is not constrained by subgroups represented by ethnicity, MI subphenotype, or gender. We are the first to show allele-specific VAMP8 expression, which provides a plausible mechanism for the genetic risk conferred by the gene.

1720/W

USF1 influences lipid and metabolic traits in subjects with FCHL and CAD in a sex-dependent manner. *D. Weissglas[1], J.C. Lee[1], J.S. Sinsheimer[1], T.W.A. de Bruin[2], A.J. Lusis[1], M. Brennan[3], M.M.J. van Greevenbroek[2], C.J.H. van der Kallen[2], S.L. Hazen[3], P. Pajukanta[1].* 1) Department of Human Genetics David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA; 2) Department of Medicine and the Cardiovascular Research Institute Maastricht, Academic Hospital, Maastricht, the Netherlands; 3) Cleveland Clinic, Departments of Cardiovascular Medicine and Cell Biology, and Center for Cardiovascular Diagnostics & Prevention, Cleveland, OH.

Familial combined hyperlipidemia (FCHL) is a common dyslipidemia predisposing to coronary artery disease (CAD). FCHL is characterized by elevated levels of serum total cholesterol, triglycerides (TGs), or both. Recently, the upstream transcription factor 1 (USF1) was identified to be linked and associated with FCHL and elevated TGs in Finnish FCHL families. The strongest association was observed in TG affected males for the common alleles of a single nucleotide polymorphism (SNP) rs3737787 or SNPs in linkage disequilibrium with this SNP. The aim of this study was to evaluate the sex specific effect of rs3737787 in Dutch FCHL families with 532 family members and in a cohort of 1367 U.S. Caucasian subjects who underwent diagnostic coronary angiography. Significant sex-dependent association with serum TGs and metabolic syndrome related traits were observed in both studies (P_{FCHL}=0.03-0.006, P_{CAD}=0.05-0.002). Furthermore, using two factor ANOVA in the unrelated subjects with CAD and the penetrance option of the Mendel package in the Dutch FCHL families, we observed a significant genotype-sex interaction with serum TGs (P_{FCHL}=3x10^{-4}, P_{CAD}=5x10^{-3}) in both studies as well as with body mass index in the unrelated subjects with verified CAD (P=4x10^{-3}). In conclusion, the SNP rs3737787 influences serum TG levels in FCHL and in subjects with verified CAD. Furthermore, the detected significant sex-genotype interaction highlights the importance of investigating sex-specific differences in CAD.

1721/W

Haplotype diversity in the angiotensinogen and Beta-1 adrenergic receptor in Mexican Mestizo populations. *E. Balam, K. Carrillo, A. Contreras, L. Alfaro, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Cardiovascular diseases are the leading cause of death in Mexico. Genetic variation in B_1-adrenergic receptor *ADRB1*and angiotensinogen *AGT*genes, influence the risk for cardiovascular disease. Here we describe the haplotype structure of 7 SNPs in *ADRB1*and 5 SNPs in *AGT*in Mexican Mestizo populations, and we compare them with other populations including those from International HapMap Project. We obtained genomic DNA from Mexican Mestizo blood simples. Volunteers were from 6 geographically distant states in Mexico: Zacatecas (ZAC), Sonora (SON), Yucatan (YUC), Veracruz (VER), Guerrero (GRO) and Guanajuato (GTO). We genotyped 5 polymorphisms in AGT: -218G>A (rs5049), -20A>C (rs5050), -6G>A (rs5051), 3889C>T (rs4762) and 4072C>T (rs699); and 7 polimorphisms in ADRB1: 145A>G (1801252), 1165C>G (1801253), (rs7093444), (rs2429511), (rs3813720), (rs2183378), (rs7919873) in 184 individuals from each population (n=1,104) using direct allelic discrimination with TaqMan probes on a 7900HT RT-PCR System (AB, USA). Haplotypes blocks founded in AGT gene in 6 Mexican Mestizo populations shows differences among mestizo populations, basically populations in the North of the country (SON and ZAC) preserves strong LD on 4 SNPs (rs699, rs 4762, 5051, 5050) with D'>97 and r^2 >0.8. Two SNPs in the regulatory regions (rs699 and rs4762) shows strong LD with those in coding regions (rs 5051 and 5050); the Mestizo Populations located at south of country shows low degree of LD between SNPs at regulatory region and codin region with D' < 0.8 and R^2 < 0.8. Comparison between haplotype blocks from Mexican Mestizo populations (SON and ZAC) and International HapMap populations (CEU, CHB, JPT and YRI) shows at these loci, shows that only in Mexican Mestizo populations is founded strong LD between SNPs in regulatory region and coding regions. Our data support the notion that some Mestizo populations in Mexico conserve haplotype blocks at these genes (*AGT*and *ADRB1*) that differ from those in other populations, that could be important issue in the genomic medicine.

1722/W

Novel familial duplication 10q23.2-q23.32: clinical manifestations and further delineation of chromosome 10q22-24 atrial fibrillation locus. *J. Buchholz, H. Ardinger, R. Ardinger.* Sections of Medical Genetics and Molecular Medicine and Cardiology, Children's Mercy Hospitals and Clinics, Kansas City, MO.

We report a non-dysmorphic 2 year-old female with a history of a secundum atrial septal defect and branch pulmonary artery stenosis requiring surgery, mild speech delay, intrauterine growth restriction, and postnatal growth delay. High resolution chromosome analysis revealed a 10q23 duplication. Subsequent 1MB microarray analysis was positive for a 4.5-7.7 Mb duplication of 9 clones at 10q23.3-q23.32. Family studies revealed the same duplication in the proband's father and sister. The proband's 5 year-old sister has a similar history of intrauterine growth restriction, postnatal growth delay, speech delay, a functional heart murmur with normal echocardiogram and electrocardiogram, and is otherwise non-dysmorphic. The father's history is remarkable for problems gaining weight, learning difficulties, and atrial fibrillation which was symptomatic at age 23 and diagnosed at age 30. His echocardiogram revealed a structurally normal heart.

While the distal trisomy 10q syndrome is well described in the literature to involve varying degrees of ocular, limb, renal, cardiac, hearing, and speech abnormalities along with mental retardation and facial dysmorphism depending on the chromosomal segment involved, most of these described chromosomal aberrations begin at or distal to the 10q24 region. There are no patients reported to have the same duplication as seen in the present family which appears to be associated with a remarkably mild phenotype.

Of considerable significance is the presence of the father's early onset atrial fibrillation and the fact that the 10q23.3-q23.32 region overlaps the 10q22-24 familial atrial fibrillation locus discovered in linkage studies in 3 Spanish families by Brugada et al (1997). The causative gene for this type of familial atrial fibrillation has not yet been identified. We believe that the chromosome findings in our report could be of critical importance in narrowing the chromosomal region of interest in screening for candidate genes related to atrial fibrillation on 10q.

1723/W

SNPs at the INSIG2 Locus are Associated with Plasma Lipid Phenotypes. *R. Do[1], G. Paré[1,2], A. Montpetit[2], S.D. Bailey[1], K. Desbiens[3], T.J. Hudson[1,2,4], C. Bouchard[5], L. Perusse[6,7], M.C. Vohl[7,8], D. Gaudet[9], J.C. Engert[1,3,4].* 1) Dept of Human Genetics, McGill Univ, Montreal; 2) McGill Univ and Genome Québec Innovation Centre, Montreal; 3) Research Institute of the McGill Univ Health Centre, Montreal; 4) Dept of Medicine, McGill Univ, Montreal; 5) Pennington Biomedical Research Centre, Baton Rouge, Louisiana; 6) Dept of Social and Preventive Medicine, Division of Kinesiology, Laval Univ, QC; 7) Lipid Research Center, Laval Univ Hospital Research Center, QC; 8) Dept of Food Science and Nutrition, Laval Univ, QC; 9) Dyslipidemia, Diabetes and Atherosclerosis Group and Community Genomics Research Center, Univ de Montréal and Chicoutimi Hospital, QC.

Elevated cholesterol is a major risk factor for coronary heart disease (CHD). Endogenous cholesterol biosynthesis is regulated by proteins in the insig-scap pathway, particularly INSIG1 and INSIG2. Recently, a genome-wide scan identified an association between a SNP (rs7566605) 5' of INSIG2 and BMI in the Framingham Heart study as well as in another 4 out of 5 cohorts. However, some studies could not replicate these results. Based on these inconsistent results, we hypothesized that INSIG2 variants may influence plasma cholesterol levels and that such variants would thus only be inconsistently associated with BMI. In addition, we believe that a denser SNP map will help to localize the association signal. To test this hypothesis, we analyzed 10 tSNPs at the INSIG2 loci in family-based and case/control CHD study samples from the Saguenay region of Quebec. Analysis of the combined samples identified a SNP (rs2422166) 5' of the transcription start site that was significantly associated with total apoB (0.016) and LDL-C (p=0.010). In addition, we identified a SNP (rs2113485) in the 3' region of the gene that was also associated with total apoB (p=0.045) and LDL-C (p=0.027). This SNP was not in linkage disequilibrium with the 5' SNP. To identify novel SNPs, we sequenced all exons and intron-exon boundaries as well as conserved promoter and intronic regions for a total of 18,434 bp in the INSIG2 gene locus of 24 French Canadian individuals. Our sequencing identified 37 SNPs, 19 of which have a MAF > 0.05 and 15 of which are novel.

1724/W

Alpha cardiac actin mutations cause atrial septal defects. *J.S. Eason[1], H. Matsson[2], C.S. Bookwalter[3], J. Klar[2], P. Gustavsson[2], J. Sunnegårdh[4], H. Enell[5], A. Jonzon[6], M. Vikkula[7], I. Gutierrez[7], J.T. Granados Riveron[1], M. Pope[1], F. Bu'lock[8], J. Cox[8], T.E. Robinson[1], F. Song[1], J.D. Brook[1], S. Marston[9], K.M. Trybus[3], N. Dahl[2].* 1) Institute of Genetics, University of Nottingham, Nottingham, United Kingdom; 2) Department of Genetics and Pathology, The Rudbeck Laboratory, Uppsala University and University Hospital, Uppsala, Sweden; 3) Department of Molecular Physiology and Biophysics, University of Vermont, Burlington, USA; 4) The Queen Silvia Children's Hospital, Göteborg, Sweden; 5) Department of Paedriatics, County Hospital of Halmstad, Sweden; 6) Childrens Hospital, Uppsala University, Uppsala, Sweden; 7) Human Molecular Genetics (GEHU), Christian de Duve Institute & Université catholique de Louvain, Brussels, Belgium; 8) Department of Paediatric Cardiology, Glenfield Hospital, Leicester, UK; 9) National Heart and Lung Institute, Imperial College, London, UK.

Congenital heart defects are common developmental defects and a leading cause of morbidity and mortality in early life. Alpha cardiac actin (ACTC1) is essential for cardiac function and mutations in *ACTC1* have been associated with dilated and hypertrophic cardiomyopathy. We identified a missense mutation in exon 2 of *ACTC1* associated with isolated atrial septal defect (ASD) in affected members from two families. This is predicted to lead to an M123V substitution. Functional analysis of ACTC1 with a M123V substitution shows a reduced affinity for myosin with retained actomyosin motor properties. We screened a cohort of apparently sporadic CHD patients for *ACTC1* mutations and identified a patient with ASD and a 17bp deletion. We hypothesised that the mutant transcript would be non-functional due to nonsense mediated decay resulting in haploinsufficiency of ACTC1. We used a morpholino to knock down ACTC1 in early chick embryos to assess the effect on the developing heart. The treated embryos showed delayed looping and reduced atrial septal size compared to wild type. Thus, we show for the first time that ACTC1 has a crucial role in the formation of atrial septa. In conclusion ACTC1 appears to have a role in both embryogenesis and in the contractile function of the adult heart.

1725/W

Genetic variants associated with myocardial infarction (MI) risk in five ethnic groups: the INTERHEART genetics study. *J.C. Engert[1], C. Xie[2], A. Montpetit[3], D. Serre[3], B. Keavney[4], H. Cordell[4], M. McQueen[2], S. Yusuf[2], T.J. Hudson[1,3], S.S. Anand[2] for the INTERHEART genetics investigators.* 1) Depts. of Medicine and Human Genetics, McGill Univ., Montreal, QC; 2) McMaster Univ., Population Health Res Inst., Hamilton Health Sciences, Hamilton, ON; 3) McGill Univ. & Genome Quebec Innovation Centre, Montreal, QC; 4) Univ. of Newcastle upon Tyne, Newcastle-upon-Tyne, UK.

The INTERHEART case-control study showed that 9 modifiable risk factors (tobacco, diabetes, hypertension, dyslipidemia, abdominal obesity, physical inactivity, psychosocial stress, low fruit and vegetable intake, and no alcohol consumption) account for > 90% of the population attributable risk for MI globally. We investigated the association between candidate gene SNPs and MI and risk factors for MI in 8,034 individuals from 5 ethnic groups. Samples from individuals of South Asian, Arab, Iranian, Nepalese and European origin were genotyped for 1,536 tagging and other SNPs in 103 candidate genes. 1,442 SNPs were polymorphic and in HWE in all 5 ethnic groups. We used a staged design in which 1,344 of these SNPs were first assessed versus the MI risk factor(s) with which they were hypothesized to be associated. SNPs significantly associated with a risk factor (after adjusting for age, sex and ethnic group, and correcting for multiple testing) were passed to stage 2 and tested in an additive model versus MI. Cases and controls were matched by ethnic group, sex and age (+/- 5 years). An empirical p value was determined using permutation testing in stage 2. Fifteen SNPs passed stage 1 testing: 12 SNPs were associated with Apo B/A ratio, 2 SNPs with alcohol intake, and 1 SNP with fruit and vegetable intake. Three SNPs from two lipid related loci (ApoE and LDLR) were associated with MI in 5 ethnic groups. Notably, all 3 associated SNPs were protective alleles. The ApoE SNP (rs7412) is the one that defines isoform 2 and it had an odds ratio of 0.78 (95% CI: 0.70-0.89) and a p-value of 0.0004. The LDLR SNPs were both intronic: rs1433099 had an OR of 0.90 (95% CI: 0.84-0.96) and a p-value of 0.002 and rs6511720 had an OR of 0.86 (95% CI: 0.77-0.95) and a p-value of 0.004.

1726/W

Adjustment for sex and age may conceal significant sex- and age- specific genomic determinants of physiological traits. *P. Hamet[1], O. Seda[1], D. Gaudet[2], P.-L. Brunelle[1], A. Gurau[1], E. Merlo[3], L. Pilote[4], T. Kotchen[5], A.W. Cowley[5], J. Tremblay[1].* 1) CR CHUM, Montreal, Quebec, Canada; 2) Complexe Hospitalier, Chicoutimi, Quebec, Canada; 3) École Polytechnique de Montreal, Quebec, Canada; 4) MUHC, Montreal, Quebec, Canada; 5) MCW, Milwaukee, Wisconsin, USA.

Sex and age are recognized factors affecting pathogenesis of complex traits. Their effects on the underlying genetic architecture received only limited attention so far. From over 500 collected traits in 120 French-Canadian families (n = 810 subjects) from the Saguenay-Lac-St-Jean region of Quebec, Canada, about 50% were sex- and age- independent, remaining ones were sex- and age-specific to a variable degree. We assessed, among other traits, the sex- and age-specific linkage of systolic (SBP) and diastolic (DBP) blood pressures and heart rate recorded also by 24h ambulatory blood pressure measurement. Multipoint and two-point sex-specific linkage analysis was performed with a variance components approach implemented in SOLAR in 3 settings: all, males and females. Age-specific two-point linkage analysis was performed by S.A.G.E. SIBPAL in all sibpairs, younger than 55 years and older than 55 years. The genetic information was represented by 437 microsatellite markers and >58,000 single nucleotide polymorphisms by Affymetrix GeneChip 50k Xba240 array. The results of linear regression confirmed significant contribution of age to the temporal change of SBP but not DBP. Heritability was higher in women for HR and DBP, conversely, most SBP measures were more heritable in men. In age-specific analysis, we observed several significant linkage signals specific for <55-year sib-pair group, e.g. SBP (peak at D8S1100, p=5.6x10-6) or pulse pressure (peak at D12S1064, p=4.3x10-6). Sex-specific linkage analysis revealed locus on chromosome 2 linked to SBP exclusively in men with no evidence of linkage in the female and combined sets. The fine genotyping pointed to rs10497097 in an intron of STAM2 gene at the peak of male-specific signal. We report identification of several age- and sex-specific genetic determinants of blood pressure and heart rate, none of which would have been observed in fully sex- and age-adjusted models.

1727/W

Association of Renin gene polymorphisms with Essential Hypertension in Koreans. *E. Kim[1], J. Kim[1], J. Han[2], C. Park[2], D. Shin[2], Y. Jang[2], B. Han[3], S. Yoon[1].* 1) Biomedical Sciences, The Catholic University of Korea, Seoul, Korea; 2) Cardiovascular Genome Center, Yonsei University Medical Center, Seoul, Korea; 3) Division of Genome Resources, NGRI, NIH, Seoul, Korea.

Renin (Ren) is a protease that acts in the rate limiting step of the cascade in the production of angiotensin II, a potent vasoconstrictor and stimulator of aldosterone release. Renin gene plays a crucial role in the regulation of blood pressure and has been shown to be involved in the underlying cause of essential hypertension in an ethnic group. The aim of the present study was to investigate the association between human rennin gene and EH in Koreans. None of six SNPs (rs# 2368564, 1464816, 2272237, 10900555, 11240688, and 6682082) of rennin gene were in linkage disequilibrium in Koreans. We carried out a case-control study of 644 hypertensive (EH) and 1329 ethnically- and age-matched normotensive (NT) subjects with rs2368564 and rs6682082. The distribution of genotype and allele for rs2368564 did not differ significantly between two groups, whereas the overall distribution of rs6682082 was significantly different between EH and NT, specifically in females. The frequencies of G allele as well as GG genotype were significantly higher in EH (p=0.0159 and 0.0383, respectively). Logistic regression analysis indicated that the GG homozygote was strongly associated with EH in the female subjects (OR, 1.454; 95% CI, 1.047-2.018, p = 0.0253). In our study, we suggest that rennin rs6682082 polymorphism is a positive genetic marker of predisposition for EH in Koreans.

1728/W

Positional cloning of genes influencing blood pressure on chromosome 2q31-q36 in the Old Order Amish. *P.F. McArdle[1], Y. Wang[1], S. Rutherford[2], J.R. O'Connell[1], S.H. Ott[1], L.J. Reinhart[1], T.I. Pollin[1], C. Damcott[1], Y.C. Chang[1], B.D. Mitchell[1], A.R. Shuldiner[1,3], N.I. Steinle[1].* 1) Department of Medicine, University of Maryland, Baltimore, MD; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Geriatrics Research and Education Clinical Center, Veterans Administration Hospital Medical Center, Baltimore, MD.

Genome-wide linkage analysis in the Amish Family Diabetes Study revealed a single locus on chromosome 2q that was strongly linked to both diastolic (DBP LOD = 4.23; p=0.00001) and systolic (SBP LOD = 1.64; p=0.003) blood pressure. Peak evidence for linkage occurred between positions 181,883,021 and 220,376,982 (Mar2006 Build). This same region has been shown to be linked to hypertension in several other populations including Caucasians and African Americans. Here, we present the findings from association mapping with 2,831 SNPs placed approximately every 5 kilobases in the 1-LOD linkage interval in 762 Amish individuals. Initial screening efforts identified seven distinct regions containing genes highly associated with blood pressure, including SLC4A3 (p=0.0000002 for DBP and p=0.00004 for SBP), PPIL3 (p=0.000002 for DBP and p=00004 for SBP), FAM126B (p=000006 for DBP and p=0.0001 for SBP), ABCA12 (p=00001 for DBP and p=0.007 for SBP), ERBB4 (p=00003 for DBP and p=0.0001 for SBP), ORC2L (p=00001 for DBP and p=00003 for SBP) and BARD1 (p= 00003 for DBP and p= 0.008 for SBP). We also analyzed SNPs from these genes in a second Amish sample of 861 individuals for which Affymetrix 500K genotypes were available. Strong association between blood pressure levels and SNPs in ABCA12, which belongs to the large family of energy dependent ATP binding cassette proteins, (p=0.003 for SBP) and ERBB4, a member of a threonine protein kinase family involved in fetal cardiac development and in maintaining normal adult blood pressure, (p=0.002 for DBP and p=0.03 for SBP) was further demonstrated. Additional analysis in other populations and elucidating the functional consequences of variation in these genes may provide new insights into the genetic basis of hypertension.

1729/W

Assessment of genes involved in inflammation in coronary artery disease in Asian Indians. *N.U. Mehta[1,2], G. Mendoza-Fandino[1,2], T.J. Pemberton[1], J. Hartiala[1], D. Conti[3], P. Kotha[4], H. Allayee[1,3], P.I. Patel[1,2].* 1) Inst for Genetic Medicine, Univ of Southern California, Los Angeles, CA; 2) Dept of Biochemistry and Mol Biology, Univ of Southern California, Los Angeles, CA; 3) Dept of Preventive Medicine, Univ of Southern California, Los Angeles, CA; 4) RICADIA, 5555 Reservoir Drive Suite 309, San Diego, CA.

Our long term goal is to identify genetic risk factors underlying coronary artery disease (CAD) in Asian Indians. The prevalence and severity of CAD in individuals of Asian Indian origin is four- to five-fold higher when compared to other ethnic groups. In Asian Indians, CAD is severe, extensive and follows an apparently much more aggressive course than in other ethnic groups. In particular, CAD rates are unusually high in Asian Indian women and natural protection from CAD seen in Caucasian premenopausal women is apparently not present in Asian Indian women. We have conducted community-based sampling of Asian Indians with CAD and gender-matched control Asian Indian subjects >60 y of age without CAD. Blood samples were collected for serum and plasma chemistries, and for RNA and DNA isolation. Previous studies have shown an association between the gene encoding arachidonate 5-lipoxygenase (5-LO), the rate-limiting enzyme in the production of leukotrienes (LTs) and atherosclerosis in various populations. Primarily, deviation away from the common 5-allele of a 5-LO promoter repeat has been associated with increased risk of atherosclerosis. We have initially examined the prevalence and allele frequencies of the various promoter alleles in a cohort of Asian Indians sampled for population genetics studies. Six promoter alleles were noted in the Asian Indian cohort, with 56.4% of individuals homozygous for the common allele of five Sp1-binding sites, 92.5% of individuals had at least one 5 repeat allele, and the remaining 7.5% had two variant alleles. This is higher than the 5.96% reported for a cohort of individuals of mixed-ethnicitye. Current studies are examining 5-LO and other genes in the inflammatory LT pathway, including 5-LO-activating protein and LTA4 hydrolase, in this case-control cohort.

1730/W

Variants in the LDL receptor gene are associated with LDL cholesterol in the Multi-Ethnic Study of Atherosclerosis. *W. Post[1], J.C. Mychaleckyj[2], L. Raynor[3], K.D. Taylor[4], X. Guo[4], K.E. Watson[5], C. Hedrick[2], J. Polak[6], M. Tsai[3], S.S. Rich[2], J.I. Rotter[4], J.S. Pankow[3].* 1) Johns Hopkins Univ, Baltimore, MD; 2) Univ of VA, Charlottesville, VA; 3) Univ of Minnesota, Minneapolis, MN; 4) Cedars-Sinai, Los Angeles, CA; 5) UCLA, Los Angeles, CA; 6) Tufts, Boston, MA.

LDL cholesterol (LDL-C) is a complex trait influenced by multiple genes. Rare mutations in the LDL receptor gene (LDLR) lead to familial hypercholesterolemia and premature CVD, but account for little variability in LDL-C in the general population. We hypothesized that common variants in LDLR influence LDL-C. Eleven tagging SNPs and one nonsynonymous coding SNP spanning 48 kb were selected in LDLR. Genotyping was completed using Illumina GoldenGate Assay in 2880 men and women, age 45-84 yrs, without known CVD from the Multi-Ethnic Study of Atherosclerosis (MESA), including equal numbers of White(W), Black(B), Hispanic(H) and Chinese(C) US subjects. Associations between each SNP and LDL-C were determined using multivariate regression, adjusting for age, gender and lipid medications, stratified by race/ethnicity and combined. All SNPs met HWE assumptions within each ethnic group. The minor allele of rs2228671 was associated with lower LDL-C in W(12% MAF), H(6% MAF),and B(1% MAF)(p<0.03, 0.02, 0.06, additive model). In the combined cohort, this allele was associated with a 5 mg/dL lower mean LDL-C (p<0.004). In the combined cohort, the minor alleles of 3 additional SNPs (rs8104576,rs2304182,rs1433099) that were not in significant LD were also associated with lower LDL-C (p<0.007, 0.002, 0.03) We also observed a rare nonsynonomous SNP(rs5928)in these populations, with only 6 heterozygotes (HETs) in the combined cohort. For this SNP the mean LDL-C was 19% higher in the HETs, compared to homozygotes for the major allele (p<.03). In summary, minor alleles of 4 common SNPs in LDLR are associated with lower LDL-C in the general population. Heterozygosity for a rare nonsynonymous coding SNP is also associated with higher LDL-C. We conclude that common variants in LDLR influence LDL-C variation in the general population across multiple ethnic groups.

1731/W

Family-based association studies of congenital heart defects. *L. Scheinfeldt[1], E. Goldmuntz[2], J. Campanile[1], M. Devoto[1,3], D.A. Driscoll[1,4].* 1) Department of Human Genetics, CHOP, Philadelphia, PA; 2) Department of Cardiology, CHOP, Philadelphia, PA; 3) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 4) Department of OBGYN, University of Pennsylvania, Philadelphia, PA.

Congenital heart defects (CHD) are the most common birth defects occurring in as many as 1% of live births, and approximately 15% of these are outflow tract malformations. Studies have identified several genes and chromosomal regions associated with syndromic CHD; however, the genetic contribution to isolated CHD remains to be elucidated. We utilized a family-based candidate gene association study to identify genes involved in cardiac outflow tract malformations. Initially, 47 tag single nucleotide polymorphisms (SNPs) in 18 candidate genes were studied in 355 trios with outflow tract malformations; the transmission disequilibrium test was used to test for association. We found significant association in one Jagged 1 (JAG1) SNP (rs1997814) with a corrected p-value of 0.012. However, an expanded validation study with 7 additional SNPs and 120 additional trios failed to confirm an association. We did not find association between JAG1 SNPs and outflow tract malformations upon replication, but have not excluded the possibility of an association. The sample size may have been too small to detect a modest genetic contribution to CHD. Further, the population is heterogeneous and until we examine sub-groups of outflow tract malformations we cannot exclude an association with JAG1 variants. The lack of association with JAG1 SNPs is surprising given previous animal and human studies. JAG1 is one of two serrate-like ligands involved in the Notch gene pathway and is expressed in the aorta. Notch2 and JAG1 double heterozygote mice have cardiac defects, and human studies have found JAG1 mutations in patients with Alagille syndrome as well as patients with isolated tetralogy of Fallot, a classic outflow tract anomaly. We conclude that further studies with larger and more homogeneous populations of patients with cardiac defects will be required to successfully identify the genes responsible for isolated CHD.

1732/W

Copy Number Variation in Individuals with Hypoplastic Left Heart. *J.T.C. Shieh[1,3], G.M. Shaw[4], D. Srivastava[2,3].* 1) Div. Medical Genetics,; 2) Div. Pediatric Cardiology, Dept. Pediatrics, University of California San Francisco; 3) Gladstone Institute of Cardiovascular Disease, San Francisco, CA; 4) California Birth Defects Monitoring Program, Berkeley, CA.

Hypoplastic left heart (HLH) syndrome is a form of congenital heart disease with high morbidity and mortality despite advances in surgical techniques. Although many of the molecular mechanisms underlying normal cardiogenesis are known, it is still unknown why the left ventricle fails to develop in this condition. Furthermore, genes whose expression is specific for the left ventricle are lacking. We have performed genome-wide screening for critical genes that underlie hypoplastic left heart.

To determine susceptibility genes in HLH, we collected blood samples from affected individuals and examined them for unique regions of copy number variation (CNV). We ascertained cases of HLH and parental samples from Cardiology clinic. Of those with normal karyotypes, we determined regions of copy number variation using high-resolution oligonucleotide-based comparative genomic hybridization (CGH). The size of CNV ranged from 20kb to 650kb. Using bioinformatics, we determined unique regions of CNV that have not been reported in normal individuals. By testing parents, we demonstrate that although some of these regions are inherited, some represent unique regions of relative copy number gain or loss. These regions encompass potential candidate genes for disease. To aid in evaluating candidate loci, we reviewed 719 HLH cases from the California Birth Defects Monitoring Program. In this cohort of HLH cases, nearly half of the cases included septal defects or potential laterality defects affecting the heart and blood vessels; 6 percent of cases had chromosome abnormalities such as Trisomy 21, 18, 13 and Turner syndrome. The remainder consisted of 334 cases of classic HLH often associated with outflow tract abnormalities. Some of these cases demonstrate specific chromosome abnormalities that will be correlated with regions of copy number variation. This combined approach has the potential to reveal novel candidates for hypoplastic left heart.

1733/W

Polymorphisms in the Tissue Plasminogen Activator gene (*PLAT*) are associated with multiple measures of coronary artery disease. *A.V. Smith[1], T. Aspelund[1], L. Launer[2], T. Harris[2], V. Gudnason[1,3].* 1) Icelandic Heart Association, Kopavogur, Iceland; 2) National Institute on Aging, Bethesda, MD, USA; 3) University of Iceland, Reykjavik, Iceland.

The Tissue Plasminogen Activator gene (*PLAT*) has been implicated in atherothrombotic disease. To investigate the genetic role of *PLAT* into the development and progression of cardiovascular disease, individuals from the Age, Gene/Environment Susceptibility (AGES Reykjavik) Study were typed with polymorphisms from the *PLAT* gene as part of a larger examination of multiple candidate genes. AGES Reykjavik is an extensive and detailed phenotyping of surviving participants (now 67 and older) of the 40 year long Reykjavik study. 2,300 individuals were typed with seven polymorphisms spanning the gene. A single SNP (rs2020919; MAF 0.075) located immediately upstream to the *PLAT* transcript is significantly associated with multiple related phenotypes combined as coronary events (CE) (OR 1.81; p= 6.9e-6) consisting of myocardial infarction (MI) (OR 2.04; p=1.1e-5), narrowing of the arterial lumen resulting in bypass surgery (OR 2.32; p=4.2e-7), and percutaneous coronary intervention (1.35; p=0.15). Significant associations are observed in both males and females with similar odds ratios and an overall population attributable risk of 6%. This polymorphism is suggestively associated with coronary artery calcium levels (p=0.01 for top bottom differences), while no association was detected with conventional cardiovascular risk factors currently measured, such as cholesterol levels. These results are consistent with a hypothesis that the polymorphisms in the *PLAT* gene are associated with progression and complications of cardiovascular disease rather than the initiation of disease.

1734/W

Identification of a modifier gene in heart failure. *F.C. Wheeler[1], T.N. Hadnott[1], O. Marks[1], M.P. Donahue[2], H.A. Rockman[2], D.A. Marchuk[1].* 1) Dept Mol Genet & Micro, Duke Univ, Durham, NC; 2) Dept Medicine, Duke Univ, Durham, NC.

Cardiomyopathy and its resultant clinical outcome of heart failure is a significant cause of death worldwide. Disease progression is highly variable, due in part to undiscovered genetic differences in the population. We have taken an unbiased genetic approach to identify novel genes that contribute to heart disease. In a well-studied calsequestrin ovexpressing transgenic mouse model of dilated cardiomyopathy, we discovered dramatic strain-specific differences in disease progression and survival. Using QTL mapping in multiple crosses, we have identified 7 distinct genetic loci, *Hrtfm1-7* (Heart failure modifier), that modify disease progression and the final outcome of heart failure. Significantly, the phenotypic effects of these loci recapitulate the complexities of human heart disease, with some loci affecting both heart function and survival, and others separately influencing these two phenotypes. In this study, we report the identification of a strong candidate gene for one Hrtfm locus, which affects both heart function and survival. *Hrtfm2* was mapped to the identical location in two different crosses, allowing us to use ancestral haplotype patterns to narrow the candidate interval to 1 Mb. Transcript levels of one gene in the interval were found to be 20-fold different between the strains used to map the locus. Tnni3k is a tyrosine kinase that interacts with cardiac Troponin I, although its precise biochemical function is unknown. We have identified a *Tnni3k* SNP that modulates the observed differences in transcript levels. This SNP, located at the +9 position of intron 19, creates a cryptic splice donor site that leads to a frameshift and a premature stop codon, targeting the message for nonsense-mediated decay (NMD). Experiments in vitro have validated the role of this SNP in aberrant splicing, and blocking NMD restores near normal message levels. We have created a transgenic mouse that expresses high levels of human TNNI3K to study the effect of increasing TNNI3K expression in the original transgenic model of cardiomyopathy. We are also currently investigating the predictive value of *TNNI3K* SNPs in a large human cohort with cardiovascular disease.

1735/W

SNPs in SCN5A for risk of arrhythmias in the context of myocardial infarction. *Q. Xi, L. Li, Q.K. Wang.* Molecular Cardiology, Lerner Research Institute / Cleveland Clinic/, Cleveland, OH.

Introduction: The cardiac sodium channel gene SCN5A is critical for generating the cardiac action potential and for conduction of electrical pulse in the heart. Many mutations in SCN5A have been found in patients with ventricular arrhythmias (VT) and sudden cardiac death due to long QT syndrome, Brugada syndrome and cardiac conduction disease. Coronary artery disease (CAD) and myocardial infarction (MI) causes >70% of sudden cardiac death. In the present study, we tested the hypothesis that single nucleotide polymorphisms (SNPs) in SCN5A may confer the risk of arrhythmias in CAD/MI patients. Methods: We carried out a case/control association study using 143 MI patients with VT and 360 control MI patients without VT. Four SNPs, one non-synonymous SNP, one intronic SNP and two SNPs in the promoter region of SCN5A were studied. SNP allelic frequencies were compared between cases and controls by a Chi-square test. Allelic specific risks were estimated as odds ratios (ORs). Bonferroni correction was used for P-value adjustment for multiple testing. Results: The allelic frequency of one SCN5A SNP showed nominal significance for association with VT in MI patients (P=0.03) with an odds ratio of 1.43. The significance of association diminished after Bonferroni correction (P=0.12). Conclusions: One SNP in SCN5A may be potentially associated with risk of developing VT in the MI population. However, the association was marginal and became non-significant after Bonferoni correction.

1736/W

Association of SLC34A2 and Sodium-Lithium Countertransport. *X. Zheng[1], C.M. Kammerer[1], L.A. Cox[2], A. Morrison[3], R.E. Ferrell[1].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Human Genetics Center, School of Public Health , University of Texas Health Science Center at Houston, Houston, TX.

Sodium Lithium Countertransport (SLC), which is a premorbid marker of essential hypertension, has been linked to a region of baboon chromosome 5, homologous to the region of human chromosome 4. The specific aim of our study is to examine the relationship between a positional candidate gene SLC34A2 (Type II Na/Pi-cotransporters IIb) and SLC by sequence analysis of SLC34A2 in baboons of known phenotype and of its' human homolog, and to conduct association analysis between variation in SLC34A2 and SLC phenotype. We sequenced the SLC34A2 gene, including coding exons, splice junctions and predicted promoter sequences in 24 baboon founders and 94 human samples. Strong homology was established in exonic organization and sequence between the human and baboon SLC34A2 genes and extensive variation in both species was identified. A total of 17 exonic single nucleotide polymorphisms (SNP) were observed in the baboon compared to five in the human, no SNP were shared between the two species. Association studies between SLC and SLC34A2 were carried out in 1856 RFHS phase II samples and 634 baboons. Significant association of SLC with human SNP rs3775909 (p=0.03) in SLC34A2 and haplotype block 2 (p<0.005) were observed. Strong evidence for association of SLC34A2 with SLC were from baboon SNP Asn136Asn (p=0.0001) in SLC34A2. This single SNP explained about 5% of variance in SLC. Consistent findings in two different species implied that SLC34A2 may be one of the genes involved in SLC. However, linkage analyses conditional on genotypes of baboon Asn136Asn suggest that Asn136Asn is not the primarily functional site responsible for SLC, there might be other variants with larger effect in or near SLC34A2 accounting for the linkage signal in baboon. We conclude that SLC34A2 is associated with the phenotypic variation of SLC, though it may not be the major effect gene.

1737/W

Novel KCNH2 Mutatuion in an Iranian Family with LQTS. *K. Banihashemi[1], S. Saber[3], E. Zaklyazminskaya[2], M. Houshmand[3], M. Eftekharzadeh[4], M. Rostami[3], T. Majidizadeh[3], M. Dehghan[3].* 1) Dept Medicine, Great Persian Encyclopedia Fnd, Tehran, Iran; 2) russian academy for medical sciences; 3) national research institute for genetic engineering and biotechnology; 4) tehran arrhhthmia center.

K Channels made with the KCNH2 protein are active in the heart muscle, where they transport potassium ions out of cells. The gene contains 15 exons spanning approximately 19 kb on chromosome 7q35. A feature of K channelopathy is pronounced prolongation of the QT interval in the supraventricular beat preceding the arrhythmia that can lead to sudden death. The subjects studied included a 6 members family with 3 out of them involved with LQTS. The criteria to identify patients were symptomatic individuals with QTc of ≥450 ms and asymptomatic individuals with QTc of ≥470 ms were classified as affected, and symptomatic individuals with QTc of ≤440 ms . Peripheral blood samples were collected from the patients after obtaining informed consent, and genomic DNA was extracted according to a standard method, and then KCNH2 were PCR-amplified and sequenced for identifying LQTS-causing mutations. We identified intragenic mutations of KCNH2 in the proband as a frameshift in the exon 15 which was present also both in his mother and his elder sister. Bidirectional sequence analyses were carried to be sure that mutations are authentic. The one more mutations included also a novel intronic mutation. All the findings were judged after sequencing the parental DNA to find not to have occurred de novo but inherited from mother to the siblings. The proband a 16 years old boy with a frame shift mutation in exon 15, showed all the features of LQTS and needed an ICD which inserted.There were no such a mutation a other healthy members of the family. Additionally there was an intronic heterozygote mutation which has not been reported although predicted to have no effect on protein truncations. this is the first frameshift mutation in Iranian population and we have provided additional data of KCNH2 mutations in LQTS patients. These findings will contribute to further understanding of the function and structure of KCNH2 and the phenotype-genotype correlation in hereditary LQTS.

1738/W

Analysis of single nucleotide polymorphisms and haplotypes in TBX20 gene within the susceptible region 7p14-15 of Fallot's tetralogy. *G.R Qiu[1], N. Xin[1], L.G. Gong[1], Y.H. Yuan[2], X.M. Han[3], H.B. Liu[4], X.Y. Xu[1], K.L. Sun[1].* 1) Department of Medical Genetics, Basic Medicine, China Medical University, Shenyang, Liaoning, China; 2) Department of Cardiac surgery, the 1st affiliated Hospital, China Medical University; 3) Department of Cardiavascular medicine, Military District Hospital; 4) Department of Health Statics, College of Public Health, China Medical University.

Objective:In the candidate region 7p14-15 where the susceptibility gene of Fallot's tetralogy might be according to our previous studies, we chose four single nucleotide polymorphisms (SNPs) in TBX20 gene to investigate single SNP and haplotypes distribution in patients and normal people in order to confirm whether or not TBX20 gene is the susceptibility gene in the candidate region. Methods: Four SNPs in the coding-region of TBX20 gene, including rs3999941, rs6950175, rs13237089 and rs336283, were chosen, and the genotypes of 4 SNPs in 215 patients and 300 normal people were analyzed by denaturing high performance liquid chromatography. Legally constituted authority statistical analysis was applied to analyze SNP genotype frequency and gene frequency in patients and control group. Then we established haplotypes and analyzed their frequency in two groups by PHASE software. Results: C/T polymorphism at rs336283 was not detected; A/G polymorphism at rs3999941 and G/T polymorphism at rs6950175 had significant difference between patients and normal people, the A allele frequency at rs3999941 and the G allele frequency at rs6950175 in patients were higher than those in healthy controls (chi square=9.39 P<0.005;chi square=9.78 P<0.005); the distribution of frequencies of 6 haplotypes showed significant difference (chi square=22.78 P<0.005) between two groups, and AGG haplotype was more common in patients. Conclusion: rs336283 and rs3999941 located in the coding-region of TBX20 gene were associated with Fallot's tetralogy, the risk of Fallot's tetralogy in the persons with A allele at rs3999941 and the G allele frequency at rs6950175 was higher. The AGG haplotype might be linked with the susceptibility gene of Fallot's tetralogy.

1739/W

New role for the neuropeptide galanin in regulation of triglyceride levels. *M. Kyttala[1], C.L. Plaisier[1], A. Huertas-Vazquez[1], B. Aouizerat[2], T.W.A de Bruin[3], C. Aguilar-Salinas[4], T. Tusie-Luna[4], M.-R. Taskinen[5], C. van der Kallen[3], P. Pajukanta[1].* 1) Human Genetics, UCLA, Los Angeles, CA; 2) School of Nursing, UCSF, San Francisco, CA; 3) Dept. of Internal Medicine, Maastricht University, Maastricht, NL; 4) INCMNSZ-UNAM, Mexico City, Mexico; 5) Dept. of Medicine, University of Helsinki, Helsinki, Finland.

Familial combined hyperlipidemia (FCHL) is a common dyslipidemia predisposing to coronary artery disease. Patients with FCHL exhibit high levels of serum total cholesterol and/or triglycerides (TGs). A previous genome-wide scan with Dutch FCHL families identified a region on chromosome 11 linked to FCHL and high TGs. This region was recently replicated in British FCHL families. Our objective was to test the neuropeptide galanin (GAL) which is a regional positional candidate gene for association. Recent findings strongly implicate GAL in pathways involving TGs, although the details of the mechanism have not been completely elucidated. Furthermore, the expression of GAL in the hypothalamus is known to be specifically upregulated in rodents due to a high-fat diet induced increase in TG levels. To investigate GAL as an FCHL candidate gene, we genotyped four tagging SNPs and singletons that covered the common variation of GAL in four different study samples: Dutch, Finnish and Mexican FCHL families and a Caucasian combined hyperlipidemia case/control sample, comprising a total of 2032 subjects. The initial association was tested in Dutch and Finnish FCHL families as well as in the Caucasian combined hyperlipidemia case/control sample. We identified the same haplotype for high TG in all of the initially tested samples ($p = 0.009$ in the Dutch and Finnish FCHL families, and $p = 0.001$ in the case/control study sample). Importantly, this same haplotype was replicated in Mexican FCHL families ($p = 0.04$), and when all the FCHL family study samples were combined, the association with TG became more significant ($p = 0.001$). In conclusion we have identified GAL as a new FCHL candidate gene, influencing serum TG levels in humans. Our future plan is to identify the functional mechanisms by which GAL regulates TG levels.

1740/W

Analysis of genetic variation in the *SCN5A* sodium channel for association to common forms of arrhythmias. *C. Lefebvre[1,2], E. Lizotte[1,2], P. Goyette[1,2], M.J. Junttila[3], H.V. Huikuri[3], R. Brugada[1,2], J.D. Rioux[1,2,4].* 1) Montreal Heart Institute, Montreal, Quebec, Canada; 2) Department of Medicine, Université de Montréal, Montreal, Quebec, Canada; 3) Department of Internal Medicine, Division of Cardiology, University of Oulu, Finland; 4) The Broad Institute of MIT and Harvard, Cambridge, MA, USA.

The *SCN5A* gene encodes the α-subunit of the human cardiac sodium channel and is the main protein responsible for the inward cardiac sodium current (I_{Na}). Several lines of evidence have shown that I_{Na} may modulate the risk for sudden cardiac death (SCD). Genetic defects in SCN5A have been identified in several arrhythmogenic disorders, such as the Brugada syndrome and progressive conduction disturbances where there has been a link with increased risk of SCD. Given the role of *SCN5A* in these monogenic disorders, we speculated that there may be a genetic predisposition to SCD and arrhythmias in the population affected by acute myocardial infarction (AMI). We therefore initiated a study of the genetic variation in *SCN5A* with the aim of examining its potential role in susceptibility to common forms of arrhythmia. Specifically, we conducted an association study of the *SCN5A* gene region on 1200 subjects from the Finnish FinGesture cohort and 550 Finnish healthy controls. This cohort consists of survivors of AMI and SCD victims with an AMI event verified at autopsy. We selected 39 informative SNPs capturing all of the common genetic variation in the *SCN5A* region, as well as the known coding and promoter variants. We observed similar haplotype structure between the FinGesture cohort and the CEU samples from the International HapMap project. Preliminary analyses identified significant association (ChiSq=7.198; p=0.0073) between a common promoter haplotype and the SCD phenotype. We are currently in the process of replicating this finding in two independent cohorts (MSIQue and ICM) and will present the complete results, including genotype-phenotype analyses.

1741/W

Genetic variants in selenoprotein S are associated with inflammatory biomarkers and vascular calcified plaque in families from the Diabetes Heart Study. *A.B. Lehtinen, Y. Liu, J.T. Ziegler, C.D. Langefeld, B.I. Freedman, J.J. Carr, D.W. Bowden.* Wake Forest University School of Medicine, Winston-Salem, NC.

Inflammation plays a role in the development and progression of common diseases such as diabetes and cardiovascular disease (CVD). Genetic variants in selenoprotein S (*SELS*) have been reported to be associated with plasma levels of inflammatory biomarkers in individuals of European ancestry, and *SELS* gene expression has been shown to be dysregulated in polygenic animal models with diabetes and glucose intolerance. We sought to replicate the association of *SELS* variants with inflammatory biomarkers in families enriched for type 2 diabetes mellitus (T2DM). Five single nucleotide polymorphisms (SNPs) in the *SELS* gene, G-105A, rs4965814 (3705G→A), rs9874 (6218A→G), rs7178239, and rs13313503, were genotyped in 937 European Americans from 375 families containing at least 2 siblings with T2DM. Four SNPs (rs4965814, rs9874, rs7178239, rs13313503) were significantly associated with IL-6 levels ($0.004<p<0.049$), 3 SNPs (G-105A, rs4965814, rs7178239) were significantly associated with ICAM1 levels ($0.0001<p<0.014$), and 2 SNPs (rs7178239, rs13313503) were significantly associated with MCP1 levels ($0.0009<p<0.042$) after adjusting for age, gender, diabetes affection status, smoking, and use of lipid-lowering medications. Because of the role of inflammation in the pathogenesis of CVD, we also evaluated the 5 *SELS* SNPs for association with quantitative measures of vascular calcified plaque as measured in the carotid artery (CarCP) and coronary artery (CCP), which show significant genetic correlation (0.52±0.11; $p<0.05$) in the study population. All 5 SNPs were significantly associated with CarCP ($0.005<p<0.042$), and 3 SNPs were trending towards association with CCP ($0.071<p<0.109$). Taken together, these data suggest that *SELS* is involved in mediating inflammation and is likely involved in the inflammatory processes of CVD leading to calcification in the carotid and coronary arteries. The observation of these associations in a diabetes-enriched population suggests that this patient subset may be particularly susceptible to genetic variants that influence the inflammatory response.

1742/W

Association between ACE pathway genes and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *X. Li[1], Y. Chen[1], T. Howard[2], J. Mychaleckyj[3], Y.I. Chen[1], S.J. Shea[4], J. Polak[5], J.R. Rotter[1].* 1) Cedars-Sinai Medical Center; 2) Wake Forest University; 3) University of Virginia; 4) Columbia University; 5) Tufts-New England Medical Center.

Angiotensin I-converting enzyme (ACE) is a key factor involved in blood pressure (BP) regulation. Since hypertension is a risk factor for atherosclerosis, variations in ACE pathway genes (ACE, AGT, AGTR1, and NOS3) may be associated with atherosclerosis development. In the MESA study, subclinical atherosclerosis is evaluated by carotid ultrasound of intima-media thickness (IMT) of the common carotid artery (CCA IMT) and of the internal carotid artery (ICA IMT). We genotyped 2847 subjects, including all 4 ethnic groups (712 African Americans, AFA, 712 European Americans, EUA, 718 Chinese Americans, CHN, and 705 Hispanic Americans, HIS), to examine the association between 61 tag SNPs of ACE pathway genes and IMT. Association tests were considered for additive models if the generalized model (2df) was significant. Multiple linear regression was first adjusted for age, gender, BMI, and then adjusted for BP. ACE gene's variation was associated with CCA IMT in CHN for 2 SNPs (rs4459610, p=0.007 and rs8066276, p=0.008). AGT did not yield any significant associations. AGTR1 was associated with IMT differentially in different ethnic groups. In AFA, 1 SNP was associated with CCA IMT (rs4488792, p=0.005) and 1 SNP was associated with ICA IMT (rs6801836, p=0.007). In EUA, 1 SNP was associated with CCA IMT (rs275645, p=0.021). And in HIS, only after adjusting for BP, 2 SNPs were associated with ICA IMT (rs275645, p=0.033 and rs2638363, p=0.038) and one was overlapping with CCA IMT (rs275645, p=0.030). Most interestingly, 1 specific NOS3 SNP was significantly associated with CCA IMT in AFA (rs743507, p=0.005) and in all ethnic groups combined (p=0.003). In summary, the association between IMT and ACE pathway genes was different across ethnic groups for most of genes studied. However, the NOS3 SNP rs743507 was associated with CCA IMT in all ethnic groups combined. The association was independent of any BP effect. These results suggest different susceptibilities to atherosclerosis at different artery segments.

1743/W

High-Density Association Analysis of the CETP Locus and HDL-C in Families of African Ancestry. *I. Miljkovic-Gacic[1], X. Wang[2], C. Kammerer[2], C. Nestlerode[1], L. Yerges[1], A. Kuipers[1], L. Goodrich[1], L. Kuller[1], C. Bunker[1], A. Patrick[3], V. Wheeler[3], R. Evans[1], J. Zmuda[1].* 1) Department of Epidemiology, University of Pittsburgh , Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) The Tobago Health Studies Office, Scarborough, Tobago.

Decreased HDL cholesterol is a major risk factor for cardiovascular disease. A recent genome-wide association analysis identified a single nucleotide polymorphism (SNP) near cholesteryl ester transfer protein (CETP) gene as the most promising locus for HDL-C in Caucasians, but the role of genetic variation in CETP as a determinant of HDL levels in African populations is still unclear. We assessed the heritability of fasting HDL-C and its association with 19 tagging and 2 SNPs from the literature across a 32 kilobase region encompassing CETP among 402 Afro-Caribbeans (mean age=42yrs) from 7 multigenerational families (median family size 51 individuals; 3535 relative pairs). Residual heritability for HDL-C after adjusting for age, sex, BMI, current smoking, alcohol intake, and physical activity was 0.55±0.11 ($P<0.001$). Residual heritability of HDL-C was higher in men (0.58±0.18, $p<0.001$) than in women (0.36±0.15, p=0.002), although this difference did not achieve statistical significance. After adjusting for significant covariates, individuals homozygous for the rs9926440 minor G allele (frequency,0.28) and for the rs289717 minor A allele (frequency,0.12) had 17.2% and 17.7% lower HDL-C, respectively, whereas individuals homozygous for the rs708272 minor A allele (frequency,0.26) had 13.5% higher HDL-C compared to the individuals homozygous for the major allele ($P<0.01$).The pairwise correlation coefficient (r2) among the 3 SNPs ranged 0.01-0.24, suggesting independent effects. Genotypes for rs9926440, rs289717 and rs708272 accounted for 3%, 1.4% and 1.1% of the total phenotypic variability in HDL-C, respectively. Our findings support the hypothesis that genetic factors are a major determinant of HDL levels among individuals of African origin, especially in men, and also suggest an association of the CETP locus with HDL-C in this population.

1744/W

Association of Polymorphisms in Cyclooxygenase (COX)-2 with Coronary and Carotid Calcium in the Diabetes Heart Study. *M.E. Rudock[1], J. Ziegler[2], S.G. Allen[3], A.B. Lehtinen[1,5], J.J. Carr[4], C.D. Langefeld[2], D.W. Bowden[1,5], Y. Liu[2,3].* 1) Center for Human Genomics; 2) Departments of Public Health Sciences; 3) Internal Medicine; 4) Radiology; 5) Biochemistry, Wake Forest University, Winston Salem, NC.

BACKGROUND: Cardiovascular Disease is the leading cause of death among Americans. Inflammation is a hallmark feature in the development of atherosclerosis and is mediated by prostaglandins, catalyzed by cyclooxygenase (COX)-2. We sought to determine if variants in the COX-2 gene were associated with measures of cardiovascular disease in a primarily type 2 diabetic population. METHODS: Eight polymorphisms in COX-2 were genotyped and vascular calcified plaque measured in the coronary, carotid, and aortic arterial beds in 978 Caucasian siblings (83% with T2DM) from 369 Diabetes Heart Study families. Tests for single SNP and haplotypic association were performed using SOLAR and QPDT, respectively (results adjusted for age, gender, diabetes affection status, smoking, and use of lipid altering medications). RESULTS: All eight SNPs genotyped were found to be in strong pairwise linkage disequilibrium (D'=1.0). Three SNPs (rs689466, rs2066826 and rs20417) are associated with either coronary or carotid calcified plaque. Subjects homozygous for the G allele of rs689466 (n=31) or the A allele of rs2066826 (n=16) had a 32% (p=0.02) and 47% (p=0.04) increase in coronary calcified plaque, respectively. Similarly, subjects homozygous for the C allele of rs20417 (n=22) or the A allele of rs2066826 (n=16) had increased carotid calcified plaque (p=0.011, p=0.014). Furthermore, the overtransmission of the rs20417 + rs689466 GA haplotype was correlated with increased coronary calcified plaque (p=0.002), while the GG haplotype was correlated with decreased coronary calcified plaque (p=0.004). CONCLUSIONS: Polymorphisms in COX2 were associated with increased coronary or carotid calcium. Individuals with these variants may be at higher risk for developing cardiovascular disease.

1745/W

Combined effect of hemostatic gene polymorphisms and the risk of myocardial infarction in patients with advanced coronary atherosclerosis. *E. Trabetti[1], M. Biscuola[1], N. Martinelli[2], U. Cavallari[1], M. Pinotti[3], O. Olivieri[2], S. Cheng[4], M. Sandri[2], S. Friso[2], F. Pizzolo[2], C. Bozzini[2], P. Caruso[3], F. Bernardi[3], R. Corrocher[2], D. Girelli[2], P.F. Pignatti[1].* 1) Dept Mother-Child & Biol-Genetics, Univ Verona, Italy; 2) Dept Clinical and Experimental Medicine, Univ Verona, Italy; 3) Dept Biochemistry and Molecular Biology, Univ Ferrara, Italy; 4) Dept Human Genetics, Roche Molecular Systems, Inc., Alameda, CA, United States.

Relative little attention has devoted until now to the combined effects of gene polymorphisms of the hemostatic pathway as risk factors for Myocardial Infarction (MI), the main thrombotic complication of Coronary Artery Disease (CAD). We studied a total of 804 subjects, 490 of whom with angiographically proven severe CAD, with or without MI (n=306; n=184; respectively). An additive model considering ten common polymorphisms [F2 20210G>A, PAI1 4G/5G, FGB -455G>A, F5 Leiden and R2, F7 -402G>A and -323 del/ins, Platelet ADP Receptor P2Y12 -744T>C, ITGA2 873G>A, and ITGB3 1565T>C] was tested. The prevalence of MI increased linearly with an increasing number of unfavorable alleles ($\chi 2$ for trend = 10.68; P = 0.001). In a multiple logistic regression model, the number of unfavorable alleles remained significantly associated with MI after adjustment for classical risk factors. As compared to subjects with 3-7 alleles, those with few (≤2) alleles had a decreased MI risk (OR 0.34, 95%CIs 0.13-0.93), while those with more (≥8) alleles had an increased MI risk (OR 2.49, 95%CIs 1.03-6.01). The number of procoagulant alleles correlated directly (r=0.49, P=0.006) with endogenous thrombin potential. The combination of prothrombotic polymorphisms may help to predict MI in patients with advanced CAD.

1746/W

Study of Genetic Susceptibility to Myocardial Infarction in a Genetic Isolated NF Population. *Y.G. Xie[1,2,3], J.X. Ciu[1], E. Randell[1], J. Renouf[4], G. Sun[2], C. Butt[1], F.Y. Han[1].* 1) Dept Laboratory Medicine, Memorial Univ, St John's, NL, Canada; 2) Dept Genetics, Memorial Univ, St John's, NL, Canada; 3) Dept Pediatrics, Memorial Univ, St John's, NL, Canada; 4) Laboratory Medicine Program, Eastern Health, NL, Canada.

Myocardial infarction (MI) is a complex disease that results from a life-long interplay between genetic and environmental factors. Being a multifactorial disorder, the genetic components of MI may be combined effects of a number of genes with each playing only a small role. Case-control association analysis is a commonly used study design in the field of complex trait genetics. However, the genetic associations with MI in most studies are not consistently reproducible due to inadequate sample size, population heterogeneity, confounding gene-gene and gene-environment interactions, and the complex dependency of the associations. Case control studies using a genetically isolated population are of advantage due to a relatively homogenous genetic background. Furthermore, large sample sizes help to identify weak genetic risk factors which may be the main factors imparting genetic susceptibility in MI. Recent advances in high-throughput genomic technology make it possible to study multiple gene polymorphisms in large populations. Taking advantage of the genetic isolated Newfoundland population, we carried out a large case-control study that involved genotyping 18 gene variants from 11 selected candidate genes in 1,000 patients with MI and 1000 healthy controls. Genotyping of SNPs was conducted using Taq Man SNP genotyping technology on real-time PCR. Our results showed an association between MI and two gene variants THB4 1186C* (OR=1.58, P=0.023) and MTHFR 1298C (OR=1.369, P<0.001). Potential gene-gene interactions were also evident which will be further validated by using a larger sample number. *date has been published.

1747/W

Evidence that a region of chromosome 10 linked to sodium-lithium counter-transport also influences plasma triglyceride level. *K.L.E. Klos[1], R. Ferrell[2], A.C. Morrison[1].* 1) Human Genetics Center, Univ Texas Health Science Center Houston, Houston, TX; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Measures of sodium-lithium countertransport (CNT), a risk factor for hypertension, are highly correlated with plasma triglyceride (TG) levels. We hypothesized that genomic regions containing CNT quantitative trait loci (QTL) may contain common genetic variation that also influences inter-individual variation in plasma TG level. We identified at least one common SNP (minor allele frequency > 0.05) in each of the 55 genes within a 35 Mb region of chromosome 10 linked to CNT. We genotyped 1,582 Caucasian individuals ascertained without regard for health through households with two or more children enrolled in primary and secondary schools of Rochester, MN as part of the community-based Rochester Family Heart Study. Plasma TG was measured by standard enzymatic methods and log transformed prior to analysis. Individuals who had not fasted for at least 8 hours prior to the clinic evaluation were excluded. TG was adjusted for age and BMI within gender (children separately from adults) prior to analysis. A generalized estimating equation (GEE) approach was used to test for association among related individuals. Variation in the two-handed zinc-finger homeodomain transcription factor 8 (TCF8), was associated (p<0.01) with TG level in this sample. A repeat polymorphism in TCF8 has been previously linked to obesity, while mutation in this gene may cause posterior polymorphous corneal dystrophy. TCF8 has been previously shown to be involved in smooth muscle cell differentiation and to repress t-lymphocyte-specific IL-2 gene expression. Since application of IL-2 to natural killer cells stimulates secretion of lipoprotein lipase, a key enzyme of triglyceride metabolism, the association of TCF8 variation with plasma TG level may provide a link in the connection between inflammatory pathway genes and lipid metabolism.

1748/W

Molecular genetic analysis of long QT syndrome patients and identification of one novel mutation in KCNH2. *X. Zhang[1,2], S. Chen[2], L. Zhang[3], C. Oberti[1], G.M. Vincent[3], Q.K Wang[1,2].* 1) Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; 2) Center for Human Genome Research, Huazhong University of Science and Technology, Wuhan, P. R. China; 3) LDS Hospital and University of Utah, Salt Lake City, UT.

Long QT syndrome is a cardiac disorder characterized by prolonged QT interval, ventricular arrhythmias and sudden death. To date, eight genes have been found for LQTS, including KCNQ1, KCNH2, SCN5A, Ank2, KCNE1, KCNE2, KCNJ2 and CACNA1C. In the past few years, we have been studying 89 independent families and patients affected with LQTS and ventricular arrhythmias. Ten mutations in KCNQ1, including five novel mutations, were previously reported (Chen S. et al. Clinical Genetics 2003;63:273-282). In this study, we performed linkage and mutation analyses for the rest of known LQTS genes in this population of patients. For two LQTS families, linkage analysis with polymorphic marker that span the LQTS genes linked one family to KCNH2, and the other family to SCN5A. Direct DNA sequence analysis identified two cis-variants, K897T and A490T in KCNH2, that co-segregated with all affected individuals in family 1. In family 2, we found that the E1784K mutation in SCN5A was present in all affected individuals, but some affected individuals also carried a G38S polymorphism in KCNE1. The patients who carried both the SCN5A E1784K mutation and KCNE1 G38S polymorphism had statistically longer QT interval than those with only the SCN5A E1784K mutation. Furthermore, the autopsy report of the proband who died suddenly at age 31 revealed dilated cardiomyopathy-like phenotype and fat infiltration and fibrosis, implicating that some LQTS patients may have the potential risk of developing dilated cardiomyopathy. Six other mutations were also identified and all in the KCNH2 gene. These mutations include one novel mutation 2040insAG, and five known mutations, A561T, D609N, A614V, D629S, and R366X. In summary, we report one novel KCNH2 mutation that causes LQTS. Further, our results suggest that polymorphisms in a known LQTS gene can modify the phenotype of LQTS patients carrying mutations in a different LQTS gene.

1749/W

Genotype-phenotype correlation in patients with bicuspid aortic valve and aneurysm. *K.C. Kent[1], M.L. Loscalzo[1], D.LM Goh[1], A.L. Cutting[1], H.C. Dietz[1,2].* 1) Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 2) HHMI, Baltimore, MD.

Bicuspid aortic valve (BAV) is the most common congenital cardiac defect occurring in 1-2% of individuals. BAV segregates in an autosomal dominant manner with decreased penetrance. BAV is often associated with ascending aortic aneurysm (AscAA). Four BAV/AscAA families with *NOTCH1* mutations have been described. In this study, we sought to determine the contribution of *NOTCH1* mutations to familial BAV/AscAA. We sequenced all exons and splice junctions of *NOTCH1* in probands from 13 affected families. Only 2 changes were identified that were not previously reported as polymorphic variants. Both were synonymous substitutions that did not have an intuitive affect on mRNA processing or stability. There are important phenotypic differences that distinguish families with and without *NOTCH1* mutations. Those with *NOTCH1* mutations show highly penetrant BAV, with uniform presence of early valve calcification and aortic stenosis (AS) and low incidence of aneurysms. In contrast, the more typical presentation of BAV/AscAA, as exemplified in all of our families, shows highly penetrant aortic aneurysm and absent aortic valve calcification. Furthermore, many relatives of individuals with BAV/AscAA show the predisposition for early onset AscAA and dissection without associated BAV or AS. These data suggest that perturbation of Notch signaling has a predominant effect on valve calcification, morphology and function, and that aortic aneurysm may occur secondary to hemodynamic perturbations in this setting. The more common presentation of BAV and aneurysm does not relate to *NOTCH1* and aortic aneurysms in this context are a direct manifestation of an underlying gene defect, which still remains to be identified. Given the variable age of onset of AscAA, these data also mandate ongoing imaging follow-up of relatives of affected individuals with or without BAV.

1750/W

A Genetic Association Between Angiotensinogen Genotype and Plasma Angiotensinogen Endophenotype in CEPH Families. *W.S. Watkins, W. Tolpinrud, A. Rohrwasser, Y. Zhang, A. Peiffer, M. Leppert, J-M. Lalouel, L.B. Jorde.* Department of Human Genetics, Salt Lake City, UT 84112.

Many previous studies have documented an association between hypertension and alleles of the angiotensinogen locus (*AGT*), one of several loci implicated in hypertension by linkage and functional studies. However, the nature and strength of this association is still debated. To help resolve this issue, we have analyzed the relationship between *AGT* alleles and the key endophenotype, plasma angiotensingen, in mostly normotensive individuals from 42 CEPH pedigrees. Plasma AGT levels were assessed in 393 samples by complete conversion of AGT to angiotensin I (AI). AI was then assayed with a modified enzyme immune assay. Using the FBAT software package, an association between *AGT* genotypes and plasma AGT levels could be detected for *AGT* SNPs -1178A, -6G, 6065C, and 6232T ($p < 0.05$). The -6A allele has been implicated previously in increased rates of *AGT* transcription and is associated with increased risk of hypertension. Using HBAT, the promoter haplotype carrying the -1178A and -6G alleles also showed an association with plasma AGT levels ($p < 0.027$). Other studies, using hypertensive subjects, have shown that elevated plasma AGT is associated with hypertension. Our results suggest that common polymorphisms at the AGT locus are associated with plasma AGT levels in families of predominantly normotensive status. Support: NIH grant HL070048.

1751/W

Fine mapping of a chromosome 16 locus associated with low high-density lipoprotein cholesterol level (HDL-C) in French Canadians. *Z. Dastani[1,2], M. Marcil[2], J.C. Lee[3], P. Pajukanta[3], D. Gaudet[4], J. Genest[1,2], J.C. Engert[1,2].* 1) Dept. of Human Genetics, McGill Univ., Montreal; 2) Cardiovascular Genetics Laboratory, McGill Univ. Health Centre, Montréal; 3) Dept of Human Genetics, David Geffen School of Medicine, Univ. of California, Los Angeles; 4) Dyslipidemia, Diabetes and Atherosclerosis Group and Community Genomics, Chicoutimi.

Low HDL-C is an independent risk factor for coronary heart disease. We previously performed quantitative linkage analysis in two independent studies in French Canadians: a Quebec-wide study (QUE) consisting of 362 individuals families and 410 individuals from the Saguanay-Lac St-Jean region (SLSJ) and showed linkage to 16q23-24 in both studies. This locus was implicated in multiple previous linkage scans for HDL-C in different populations and resulted in linkage peaks that are less than 12 cM far from our peak. We performed SNP fine mapping using family based association and case-control association analysis. Using families from the QUE study, defining HDL-C < 5th%(age/gender-matched) as cases, the region was narrowed from 25 cM to 18.1 cM. Affected from four families share a 2 microsatellite haplotype. SNP genotypes narrowed the shared haplotype to 4Mb. A SLSJ case-control study identified several significant SNPs, one located in the same region as the shared haplotype from the QUE families. A haplotype containing this SNP and another increased the evidence for association ($p = 0.016$ to 0.0097). The CHST6 gene, found within this region, was previously associated with macular corneal dystrophy. Because of the presence of occular manifestations with other genes associated with low HDL-C (e.g. LCAT, ApoA1), we sequenced the CHST6 gene and its nearby homologue CHST5, and found two missense variants. The variant in the CHST5 gene did not segregate with low HDL. However, a variant in the CHST6 gene showed strong segregation in the 4 families sharing the microsatellite haplotype. This same variant also demonstrated an odds ratio >2 in the case/control sample, but this was not significant due to the small sample size. Our data present strong evidence for a HDL-C gene on chromosome 16q23-24 that may be related to the CHST6 or CHST5 loci.

1752/W

Association of AGGF1 gene polymorphisms with susceptibility for Klippel-Trenaunay syndrome. *Y. Hu[1], S.B. Seidelmann[1], L. Li[1], A.A. Timur[1], D.J. Driscoll[2], Q.K. Wang[1].* 1) Molecular Cardiology, Cleveland Clinic, Cleveland, OH; 2) Department of Pediatrics, Mayo Clinic, Rochester, MN.

Klippel-Trenaunay syndrome (KTS) is a severe congenital disorder that results in mixed vascular malformations. It is characterized by capillary malformations, venous malformations or varicose veins, and hypertrophy of the affected tissues. Our molecular genetic dissection of one KTS- associated translocation involving chromosomal segments 5q and 11p identified a strong candidate gene, AGGF1 (previously known as VG5Q), which increases KTS susceptibility. To further analyze the genetic relationship between AGGF1 and KTS, we examined whether common variants in AGGF1 were associated with susceptibility to KTS. We analyzed HapMap data and selected two SNPs, rs13155212 in exon 7 and rs7704267 in intron 11 that capture information for all common variants in the one haplotype block that covers the entire AGGF1 gene. The two SNPs were genotyped in 173 Caucasian KTS patients and 477 Caucasian non-KTS controls. AGGF1 variants rs7704267 and rs13155212 were significantly associated with susceptibility for development of KTS (P = 0.004 and 0.013, respectively). Permutation testing also showed a significant empirical P value for the association (empirical P = 0.006 and 0.015, respectively). These results suggest that common AGGF1 variants confer risk of KTS.

1753/W

Systematic evaluation of genetic defects in 220 patients with Tetralogy of Fallot. *A. Rauch[1], R. Rauch[2], S. Zink[3], C. Zweier[1], C. Purmann[1], J. Hoyer[1], P. Nürnberg[4], A. Reis[1], H. Singer[3], M. Hofbeck[2].* 1) Institute of Human Genetics, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; 2) Pediatric Cardiology, University of Tuebingen, Tuebingen, Germany; 3) Pediatric Cardiology, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; 4) Cologne Center for Genomics (CCG), University of Cologne,Cologne, Germany.

Tetralogy of Fallot (TOF) is the most common complex congenital heart defect accounting for about 5-6% of patients. Various chromosomal anomalies, microdeletion 22q11.2 and mutations in NKX2.5 have been described as recurrent causes. In order to address the question about the incidence of these known causes in a larger cohort and to prove the role of JAG1 and TBX1 mutations as candidate genes known to cause isolated TOF in single families and the 22q11.2 microdeletion phenotype, respectively, we performed chromosomal analysis, 22q11.2 microdeletion testing and sequencing of NKX2.5, JAG1 and TBX1 in a cohort of 220 unselected patients with TOF. 80 randomly selected patients were also analysed for chromosomal microaberrations with molecular karyotyping using 100 K SNP arrays. 17 patients (7.7%) showed the common 3 Mb microdeletion 22q11.2, 6 patients (2.7 %) had trisomy 21, 3 (1.2%) had other chromosomal aneusomies. Two patients (1%) each had known mutations in NKX2.5 and JAG1. Two patients showed rare variants in TBX1, one of which did not show any functional alteration in a transcriptional reporter assay while results in the second are pending. Surprisingly, 4 of 80 patients tested (5%) showed causative microaberrations detected by molecular karyotyping. These 4 patients had mental retardation in addition to TOF. Another 4 patients had small microdeletions containing candidate genes for heart development, which are under further investigation. Our results confirm that microdeletion 22q11.2 and trisomy 21 are the most common causes of TOF, while NKX2.5 and JAG1 are only rare causes of TOF. TBX1 apparently does not significantly contribute to the aetiology of TOF. In contrast, microaberrations detectable by molecular karyotyping seem to play a major role in congenital heart defects.

1754/W

FTO genotypes and weight gain in early life in two prospective birth cohort studies from Finland and the UK. *M-R. Jarvelin[1,2,3], P. Elliott[1], T.M. Frayling[4], R.M. Freathy[4], U. Sovio[1], A.J. Bennett[5], A. Ruokonen[2], A. Pouta[3], J. Laitinen[6], A-L. Hartikainen[2], D.A. Lawlor[7], E. Zeggini[5], C.M. Lindgren[5], S.M. Ring[7], A.R Ness[7], A.T. Hattersley[4], M.I. McCarthy[5], G. Davey Smith[7], N.J. Timpson[5,7].* 1) Imperial College London, United Kingdom; 2) Oulu University, Finland; 3) National Public Health Institute, Finland; 4) Peninsula Medical School, Exeter, UK; 5) Oxford University, UK; 6) Finnish Institute of Occupational Health, Finland; 7) Bristol University, UK.

In large-scale association studies on nearly 40,000 individuals, we have recently shown that a cluster of variants within the FTO gene on chromosome 16 is strongly associated with overweight/obesity. This association was not seen at birth but from age 7y onwards. We studied here the effect of FTO variant (rs9939609) on body mass until age 4y. We analyzed data on singletons in Northern Finland Birth Cohort born in 1966 (NFBC, n= 3849) collected at ages 6 and 12mo and in the Avon Longitudinal Study in Parents and Children born in 1991-2 (ALSPAC, n=7126) collected at 6 wks, 9mo, 18mo and 4y, adjusting for gestational age until 1y and for sex. In ALSPAC, at ages 4 and 11y, 19% were overweight or obese by IOTF age-gender specific criteria, and in NFBC at 14y (assessed about 20y earlier than ALSPAC) 7% were overweight or obese. In neither cohort was the A allele at rs9939609 associated with higher body mass index (BMI, log-transf.) during the first year: in NFBC, percentage change in BMI varied from -0.06% (95%CI -0.53,0.40) to 0.20% (-0.21,0.61) and in ALSPAC from -0.34% (-0.68,-0.0004) to -0.15% (-0.51,0.21) per allele. In ALSPAC, there was no evidence of weight gain by increasing number of A alleles from second year up to 4y; between-homozygote-groups differences in BMI (geometric means, AA-TT) were -0.11 kg/m2 at 18mo (p 0.04) and -0.02 kg/m2 at 4y (p 0.69). Taken together with our earlier publication, these studies suggest that this variation in FTO gene is not associated with excessive weight gain in utero or early life. They indicate that the variant is associated with an acceleration in weight gain during pre-school and early school years between age 4 and 7 years.

1755/W

MTHFR and E-selectin gene polymorphism towords genetic predisposition of coronary artery disease (CAD). *R. Tripathi[1,2], S. Agarwal[2].* 1) Genetics, SGPGIMS, Lucknow, India; 2) Genetics prof.

MTHFR (5,10 methylene tetrahydrofolate reductase) is a regulatory enzyme of homocysteine metabolism whereas E-selectin (CD62E) mediates the adhesion of circulating leukocytes and play a role in pathogenesis of atherosclerosis. The aim of the study to determine the influence of C677T polymorphism of MTHFR gene and A561C polymorphism of E-selectin gene in North Indian population.The C677T polymorphism and A561C polymorphism were genotyped by PCR-RFLP in n=112 angiographically documented CAD patients and n=127 age/sex matched healthy individuals as control.The T allele frequency of MTHFR gene were 13.56% in CAD patients Vs 7.4% in control,this indicate significant association (p<0.05, OR=1.94, 95%CI=1.076-3.778) of MTHFR gene C677T polymorphism with CAD. The C allele of E-selectin gene (p>0.05, OR=1.15) shows insignificant association.Our study shows that MTHFR can be used as genetic marker for predisposing CAD. However, largesample size is needed to confirm the results.

1756/W

Promoter polymorphism of iNOS gene and their influence on essential hypertension in Chinese. *Y. Zhao[1], L. Fu[1,2], Y. Gao[1], J. Shi[2], J. Liu[1], M. Qi[1], H. Liu[1].* 1) Dept Medical Genetics, China Medical Univ, Shenyang, Liaoning, China; 2) Dept Clinical Epidemiology, First Affiliated Hospital, China Medical University, Shenyang, China.

Inducible nitric oxide synthase (iNOS) catalyzes L-arginine to NO, a potent vasodilator which participates in the development of hypertension. iNOS expression is induced by many factors in various tissues including brain, heart, vessel and kidney. To determine the relationship of genetic variation in the regulatory region of the iNOS gene with hypertension in Chinese population, we performed case-control study with 610 subjects, 308 normal controls and 302 hypertensives. The iNOS-1026C/A polymorphism was detected by real-time PCR. The -1026A allele and the -1026AA genotype had significantly lower frequency in hypertensives than in controls (P<0.05). After the non-conditional Logistic analysis, -1026AA genotype was an independent predictor for hypertension (OR=0.129, 95% CI 0.053~0.318), and it would be a protective factor for hypertension. We reported for the first time that iNOS -1026C/A is associated with hypertension. Further research is necessary to identify the functional consequence of the variant that modify the susceptibility to hypertension.

1757/W

Whole genome maps of USF1 and USF2 binding and histone 3 acetylation reveal new aspects of promoter structure and candidate genes for common human disorders. *C. Wadelius[1], A. Rada Iglesias[1], A. Ameur[2], P. Kapranov[3], S. Enroth[2], J. Komorowski[2], T. Gingeras[3].* 1) Dept Genetics & Pathology, Uppsala Univ, Uppsala, Sweden; 2) Linnaeus Centre for Bioinformatics, Uppsala, Sweden; 3) Affymetrix, Inc., Santa Clara, USA.

USF1 is a transcription factor associated with familial combined hyperlipidemia (FCHL) and binds as a heterodimer with USF2. Binding sites for USF1, USF2, and regions of histone 3 acetylation (H3ac) were mapped in HepG2 cells across the genome using ChIP and tiling arrays. High resolution was achieved by sonicating the enriched DNA to average sizes of 300 bp and the array design. Regions with signal significantly higher than negative controls were identified and subsequent Q-PCR in such regions showed <1% false positives for each factor. Regions bound by USF1, USF2 and H3ac were characterized against a range of annotated genomic features. Genes with promoters bound by USFs were analyzed using gene ontology classification and three biological pathways were identified that contained genes that are excellent candidates for FCHL. H3ac signal was found at 10.900 regions, 50% of them <1kb from TSS of protein coding genes (PCG). The footprint of this signal showed a bimodal pattern with a major peak downstream of TSS at +600 - +800bp and a smaller peak symmetrically located upstream of the TSS. There is a high frequency of bidirectional promoters on the genome and footprints for unique and bidirectional promoters were identical downstream of the TSS but the upstream signal was significantly lower for unique promoters. The ChIP-chip signals were compared to the expression pattern of 18 000 genes and the height of the downstream peak was positively correlated with gene expression. USF1 and USF2 preferentially bound -300 to -100bp i.e. upstream of TSS of PCG. In bidirectional promoters USF1 and USF2 bind between the two TSS and the peak of H3ac is downstream of each TSS. Footprints of H3ac around the TSSs of mRNAs/spliced ESTs for CpG island positive transcripts showed a peak downstream of TSS which suggests that they are true promoters. These lines of high resolution analysis will aid annotation of promoters in the genome.

1758/T

Cigarette Smoking Modulates Genetic Effects on Chromosome 3q13-21 in Early-Onset Coronary Artery Disease. *B.D. Horne[1], L. Wang[2], J.B. Muhlestein[3], J.L. Anderson[3], J.F. Carlquist[3], E.R. Hauser[2], S. Shah[2,4], W.E. Kraus[4], J.M. Vance[5], P.J. Goldschmidt-Clermont[6].* 1) Cardiovasc Dept, LDS Hosp & Genet Epidemiol, Univ Utah, SLC, UT; 2) Dept Medicine & Cent Human Genet, Duke Univ, Durham, NC; 3) Cardiovasc Dept, LDS Hosp & Cardiol Div, Univ Utah, SLC, UT; 4) Dept Medicine & Div Cardiology, Duke Univ, Durham, NC; 5) Miami Inst Human Genomics, Miami, FL; 6) Miller School Med, Univ Miami, Miami, FL.

Consistent linkage evidence has been found for coronary artery disease (CAD) at chromosome 3q13-21. We previously reported genes within the linkage peak associated with early-onset CAD in the CATHGEN dataset, implicating the Kalirn-RhoGTPase pathway (KALRN, CDGAP, and MYLK) and the transcription factor GATA2. We sought to validate associations in those genes in a large independent dataset of early-onset CAD cases and controls (IHCS, N=1325). Eleven previously studied single nucleotide polymorphisms (SNPs) in KALRN, CDGAP, MYLK, and GATA2 were examined. Single SNP association was evaluated adjusting for classic risk factors. Given the much higher prevalence of smoking in CATHGEN than IHCS (41% vs 11% in controls, 74% vs 29% in cases), stratified analysis on cigarette smoking and genotype-smoking interaction analysis were performed. Overall, suggestive association was found at SNP rs2713604 in GATA2 (p=0.057, OR=1.2). Among smokers in IHCS, significant associations were found at rs10934490 in CDGAP (p=0.019, OR=1.6) and rs12637456 in KALRN (p=0.011, OR=2.0) and suggestive association at rs16834871 in MYLK (p=0.051, OR=1.8, adjusting for gender only). No association was found in any SNPs among non-smokers in IHCS. Strong interactions between SNP genotype and smoking status were detected in CDGAP (rs10934491, p=0.017) and KALRN (rs12637456, p=0.010) with suggestive interaction in MYLK (rs16834817, p=0.08, adjusting for gender only). We validated the associations with early-onset CAD in KALRN, CDGAP, MYLK, and GATA2. These data suggest that the genetic risk conferred by those genes may be modified by cigarette smoking. Interaction between smoking and genotype could explain all or part of the difference in genetics effects observed in different datasets.

1759/T

Daily physical activity modifies the association between endothelial nitric oxide synthase gene variant and blood pressure. *V. Karani Santhanakrishnan[1], P.W Franks[2], I. Barroso[3], S. Brage[1], U. Ekelund[1], N.J Wareham[1], R.J.F Loos[1].* 1) MRC Epidemiology unit, Cambridge, UK; 2) Genetic Epidemiology & Clinical Research Group, Department of Public Health & Clinical Medicine, Division of Medicine, Umeå University Hospital, Umeå, Sweden; 3) The Wellcome Trust Sanger Institute, Metabolic Disease Group, The Wellcome Trust Genome Campus, Hinxton, UK.

The endothelial nitric oxide synthase (NOS3) gene encodes the enzyme (eNOS) that synthesises the molecule nitric oxide which facilitates endothelium-dependent vasodilation in response to exercise. Thus, variation at NOS3 may modify the association between physical activity and blood pressure. To test this hypothesis, we genotyped 11 NOS3 polymorphisms, capturing all common variations, in 726 men and women from the MRC Ely Study (age (mean ± SD): 55±10 years, BMI: 26.4±4.1 kg/m2). Free-living total energy expenditure (TEE) was assessed via individually calibrated heart rate monitoring over 4 days. The intronic variant, IVS25+15 G→A, was significantly associated with blood pressure; GG homozygotes had significantly lower levels of diastolic blood pressure (DBP) (- 2.8 mmHg; p= 0.016) and systolic blood pressure (SBP) (- 1.9 mmHg; p= 0.018) than A-allele carriers. The interaction between TEE and IVS25+15 was also significant for both DBP (p= 0.006) and SBP (p= 0.026); i.e the association between the GG-genotype and blood pressure was only significant in the individuals with the highest energy expenditure (DBP: - 4.9 mmHg, p=0.02. SBP: -3.8 mmHg, p= 0.03). Similar results were observed when the outcome was dichotomously defined as hypertension. In summary, the NOS3 IVS25+15 is directly associated with blood pressure and hypertension in UK Europids. However, the associations are most evident in physically active individuals. These results may be informative for targeted disease prevention, where the selection of individuals for lifestyle intervention programs could be guided by knowledge of their genotype.

1760/T

CD36 as a Candidate Gene for the Metabolic Syndrome. *L. Love-Gregory, R. Sherva, L. Sun, J. Wasson, R. Neuman, M.A. Permutt, N.A. Abumrad.* Washington University School of Medicine, St. Louis, MO 63110.

A genome scan in African American (AA) families of the Hypertension Genetic Epidemiology study (HyperGEN) identified a QTL for beta cell function and another for insulin sensitivity on chromosome 7q near a 1.2Mb region encompassing the CD36 gene. CD36 is a transmembrane glycoprotein that facilitates fatty acid (FA) uptake into adipose and skeletal muscle tissues. Impairment of FA metabolism is implicated in the etiology of obesity, insulin resistance, and hypertension (central components of the metabolic syndrome, MS). AA have a high incidence of variability at the CD36 locus and this may play a role in the susceptibility to obesity and related diseases in this population. We obtained DNA and clinical parameters from 2300 AA HyperGEN participants. We initiated genotyping of tag SNPs across CD36 and its promoter regions to determine if common variants in this region are associated with MS and how they impact CD36 expression. Of 40 SNPs genotyped in 2020 subjects, 90% have minor allele frequencies (maf) ≥5%. Preliminary analyses of data adjusted for age, gender, BMI, and recruitment site identified 4 non-coding SNPs that increase risk for MS (p<0.034). However, the minor allele (m.a.) of 1 coding SNP (maf 10%; to-date only identified in subjects of African ancestry) associates with decreased odds ratio for MS, OR 0.710 (95% CI 0.52-0.95, p 0.0098), decreased triglycerides (p=0.0058), and increased HDL-C (p=0.0002) The coding SNP is predicted to cause complete CD36 deficiency in subjects homozygous for the m.a. while heterozygous subjects likely have reduced CD36 levels. Data were analyzed using PROC GENMOD in SAS (regression, Additive Model) in which SE were adjusted to for family relationships. Subsequent analysis with FBAT confirmed findings. Although these data suggest that the m.a. is protective, subjects homozygous for the m.a. (presumably CD36 deficient, n= 10) trended metabolically (higher mean TGs and lower HDL) in the opposite direction vs. heterozygotes. These findings suggest that variants in the CD36 gene influence metabolic syndrome susceptibility and that partial vs. complete CD36 deficiency may associate with different phenotypic outcomes.

1761/T

Mutation screening of NOTCH pathway genes in individuals with left ventricular outflow tract defects. *K.L. McBride[1], G. Zender[1], S.M. Fitzgerald-Butt[1], S.D. Fernbach[2], J.A. Towbin[2], J.W. Belmont[2].* 1) Center of Molecular & Human Gen, Columbus Child Res Inst, Columbus, OH; 2) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston TX.

The NOTCH signaling pathway is important for heart development. Multiple receptors (NOTCH 1-4) interact with multiple membrane bound ligands (JAGGED, SERRATE, DELTA) leading to cleavage and release of the intracellular NOTCH domain. This domain interacts with the RBPJK/CBF1/Su(H) transcription factor, changing it from a repressor to an activator of genes from the HES families (e.g. HEY2). JAG1 defects in human (Alagille syndrome) frequently cause tetralogy of Fallot, septal defects, aortic valve stenosis (AVS) and coarctation of the aorta (CoA). The cardiac defects in the mouse HEY2 knockout are similar to human Alagille syndrome, while the zebrafish homologue mutant gridlock recapitulates coarctation of the aorta. Recently, NOTCH1 mutations have been found in 2 families with calcific AVS. We postulated NOTCH pathway genes may be important in the development of congenital left ventricular outflow tract (LVOT) defects in humans. We screened 101 individuals with AVS, CoA or hypoplastic left heart syndrome (HLHS) for mutations in HEY2 and NOTCH1 by denaturing high performance liquid chromatography. Direct sequencing was performed on any amplicons demonstrating an abnormal chromatogram. None of the cases had mutations in HEY2. 64 variants in NOTCH1 were identified, 27 of which were novel. 9/27 variants cause an amino acid substitution, and could be found in the subject and one parent. The missense mutations are scattered throughout the gene, but are not present in the Notch, transmembrane, or ankyrin domains. 6/9 of these involve a highly conserved nucleotide. None of these changes have been observed in 184 control chromosomes. The presence of the variant in both the subject and a parent, and the occurrence in highly conserved amino acids among non-critical domains are characteristic of a susceptibility allele for a complex genetic disease. We believe this supports our hypothesis that NOTCH1 variants are necessary, but not sufficient, to cause LVOT defects in some subjects.

1762/T

Genetic studies in Korean patients with cardiac outflow tract anomalies. *E.J. Seo[1,2,3], M. Hong[3], K.J. Kim[3], Y.H. Kim[3,4], J.K. Ko[3,4], I.S. Park[3,4].* 1) Medical Genetics Clinic & Lab, Univ Ulsan, Asan Medical Ctr, Seoul, Korea; 2) Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center; 3) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center; 4) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Cardiac outflow tract anomalies is caused by defects of the truncal septation and the secondary heart field during embryogenesis. Many genetic interactions are thought to contribute to the formation of the cardiac outflow tract (OFT). To investigate mutations of the candidate genes, we did genetic analysis in sixty-three Korean patients with OFT anomalies including interrupted aortic arch, truncus arteriosus, coarctation of aorta, etc. Direct sequencing for seven genes such as TBX1, FGF8, FOXP1, GJA1, KCNJ2, ACVR1, and SEMA3C was performed. We identified 8 novel non-synonymous (T268S, N397H, L400Q and G483E in TBX1; T226A and V386E in FOXP1; L8P in FGF8; T562I in SEMA3C), 5 novel synonymous variations (F140, H242, G310 and A311 in TBX1; L196 in ACVR1) and 12 novel non-coding variations in promoter and untranslated regions. Among them, 6 non-synonymous (T268S, L400Q and G483E in TBX1; T226A and V386E in FOXP1; L8P in FGF8) and 6 non-coding variations from 12 unrelated patients were not detected in 100 healthy Korean individuals, suggesting that these mutations could be involved in the malformation of OFT. Particularily, total 9 novel variations of the TBX1 gene were found in this study. Further studies will be necessary to determine the precise contributions of specific mutations of these genes to OFT anomalies.

1763/T

Homozygous mutation in desmocollin-2 in arrhythmogenic right ventricular cardiomyopathy. *D. Ahnood, M.A. Simpson, S. Mansour, E.R. Behr, A.H. Crosby.* St George's University of London, London, United Kingdom.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a myocardial disorder associated with ventricular arrhythmias, heart failure and sudden death. The disease is characterised by progressive loss of cardiomyocytes and fibrofatty replacement, primarily occurring in the right ventricle though left ventricular involvement may also arise. Autosomal recessive mutations causing ARVC have been described in plakoglobin (JUP) and desmoplakin (DSP) and are characterised by the triad of ARCV with palmoplantar keratoderma and woolly hair. We present the identification homozygous single base deletion in exon 16 of the desmocollin-2 (DSC2) gene in a 30 year old male with ARVC, woolly hair and mild palmar-plantar keratoderma. Whilst 3 autosomal dominant mutations have been identified in DSC2 in patients with ARVC, this is the first reported homozygous mutation. A panel of microsatellite markers located in and around the 5 desmosomal genes previously identified in the pathogenesis of ARVC were genotyped in this individual. Heterozygosity in the proband was revealed at 4 of the 5 loci investigated (JUP, DSP, PKP2, DSG2). However, a region of homozygosity was identified in the proband at the DSC gene cluster located on chromosome 18. Sequence analysis of the DSC2 gene, revealed a single basepair deletion in exon 12 (1841delG). This mutation is predicted to lead to a frameshift and a premature termination codon at position 625 (S614fsX625).

1764/T

Circulating endothelial progenitor cells are elevated in pulmonary arterial hypertension and show distinct expression profiles consistent with increased endothelial lineage commitment. *M.A. Aldred[1], K. Asosingh[2], S.C. Erzurum[2].* 1) Genomic Medicine Institute, and; 2) Department of Pathobiology, Cleveland Clinic Lerner Research Institute, Cleveland, OH.

Pulmonary arterial hypertension (PAH) is a serious progressive lung vascular disorder characterized by proliferation and migration of endothelial and smooth muscle cells, leading to narrowing of precapillary pulmonary arteries and a sustained elevation of mean pulmonary artery pressure. Mutations of the *BMPR2* gene underlie most familial cases and 20-25% of sporadic PAH. However, the penetrance averages only 20%; one of the biggest challenges is thus to identify early markers of disease in asymptomatic carriers. Vascular repair has traditionally been thought to occur through resident cell populations, but recently the role of bone marrow-derived circulating stem cells has become apparent. Circulating endothelial progenitor cells (EPCs) are crucial to the process of vasculogenesis, homing to damaged tissues and incorporating into existing endothelium to form new vessels. We hypothesized that EPC dysfunction might contribute to proliferative vascular disorders such as PAH. EPCs were isolated from peripheral blood samples from 14 PAH patients and 9 controls. FACS analysis demonstrated significantly more EPCs in patients than controls and a correlation with mean pulmonary artery pressure. *In vitro* endothelial cell colony forming assays (CFU-EC) yielded 10-fold more colonies in PAH patients, indicating greater commitment to the endothelial cell lineage. Expression microarray analysis on CFU-EC from 10 patients and controls confirmed that CFU-EC express endothelial and angiogenic markers, such as Endoglin and VEGF. Furthermore, our data suggest that a subset of these genes are differentially expressed in PAH and may correlate with *BMPR2* mutation status. Genes in lipid and hypoxia pathways were also dysregulated in PAH CFU-EC. Our data suggest that EPCs from PAH patients have increased endothelial lineage commitment and may play a role in disease pathogenesis. With further characterization of CFU-EC gene expression, EPCs may have utility as markers of early PAH in asymptomatic mutation carriers.

1765/T

The NFKβia gene is a novel PPAR Cardiac target gene. *N. Buroker[1], J-Y. Huang[2], M. Ge[1], X-H. Ning[1,2], M. Portman[1,2].* 1) Dept Cardiology, Seattle Children's Hosp, Seattle, WA 98105; 2) Department of Pediatrics, University of Washington, Seattle, WA 98195.

The peroxisome proliferator-activated receptors (PPARs) and the retinoid X receptors (RXRs) are members of the nuclear receptor superfamily, which consists of a large number of special transcription factors whose activities are regulated by their cognate ligands. These steroid hormone receptors are important regulators of gene expression and differentiation. These receptors form homo- (RXR) and hetero- (PPAR-RXR) dimers that bind DNA at various response elements (PPAR and RXR). We identify the PPAR/RXR response elements in the promoter of the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha gene (NFKbia). In this study a known PPARactivator (WY14, 643) and DMSO (vehicle) was introduced into control and δ337T thyroid hormone receptor (TRβ transgenic mice. The δ337T TRβ transgenic mouse has been created to reproduce the human genetic disease known as resistance to thyroid hormone (RTH). Heart tissue was extracted and NFKbia gene expression was compared using Affymetrix 430_2 expression arrays and qRT PCR among four studies groups [control, control with WY14, 643, δ337T TRβ and δ337T TRβ with WY14, 643] consisting of seven mice per group. The gene expression of NFKbia in the WY14, 643 control and transgenic mouse groups was significantly up regulated over the vehicle mouse groups for both the array ($p<0.05$) and qRT PCR ($P<0.01$) studies. Duplex oligo DNAs containing the PPAR/RXR motif (aggtca/tccagt) from the NFKbía promoter were used in EMSA to verify binding of the PPAR and RXR receptors to their response elements. pGL4.0 [Luc] constructs of the NFKbia promoter with and without the PPAR/RXR motifs were co-transfected with mouse PPARα,γ into HepG2 cells and used in Lucerifase assays to verify gene activation. In conclusion our study revealed that PPARregulates the mouse cardiac NFKbia gene in both the control and transgenic mouse.

1766/T

KCNQ1 V205M missense mutation causes LQTS in a Northern Canadian community. *J. Eldstrom[1], S. Rezazadeh[1], R. Rupps[2], S. Sanatani[3], B. Casey[4], G. Tibbits[5], D. Fedida[1], L. Arbour[2].* 1) Dept of Anesthesiology, UBC Vancouver, BC, Canada; 2) Departments of Medical Genetics,; 3) Pediatrics, and; 4) Pathology, University of British Columbia, Vancouver, BC; 5) Department of Kinesiology, Simon Fraser University, Burnaby, BC, Canada.

Eight known genes are responsible for hereditary Long QT syndrome (LQTS), an autosomal dominant condition predisposing to arrhythmia and risk of sudden death. Usually rare (about 1 in 7,000), it was brought to our attention that at least 40 people were considered affected with 200 relatives at risk, all from the same semi-isolated community. Two distantly related affected women were screened for mutations. A novel missense mutation, V205M in the S3 transmembrane region of the KCNQ1 channel, the α-subunit for the slow delayed rectifier potassium channel, *Iks,* was confirmed in both. To verify pathogenesis, wild type (Wt) KCNQ1 and subunit KCNE1 for *Iks* were expressed in mammalian cells and compared to those with a V205M construct. Similar surface expression of the channels was evident, but with a depolarizing shift in the V1/2 of activation in the mutant channels. Furthermore, a slowing of channel activation (294 ± 85 ms, n=5; and 878 ± 124 ms, n=4; for Wt and V205M channels, respectively, at +70 mV), and an acceleration of deactivation (53 ± 3.6 ms, n=5; and 14 ± 3.5, n=4; for Wt and V205M channels, at -100 mV) was evident. Using a ventricular action potential voltage clamp protocol applied at 3 Hz, Wt channels, but not V205M channels, accumulated in the open state, resulting in large outward IKs currents only for Wt channels. The outward ionic charge through heteromultimeric WT/mutant channels during the action potential clamp was reduced by more than 75% suggesting a functional dominant negative effect of the V205M mutation at high heart rates. Conclusions: The changes in IKs kinetics produced by the V205M mutation are expected to decrease current and reduce the repolarization reserve during the cardiac ventricular action potential, with increased susceptibility to initiation of arrhythmias, especially during periods of high sympathetic drive. Genotyping is underway for the remainder of those at risk.

1767/T

First 503 Human Subjects of "Genetics of Left Ventricular Outflow Tract Malformation" Study. *S.M. Fitzgerald-Butt, G.A. Zender, K.L. McBride.* Center for Molecular & Human Genetics, Children's Research Inst, Columbus, OH.

Congenital heart defects are among the most common of all medically significant birth defects and are a leading cause of infant mortality. Left ventricular outflow tract (LVOT) obstruction malformations, include aortic valve stenosis (AS), coarctation of the aorta (CoA), mitral valve stenosis (MS), hypoplastic left heart syndrome (HLHS), Shone complex (SC) and bicuspid aortic valve (BAV). They are thought to arise embryologically from reduced flow through the LVOT and therefore likely have related genetic and environmental etiologies. We are actively recruiting probands with an LVOT defect and both parents in a study to define these etiologies. LVOT diagnoses, family history, pregnancy exposures, and maternal health are obtained on each proband and other affected relatives using the National Birth Defects Prevention Study questionnaire as a template. An investigator-designed Microsoft Access database is used to gather, store and analyze the data. We have enrolled 503 subjects in 183 families over 25 months of recruiting; 36% of subjects are probands (65% male and 93% white), 33% mothers, 25% fathers and 6% other relatives. 25 (14%) families have a family history of LVOT malformation and 6% of enrolled relatives are affected. Of probands, 20% have HLHS, 5% SC, 2% IAA-A, 42% CoA±BAV, 21% AS±BAV, 9% BAV and <1% MS. We have collected blood samples for lymphocyte EBV transformation on 78% of the all subjects and 77% of probands. For the last six months we have offered a $25 gift card as incentive for completion of the study which resulted in approximately a 10% increase in blood samples. The pregnancy exposure questionnaire has been completed by 124 families or 69%. Reported prenatal exposures include: medication (63%), alcohol (56%), cigarette smoking (26%), second hand smoke (16%), chemical (16%), radiation (12%), hot bath/Jacuzzi (10%), fever (6%), recreational drugs (4%) and heavy metal (2%). Similar collection of a matched control group should provide us with adequate power to define the etiologies of LVOT malformations.

1768/T

5-lipoxygenase (5-LO), dietary arachidonic acid (AA), and risk of myocardial infarction (MI). *J. Hartiala[1], A. Baylin[2], H. Wijesuriya[1], G. Mendoza-Fandino[1], P.I. Patel[1], H. Campos[3], H. Allayee[1].* 1) Institute for Genetic Medicine, USC Keck School of Medicine, Los Angeles, CA 90033; 2) Department of Community Health, Brown University, Providence, RI 02912; 3) Department of Nutrition, Harvard University School of Public Health, Boston, MA 02115.

5-LO, the rate-limiting enzyme in the biosynthesis of pro-inflammatory leukotrienes from AA, has been associated with the development of atherosclerosis in mouse models and humans. To follow up on these previous observations, we have now tested the effect of a 5-LO promoter polymorphism on risk of MI and whether dietary AA modified the observed results. We genotyped 1,885 MI cases and 1885 controls and used conditional logistic regression to estimate odds ratios and confidence intervals. Dietary intake was assessed by a validated food frequency questionnaire. No differences in the frequency of deletion (DD/WD) or addition (AA/WA) genotype groups were identified: frequencies for DD/WD were 0.29 in cases and 0.30 in controls and 0.024 and 0.031, respectively, for the AA/WA genotype group. However, a significant gene-diet interaction was found between the 5-LO repeats, AA intake, and MI ($p=0.014$). Compared to the wildtype allele (W), the D alleles were associated with increased MI risk in the high (>0.25g/d) dietary AA group (OR 1.23, CI 0.99-1.53) and with decreased risk in the low (<0.25) AA group (OR 0.79, CI 0.63-0.98). The A alleles were inversely associated with MI in both diet groups, although this association was not statistically significant perhaps due to their low frequency. To determine if functional differences between the alleles could explain the observed results, we measured relative amounts of RNA from the D alleles compared to the W allele in leukocytes from 66 heterozygous individuals from the same population using allele specific quantitation. As a control, DNA from the same individuals for each respective allele was used. Consistent with the results above, the D alleles had ~1.5 fold increased expression relative to the W allele. These results are consistent with the observation that the D alleles are associated with increased atherosclerosis and MI risk, particularly in the context of a high AA diet.

1769/T

Familial Noncompaction Cardiomyopathy: A Novel Genetic Cardiomyopathy. *Y.M. Hoedemaekers[1, 2], K. Caliskan[2], F.J. ten Cate[2], M. Michels[2], D. Dooijes[1], D.F. Majoor - Krakauer[1].* 1) Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands; 2) Thoraxcenter, Erasmus Medical Center, Rotterdam, the Netherlands.

Background: Noncompaction cardiomyopathy (NCCM) has recently been recognized as a novel cardiomyopathy characterised by an excessively thickened endocardial layer with deep intertrabecular recesses. Cardiac symptoms include heart failure, lethal arrhythmias and/or thrombo-embolic complications, mostly affecting young adults. Sporadic and familial forms of NCCM exist. Genetic NCCM is heterogeneous, mostly inherited as an autosomal dominant trait. Previously we described the occurrence of two sarcomeric gene mutations in two NCCM families. To investigate recurrence of NCCM in families, cardiologic screening of first-degree relatives of 30 NCCM patients was performed. Methods: Cardiologic screening of 52 relatives consisted of an ECG, two-dimensional echocardiography and physical examination. In some relatives contrast echocardiography or MRI and/or exercise ECG was also performed. Relevant medical records deceased relatives were ascertained to establish the cause of death. DNA analysis was performed of relatives in families with sarcomeric mutations. Results: In 17 of the 30 families (57%) a cardiomyopathy was detected in relatives; 30/52 (58%) relatives had NCCM, 8/52 (15%) of relatives had another cardiomyopathy: 2 with dilated cardiomyopathy (DCM), 4 with hypertrophic cardiomyopathy (HCM) and 2 with an unspecified cardiomyopathy. Most affected relatives were asymptomatic and severity of the cardiomyopathy was highly variable. Additional molecular screening of relatives in four families identified two mutation carriers without any cardiac features of NCCM or other cardiomyopathy and allowed exclusion of the mutation in three relatives. Conclusion: The majority NCCM is genetic. Therefore, cardiologic screening of all first-degree relatives of patients is important to reveal the variable features including NCCM, DCM and HCM. When appropriate, presymptomatic DNA testing is valuable to identify asymptomatic carriers, who are at risk developing manifestations of cardiomyopathy.

1770/T

KRAS and SOS1 mutations in Noonan and related syndromes. *K. Kalidas, A.H. Crosby, S. Jeffery, A. Shaw, M.A. Patton.* Medical Genetics Section, Clinical Developmental Sciences, St George's University of London, London, United Kingdom.

Noonan Syndrome, Cardi-facio-cutaneous syndrome (CFC) and Leopard Syndrome are a group of developmental disorders with overlapping congenital abnormalities. Mutations in the PTPN11 (protein tyrosine phosphatase) genes have been found to account for up to 60% of cases of Noonan syndrome. Mutations in the functionally related KRAS and SOS1 genes gene were recently found to cause Noonan syndrome. We have analysed the KRAS and SOS1 genes in a group of 65 individuals with Noonan syndrome, 15 with CFC syndrome and 2 with LEOPARD syndrome, all of whom have been excluded for variants in the PTPN11 gene. Variants in the KRAS gene, including novel mutations in exon 4, were identified in one CFC patient and four Noonan patients. Three SOS1 variants were also identified in Noonan syndrome, two of which were novel. No mutations were identified in the LEOPARD syndrome patients. Our results indicate that KRAS and SOS1 are responsible for only a small proportion of patients with this clinical spectrum and that mutations in other gene(s) contribute to the pathogenesis of these disorders.

1771/T

Evc and Lbn are co-expressed in structures affected by Ellis van Creveld Syndrome. *K. Lipscomb Sund[1,2], D.W. Benson[1,2].* 1) Molecular & Developmental Biology, Genetic Counseling, University of Cincinnati, Cincinnati, OH; 2) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Atrioventricular septal defects (AVSDs), limb dwarfism, and polydactyly are features of recessively-inherited Ellis van Creveld (EvC) syndrome. Linkage mapping and positional cloning led to identification of associated human mutations in two previously unknown non-homologous genes, EVC (31%) or EVC2/LBN (38%), which are encoded head-to-head on chromosome 4. Mutation analysis in a small cohort of patients with EvC syndrome confirmed loss-of-function mutations and led us to explore the role of Evc and Lbn in cardiac and limb morphogenesis. Evc and Lbn riboprobes created for in-situ hybridization ascertained endogenous expression patterns in murine hearts at 9.5-12.5 dpc, when atrioventricular (AV) septation occurs. Peptide specific antibodies generated for immunohistochemistry confirmed expression patterns and identified cellular compartmentalization of the proteins. Evc and Lbn co-localization was evident in murine NIH3T3 and ATDC5 cell lines. In the mouse long bone growth plate, there was co-expression in the pre-hypertrophic and hypertrophic zones. In the developing heart, Mf-20 and Pecam co-staining revealed Evc is expressed in endocardial, mesocardial and mesenchymal cells while Lbn is predominantly found in myocardium and mesenchyme. We identified overlap of Evc and Lbn protein in cardiac structures affected by EvC syndrome, including the atrial septum, AV junction, and AV valves. The presence of Evc and Lbn in key structures during cardiogenesis indicates their importance in contributing to vavuloseptal morphogenesis. Bone expression patterns and cellular localization provide insight into protein function because co-localization of Evc and Lbn in the pre-hypertrophic zone is followed by nuclear translocation of Evc in the hypertrophic zone. Based on human mutations predicated to result in a complete loss of protein function, a bidirectional genomic organization expected to lead to coordinate expression, and evidence of mRNA and protein co-localization in structures exhibiting EvC pathology, we believe that co-expression of Evc and Lbn is necessary for proper function.

1772/T

Association of the endothelial nitric oxide synthase gene polymorphism (4a/4b) with the risk of coronary artery disease in mexican population. *R.P. Mariaud[1,2], M. Zuñiga[3], M.P. Gallegos[2].* 1) Instituto de Investigación en Odontología, Departamento de Clinicas Odontologicas Integrales,Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara; Guadalajara, Jalisco, Mexico; 2) Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social; Guadalajara, Jalisco, Mexico; 3) Unidad de Terapia Intensiva de Cardiología, Hospital de Especialidades, Centro Medico Nacional de Occidente, Instituto Mexicano del Seguro Social; Guadalajara, Jalisco, Mexico.

BACKGROUND: The vascular endothelium is now recognized as an important participant in a healthy cardiovascular system, and dysfunction of this cellular monolayer might be an initiating event in many or most cardiovascular disease states. The eNOS gene harbours a common polymorphism in intron 4 (4a/b), and some clinical studies have suggested an association of the rare a-allele with coronary artery disease (CAD) and myocardial infarction (MI). However, contradictory results have also been reported. OBJECTIVE: Determinated the association of eNOS polymorphism 4a/4b in CAD patients from Mexican population. MATERIAL AND METHODS: We studied the association of eNOS polymorphism with CAD in 153 patients and 112 controls. For the polymorphisms analysis we amplified a 420bp segment of intron 4. RESULTS: We not observed a significant differences between patients carrying the a-allele (ba+aa) compared with control group, (odds ratio 1.01, 95% confidence interval 0.60-1.61, P>0.05). CONCLUSION: The eNOS gene 4a/b polymorphism was not associated with Mexican patients with CAD.

1773/T

Circulating TGF-beta as a prognostic and therapeutic biomarker in Marfan syndrome. *P. Matt, J. Habashi, T. Holm, E. Klein, M. Gamradt, D. Huso, J. Van Eyk, H. Dietz.* Inst of Genetic Medicine and Div of Cardiology, Johns Hopkins Univ Sch of Med, Baltimore, MD.

Aortic root dilatation is the main cause of mortality in Marfan syndrome (MFS), a disorder caused by mutations in the gene encoding fibrillin-1 and consequent dysregulation of TGF-beta signaling. Recent evidence suggests that losartan, an AT1 antagonist that blunts TGF-beta activation, may be a productive treatment for MFS. Currently there are no surrogate markers for the therapeutic effect of losartan that can be used to develop and individualize therapeutic regimens. Optimal doses of losartan for this indication may differ from those currently used to treat hypertension. Serum samples from a MFS mouse model (*Fbn1* C1039G/+) and wild-type littermates treated with losartan or placebo were collected at 10 weeks, 6 months and 10 months of age. Total TGF-beta1 serum concentrations were measured by ELISA. Echo measurements of the aortic root were obtained at 10 months of age. TGF-beta serum levels were higher in C1039G/+ mice compared to wild-type mice (p=0.01; 80.0 ng/ml (n=5) vs. 58.3 ng/ml (n=4) at 10 weeks, 117.4 ng/ml (n=11) vs. 87.0 ng/ml (n=6) at 6 months, 137.5 ng/ml (n=3) vs. 103.0 ng/ml (n=2) at 10 months, respectively). Losartan-treated C1039G/+ mice had lower mean TGF-beta serum levels compared to C1039G/+ mice treated with placebo (p=0.007; 92.9 ng/ml (n=5) vs. 117.4 ng/ml (n=11) at 6 months, 101.2 ng/ml (n=13) vs. 137.5 ng/ml (n=3) at 10 months, respectively). Mean TGF-beta levels in losartan-treated C1039G/+ mice and wild-type mice were indistinguishable (p=0.3; 92.9 ng/ml (n=5) vs. 87.0 ng/ml (n=6) at 6 months, 101.2 ng/ml (n=13) vs. 103.0 ng/ml (n=2) at 10 months, respectively). Echo analyses revealed smaller mean aortic root diameters in 10 month old wild-type and losartan-treated C1039G/+ mice compared to age-matched C1039G/+ mice treated with placebo (p=0.001; 1.94 mm (n=2) and 2.06 mm (n=13) vs. 2.4 mm (n=3), respectively). Correlation was observed between TGF-beta1 levels and aortic root diameter in untreated C1039G/+ mice (R^2=0.6). Circulating TGF-beta1 is a promising biomarker for prognostication and monitoring the therapeutic response to losartan in MFS.

1774/T

Copy Number Variations in del22q11.2 Syndrome. *S. McGhee[1], M. Suchard[2], E.R.B. McCabe[1,2].* 1) Pediatrics, UCLA, Los Angeles, CA, USA; 2) Human Genetics, UCLA, Los Angeles, CA, USA.

Genetic polymorphisms, including single nucleotide polymorphisms, small insertions, and deletions, contribute to phenotypic changes and may be associated with disease. Large-scale copy number variations (CNVs) are a part of this variability and can be determined genome-wide using representational oligonucleotoide microarray analysis (ROMA). Such CNVs are found in normal patients and new CNVs frequently arise in individual patients. We investigated the hypothesis that patients with del22q11.2 syndrome might have a broader susceptibility to larger or more frequent CNVs. CNVs and the del22q11.2 deletion both may arise from unequal crossing-over events, and we hypothesized that del22q11.2 patients may have an abnormal mechanism resulting in a predisposition to larger CNVs. 12 patients with del22q11.2 syndrome and 8 healthy controls were assessed for genome-wide copy number changes using ROMA. There was an average of 16 CNVs per genome and this average did not differ between del22q11.2 syndrome patients and controls (Wilcoxon p=0.516). The median CNV size also did not differ between patients and controls (157,555 vs. 153,157 respectively). We also determined whether patients with del22q11.2 syndrome were more likely to have larger CNVs. Here also, the frequency of CNVs greater than 500 Kb was not significantly different than controls (Fisher p=0.21, OR of 1.74, 95% CI 0.74-4.08). We conclude that patients with del22q11.2 syndrome do not differ from normal controls with respect to the frequency or size of CNVs, suggesting that the unequal crossing over event that produces both CNVs and the del22q11.2 deletion result from similar mechanisms in patients and controls.

1775/T

The Intermountain Genealogical Registry: Initial Evaluation of a Population Genealogy for an Outbred Continental Population. *J.B. Muhlestein[1,2], J.L. Anderson[1,2], T.L. Bair[1], D.G. Renlund[1,2], A.G. Kfoury[1,2], D.L. Lappe[1,2], J.F. Carlquist[1,2], R.R. Pearson[1], H.T. May[1], M.S. Hammad[1], B.D. Horne[1,3].* 1) Cardiovasc Dept, LDS Hosp, SLC, UT; 2) Cardiology Div, Univ Utah, SLC, UT; 3) Genet Epidemiol Div, Univ Utah, SLC, UT.

Cardiovascular (CV) genetic studies traditionally evaluated families from inbred isolates, close relatives, or samples of unrelated individuals. Because CV diseases are common and complex, additional approaches are needed. We record-linked publicly-available genealogical records to patient records to create an Intermountain Genealogical Registry (IMGR) for CV genetic research. In an outbred continental Caucasian population previously shown to be genetically similar to the US Caucasian population, electronic pedigree information was extracted from public records in published books and on the internet. Beginning in 2004, CV patients provided 5-generation pedigree charts (name, birthdate, death date) for 3 generations of ancestors, themselves, siblings, and children. A probabilistic matching algorithm, the Phonetic Transducer, eliminated duplicate records and curated pedigree connections, with patient-submitted pedigrees used to validate computerized record-linking. Subsequent record-linking of genealogical and medical data was performed. Pedigrees of 10.1 million individuals were included in IMGR, with ≥3 life events (birth, marriage, death) per pedigree occurring in Utah and surrounding states after 1846. Most pedigrees covered the 1800s and 1900s, with many extending to the 1500s. Among >14,000 cardiac patients who donated DNA, record-linking found 13% matched exactly to IMGR by name, sex, and birthdate, with 31% more having record-linking scores suggesting a correct match. Among 340 patients who provided pedigree charts, 66% had one or more relative among the 10.1 million in IMGR, including 15% for whom all pedigree data were included. IMGR, a population genealogy among a general, outbred population, was created and found to provide substantial information regarding cardiac patient pedigrees. This resource will provided enhanced ability to identify and study familial cardiac traits.

1776/T

Genome-wide association study of QT interval duration and staged validation. *C. Newton-Cheh[1,2,3,4], X. Yin[2,5], A-J.L.H.J. Aarnoudse[7,8], P.I.W. deBakker[1,3,4], A. Surti[1], A.G. Uitterlinden[7], M.G. Larson[6], B.H.C. Stricker[7,8], C.J. O'Donnell[2,3,4], J.N. Hirschhorn[1,4,9].* 1) Broad Inst of Harvard & MIT, Cambridge, MA; 2) NHLBI's Framingham Heart Study, Framingham, MA; 3) Massachusetts General Hosp, Boston, MA; 4) Harvard Medical Schl, Boston, MA; 5) Boston University Schl of Public Health, Boston, MA; 6) Boston University Schl of Medicine, Boston, MA; 7) Erasmus Medical Ctr, Rotterdam, Netherlands; 8) Inspectorate for Healthcare, the Hague, Netherlands; 9) Childrens Hosp of Boston, MA.

Electrocardiographic QT interval (QT), a heritable quantitative trait is associated with sudden cardiac death when prolonged or hastened. With collaborators, we identified through a modest genome-wide association study (GWAS) a common SNP in *NOS1AP* associated with QT (Arking et al *Nat Gen* 2006). We report a larger GWAS of continuous QT interval duration with staged follow up. From an initial QT GWAS of 70,987 polymorphic SNPs (Affymetrix 100K) in 1175 Framingham Heart Study (FHS) men and women, we selected the top 162 SNPs to genotype in 1531 independent FHS men and women (stg II). Of 152 SNPs genotyped successfully (95% call rate, HWE p>0.001) in the stage II sample, weighted joint analysis with stage I data identified 3 loci with $p<10^{-4}$. Locus #1 included rs1932933 in *NOS1AP* with 14.6% SD (SE 2.4%) increase in sex-, age- and RR-interval-adjusted QT per minor allele ($p=5x10^{-7}$). Locus #2 included rs10503034, associated with a 13.2% SD (SE 2.6%) increase in adjusted QT per minor allele ($p=2x10^{-5}$). Locus #3 included rs1202113 associated with 12.6% SD increase in adjusted QT per minor allele ($p=1x10^{-5}$). *NOS1AP* SNPs were recently confirmed to be associated with QT in 6571 Rotterdam Study (RS) men and women ($p<10^{-19}$, Aarnoudse, Newton-Cheh et al *Circulation* in press). We also genotyped rs10503034 and rs1202113 in RS men and women and found no evidence of association of rs10503034 with adjusted QT (p=0.98) and modest association of rs1202113 with a 3.7% SD (SE 1.7%) increase in adjusted QT per minor allele (2-tailed p=0.03). SNP rs1202113 is 54kb upstream of *KCNQ5*, a potassium channel not previously known to modulate myocardial repolarization. Further study of this gene is warranted.

1777/T

52,608 gene-based SNPs association study to identify genes related to myocardial infarction. *K. Ozaki[1], H. Sato[2], A. Iida[1], H. Mizuno[2], A. Takahashi[1], T. Nakamura[1], H. Lwin[1], S. Ikegawa[1], M. Hori[2], Y. Nakamura[1], T. Tanaka[1].* 1) SNP research center, RIKEN, Tokyo, Japan; 2) 2) Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan.

To clarify genetic backgrounds in the pathogenesis of myocardial infarction (MI), we have performed large scale case-control association study in a Japanese population using 52,608 haplotype-based single-nucleotide polymorphism (SNP) markers. We have identified two susceptible loci on chromosome 22q12.1 and 3p21.2-p21.1. Following linkage disequilibrium (LD) mapping and haplotype analyses revealed that six SNPs of novel gene on chromosome 22q12.1, all of which were in complete LD, and a SNP in the inter-alpha (globulin) inhibitor 3 gene (*ITIH3*) on chromosome 3p21.2-p21.1 showed markedly significant association with MI (χ^2=25.27, *P*=0.0000005 for one SNP in the novel gene, χ^2=24.88, *P*=0.00000061 for a synonymous SNP in exon 2 of *ITIH3*; comparison of allele frequency, approximately 3,400 affected individuals versus 3,800 controls). Within first locus, we isolated a complete cDNA of a novel gene, designated *MIAT* (myocardial infarction associated transcript). *MIAT* has five exons and in vitro translation assay showed that *MIAT* did not encode any translational product, indicating that this is likely to be a functional RNA. In vitro functional analyses for SNPs in *MIAT* and *ITIH3* revealed that the minor variant of one SNP in exon 5 of *MIAT* and the major variant of the SNP in *ITIH3* increased transcriptional level to each of the gene. Moreover, unidentified nuclear protein(s) bound more intensely to risk allele than non-risk allele in the case of *MIAT*, and two different proteins bound to each of the allele for the SNP in *ITIH3*. Our findings suggest that *MIAT* and *ITIH3* SNPs are novel genetic risk factors of MI.

1778/T

Chromosome 22q11 deletion syndrome: is MTHFR a modifier of the cardiovascular phenotype? *G.M. Repetto[1], J.F. Calderon[1], M.L. Guzman[1], A. Puga[1], C.P. Astete[2], M. Aracena[3], C. Mellado[3], T. Aravena[4], M. Arriaza[5], P. Sanz[6].* 1) Dept Genetics, Clin Alemana-Univ Desarrollo; 2) Hosp. Luis Calvo Mackenna; 3) Hosp. Clinico P. Universidad Catolica; 4) Hosp. Sotero del Rio; 5) Hosp. Gustavo Fricke; 6) Hosp. Clinico U. de Chile, Santiago, Chile.

Chromosome 22q11 microdeletion syndrome (del22q11) affects 1:4000 live borns. Its main clinical features include congenital heart defects (CHD), cleft palate and learning disabilities. Most patients share a common deletion, but clinical variability is marked. CHD is a significant cause of morbidity and mortality, and is present in 50-75% of cases reported in large series. MTHFR polymorphism 677C>T is associated with an increased risk of non-syndromic CHD, but its effect on CHD in del22q11 patients has not been studied. We evaluated the association of 2 common polymorphisms in the MTHFR gene, 677 C>T and 1298 A>C, with the presence or absence of CHD in patients with 22q11 deletion. Seventy-one unrelated patients were included in the study. CHD disease was present in 52% of them. We found a significant difference in allelic and genotypic frequencies at position 1298. Variant A had a frequency of 0.84 in patients with and 0.69 in those without CHD (p=0.04). Genotypic frequencies were AA=0.7, AC=0.27 and CC=0.03 in patients with CHD and 0.38, 0.62 and 0.0 in patients without CHD, respectively (p=0.02). No significant difference was observed in allelic or genotypic frequencies of 677C>T between patients with or without CHD or with healthy population controls. We report a significantly higher frequency of MTHFR allele 1298C in patients with 22q11 deletion and no CHD compared to those with CHD. The cause of this association remains to be explored. Funded by Fondecyt-Chile, Grant # 1061051.

1779/T

Mutation of Nkip1 in bovine cardiomyopathy woolly haircoat syndrome. *P. Solanki[1], M.A. Simpson[1], R. Cook[2], A.H. Crosby[1].* 1) Medical Genetics, St George's University of London, London; 2) NWS Agriculture, Camden, NSW, Australia.

In recent years great strides have been made in the understanding of the pathogenic mechanisms of the cardiomyopathies. In particular, genetic studies of both human cardiomyopathies and animal models have identified several molecular pathways which are crucial for normal cardiac function. We present here the identification of the causative mutation in a naturally occurring bovine cardiomyopathy inherited in an autosomal recessive fashion. In 1969, a lethal autosomal recessive cardiomyopathy, known as cardiomyopathy and woolly haircoat syndrome (CWH), was reported in Poll Hereford calves in Australia. The cardiac defect is particularly aggressive and the identification of extensive lesions of myocardial fibrosis in neonates suggests an in-utero onset of myodegeneration. Affected calves are identifiable by a woolly haircoat which cosegregates with the heart condition and death normally occurs within the first 12 weeks either due to sudden cardiac death or congestive heart failure. Assuming that a founder mutation was responsible, we undertook a homozygosity mapping approach in order to identify the underlying genetic defect. This identified a region of homozygosity in the vicinity of the bovine orthologue of the Nkip1 gene. Sequence analysis of this gene revealed the presence of a 7bp duplication in exon 6 of this gene, which cosegregates with the disease status. The duplication is predicted to disrupt the encoded protein product causing a frame shift resulting in the substitution of 55 amino acids and premature termination at position 377. These findings clearly highlight this gene as an important candidate for mutation in human forms of cardiomyopathy.

1780/T

Detection of 7q11.23 microdeletion in a Williams-Beuren syndrome patient carrying a severe supravalvular pulmonar stenosis and mild supravalvular aortic stenosis. *I. Trabelsi[1], M. Cherif[3], S. Kammoun[1], T. Rebai[2], N.B. Abdelmoula[2].* 1) Cardiology Dept, Hedi Chaker Hosp, Sfax, Tunisia; 2) Histology Lab, University of Medicine, Sfax, Tunisia; 3) Genetic Laboratory, Tunis, Tunisia.

Williams-Beuren syndrome is a multisystemic developmental disorder which usually occurs sporadically. Phenotypes include a dysmorphic face and congenital heart disease. These features are caused by deletion of the Williams-Beuren syndrome critical region at chromosomal position 7q11.23 on either the maternal or paternal chromosome 7. Only the elastin gene is associated with a phenotype which is supravalvular aortic stenosis. Here, we report a Tunisian 2-year-old boy referred to us for cytogenetic evaluation of elfin facies, growth retardation and congenital heart disease. He was the first child of healthy non-consanguineous parents. The paternal age was 26 and the maternal age was 24 years. He was born par caesarean section at 38 week's gestation of an uneventful pregnancy. Birth weight was 2400 g. Apgar scores were 6 at one minute and 10 at five minutes. He was born with the two superior middle teeth. On examination at 9 months, the boy had growth and psychomotor developmental retardation (weight at -3DS). At 2 years, he doesn't walk and only the word "papa" is spoken. Cardiac evaluation showed severe supravalvular pulmonar stenosis and mild supravalvular aortic stenosis. Clinical evaluation disclosed squint, periorbital fullness, bitemporal narrowness, small upturned nose, full nasal tip, long philtrum, full cheeks, full lips, wide mouth, and small jaw. The boy had a particular cognitive and behavioural profile including mild mental retardation, delayed expressive and receptive language abilities, attention deficit, hyperactivity and anxiety. Williams-Beuren syndrome was suspected. Chromosomal analysis demonstrated a 46,XY karyotype. Fluorescent in situ hybridization was performed using a probe directed to the elastin gene and another marker probe directed to band 7q36. Submicroscopic deletion of the williams syndrome locus was found and the diagnosis was confirmed. FISH analysis at the mother and the father did not show any deletion.

1781/T

The novel mitochondrial DNA A4401G mutation is involved in left ventricular hypertrophy in one Han Chinese pedigree. *S. Wang[1], H. Zhu[1,2], R. Li[2], L. Yang[2], Y. Liu[1], Z. Li[1], M.X. Guan[2].* 1) Dept Geriatric Cardiology, Chinese PLA General Hosp, Beijing, China; 2) 1Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.

Left ventricular hypertrophy (LVH) is one of the most important target organ damages in hypertension. Despite the involvement of multiple factors, the genetic factors including mitochondrial genomes have been implicated to play an important role in the pathogenesis of LVH. Recently, a systematic and extended mutational screening of mitochondrial genome has been initiated in a large cohort of Chinese clinical population of Geriatric Cardiology Clinic at the Chinese PLA General Hospital, Beijing. Further genetic evaluation suggested that several Chinese families with LVH appeared to be transmitted maternally. Sequence analysis of mitochondrial DNA in one Chinese pedigree identified a novel A-G transition at position 4401 (A4401G) at the junction of tRNAMet and tRNAGlu. In fact, this mutation was absent in 272 Chinese control subjects. This mutation appears to affect the processing of precursors in these mitochondrial tRNAs. Functional significance of this mutation was supported that the marked decreases in the steady-state levels of tRNAMet and tRNAGln were detected in the cells carrying this mutation. The resultant defects in mitochondrial protein synthesis may contribute to the reduction in the rate of respiration in cells carrying this mutation. These genetic and biochemical data imply that the novel A4401G mutation is involved in the pathogenesis of left ventricular hypertrophy.

1782/T

Population-based and case-control whole genome association studies confirms known genes and identifies novel transcription factors associated with atherogenic dyslipidemia. *D. Waterworth[1], K.S. Song[1], X. Yuan[1], Y.A. Kesaniemi[2], R. McPherson[3], R. Mahley[4], T. Bersot[4], P. Barter[5], D.K. Burns[1], E.H. Lai[1], P. Vollenweider[6], L.T. Middleton[1], A.D. Roses[1], S.M. Grundy[7], G. Waeber[6], V.E. Mooser[1].* 1) GlaxoSmithKline, King of Prussia, PA, RTP, NC; 2) Dept of Internal Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland; 3) University of Ottawa Heart Institute, Ottawa, Canada; 4) Gladstone Institute of CVD, UCSF, CA and American Hospital, Istanbul, Turkey; 5) The Heart Research Institute, Sydney, Australia; 6) CHUV University Hospital, Lausanne, Switzerland; 7) Center for Human Nutrition, University of Texas Southwestern Medical Center, Dallas, TX.

Atherogenic Dyslipidemia, operationally defined here as low HDL (< 25%ile for HDL adjusted for gender, age and population) and high triglyceride (> 75% triglycerides), is an important risk factor for CVD and has a genetic component. We performed a WGAS using the Affymetrix 500k chip on 923 cases and 924 controls (> 50%ile for HDL and < 50%ile for triglycerides) from the GEMS study and 633 cases and 678 controls selected from the CoLaus Lausanne population-based study. Single point analysis was performed in each sample separately using logistic regression and the Armitage trend test, as implemented in PLINK. A total of 581 SNPs were nominally significant with an allelic effect in the same direction in both studies; many of these SNPs were not independent of each other. A meta-p was also generated using Fisher's method. Multilocus methods, BEAGLE and the Bayesian Graphic Model were also applied to the data and replicated regions were retained for further evaluation. Several genes known to be involved in lipid metabolism were found to be highly significant, e.g. LPL, APOAV, GCKR and APOC1. The pool of novel genes was enriched with transcription factors, particularly those with zinc finger motifs. Testing for association against lipid levels as continuous variables in the population, contextualization of these results, replication with other datasets and pathway analyses are now underway and the results of this analysis will be described.

1783/T

Genome-wide association scan for HDL cholesterol, LDL cholesterol and triglyceride levels in 9,000 individuals. *C.J. Willer[1], A. Scuteri[2,3], L.L. Bonnycastle[4], S. Sanna[5], A.U. Jackson[1], A. Maschio[5], W.L. Duren[1], F. Busonero[5], R. Pruim[6], Diabetes Genetics Initiative[7], R.M. Watanabe[8], S.S. Najjar[2], L.J. Scott[1], M. Uda[2], J. Tuomilehto[9], G.R. Abecasis[1], F.S. Collins[4], D. Schlessinger[2], K.L. Mohlke[10], E.G. Lakatta[2].* 1) Dept Biostatistics, Univ Michigan; 2) Gerontology Research Center, National Institute on Aging; 3) Unita Operative Geriatrica INRCA, Rome Italy; 4) Genome Technology Branch, National Human Genome Research Institute; 5) Istituto di Neurogenetica e Neurofarmacologia, CNR, Cagliari, Italy; 6) Dept Mathematics & Statistics, Calvin College; 7) Broad Institute of Harvard & MIT, Lund Univ, & Novartis Institutes of BioMedical Research; 8) Dept of Physiology and Biophysics, Keck School of Medicine, Univ Southern California; 9) Dept Epidemiology & Health Promotion, Dept Biochemistry, National Public Health Institute, Helsinki, Finland; 10) Dept Genetics, Univ North Carolina.

Cardiovascular diseases (CVD) are the leading cause of death in industrialized countries. Low density lipoprotein cholesterol (LDL) is a major risk factor for CVD whereas high density lipoprotein cholesterol (HDL) protects against CVD. Triglyceride levels (TG) may also be associated with risk of coronary artery disease. Heritability of these traits is between 30 and 60%. We have combined genome-wide association data from the ProgeNIA study of 4,301 Sardinian individuals from 450 families, the FUSION study of 2,337 Finnish individuals and 2,659 Caucasian individuals from the Diabetes Genetics Initiative (DGI). To allow for meta-analysis with genotyped SNPs from two platforms (Affymetrix 500k and Illumina 300k), we imputed genotypes for untyped SNPs in the FUSION individuals. Meta-analysis provided clear association with several previously reported loci, including *APOC1* (LDL, $p = 1 \times 10^{-18}$), *GCKR* (TG, $p = 3 \times 10^{-16}$), *CETP* (HDL, 6×10^{-16}), *LPL* (TG, $p = 7 \times 10^{-15}$), *APOB* (TG, 9×10^{-10}), and *LIPC* (HDL, 2×10^{-8}). We detected second independent association signals in 5 of these genes ($p < 5 \times 10^{-6}$). We observed 15 new loci with $p < 5 \times 10^{-6}$ that we are in the process of genotyping in 7,300 individuals. The new loci appear to be involved in pathways such as cell adhesion and lipid metabolism.

1784/T

Clinical Genetic Approach for Evaluation of Noncompaction Cardiomyopathy. *M.V. Zaragoza, R. Cox.* Center for Molecular and Mitochondrial Medicine and Genetics and the Dept of Pediatrics, Division of Genetics and Metabolism, University of California, Irvine.

Noncompaction of the ventricular myocardium is a primary genetic cardiomyopathy characterized by prominent trabeculae and deep intertrabecular recesses in the myocardial wall. Noncompaction cardiomyopathy is thought to arise from a block in the embryonic process in which myofibrils compact during cardiogenesis. This is thought to result in the characteristic two layers consisting of compacted and noncompacted myocardium. Clinical manifestations are variable; patients may develop ventricular dysfunction, heart failure, arrhythmia or complications due to thromboembolism whereas others remain asymptomatic. The genetic etiology of noncompaction is heterogeneous. Mutations in *TAZ* on Xq28, *DTNA* on 18q12.1, *LDB3* on 10q23.2 and *LMNA* on 1q22 have been described in patients; genetic linkage to 11p15 has been reported in a single family. This report describes the clinical genetic approach for the evaluation of a two year-old girl who presented with new onset heart failure. Since noncompaction cardiomyopathy may be "isolated" or found as a feature of a genetic syndrome, clinical genetic examination is important and there are limited guidelines specifying how it should proceed. As illustrated in this report, noncompaction cardiomyopathy can be familial. The patient's family history revealed variable features of presentation of affected individuals and multiple family members at increased risk for inheriting the condition. Thus, we emphasize that clinical genetic examination, detailed pedigree analysis and cardiac screening of at least all first-degree relatives are essential in the evaluation of patients with noncompaction cardiomyopathy.

1785/T

Functional characterization of mutations causing disease in Familial Hypercholesterolemia. *A.C. Alves[1], S. Silva[1], M.A. Duarte[1], D. Patel[2], A.M. Medeiros[2], A.K. Soutar[2], M. Bourbon[1].* 1) UIC, INSA, Lisboa, Portugal; 2) MRC-Lipoprotein Group,CSC, London, UK.

Familial hypercholesterolaemia (FH) is an inherited disorder of cholesterol metabolism and FH patients present an increased risk of premature cardiovascular disorders. FH is caused mainly by mutations in the LDLR. Many different mutations have been identified worldwide in FH patients, but not all give rise to a defective LDLR. The Portuguese FH Study (EPHF) has identified 54 different LDLR mutations in more than 200 affected individuals. The aim of this study was the functional characterization of novel missense and putative splicing mutations in LDLR found in patients of the EPHF. Different LDLR mutants were generated by site-directed mutagenesis and expressed in CHO-ldlA7 cells lacking endogenous expression of LDLR. To determine the effects of mutations on LDLR function, saturable binding plus uptake and degradation of 125I-labelled LDL was measured at 37ºC. The putative splicing mutations were analysed by RNA extraction from patient lymphocytes and RT-PCR. Eleven novel missense mutations and 11 putative splicing mutations were found in EPHF. The functional studies for 4 missense mutations (V408L, W469R,S627P,V838M) revealed that all but V838M, are mutations causing disease showing decreased LDLR activity. It was possible to perform RNA studies for 6 putative splicing mutations c.313+6T>C, c.1359-5C>G c.1061-8T>C and c.2140+5G>A, c.2389G>T (V776L) and c.1185G>C (V374V). Alterations c.313+6T>C, c.1359-5C>G and c.2389G>T showed splicing defects and c.1185G>C was not found in RNA from the patient suggesting that only one allele is being expressed, but the gene defect in this patient does not seem to be a splicing mutation and needs further investigation. The remaining mutations are still under study. The simple finding of an alteration in the LDLR gene does not mean that it is the cause hypercholesterolaemia in a patient, as proven by our results. Despite the knowledge about the structure and function of the LDLR gene and protein, this is not enough to predict about pathogenicity of novel mutations, justifying the need and importance of functional characterization studies.

1786/T

Extension of the clinical spectrum of Danon disease. *R.H. Lekanne Deprez[1], A.J. van der Kooi[2], I.M. van Langen[1], E. Aronica[3], P.A. van Doorn[6], J.H.J. Wokke[7], E. Brusse[6], C.T. Langerhorst[4], P. Bergin[8], L.R.C. Dekker[5], M. Visser[2].* 1) Clinical Genetics, University of Amsterdam, Academic Medical Centre, Amsterdam, Netherlands; 2) Neurology, University of Amsterdam, Academic Medical Centre, Amsterdam, Netherlands; 3) Pathology, University of Amsterdam, Academic Medical Centre, Amsterdam, Netherlands; 4) Opthalmology, University of Amsterdam, Academic Medical Centre, Amsterdam, Netherlands; 5) Cardiology, University of Amsterdam, Academic Medical Centre, Amsterdam, Netherlands; 6) Neurology, University Medical Centre Dijkzigt, Rotterdam, Netherlands; 7) Neurology, University Medical Centre Utrecht, Utrecht, Netherlands; 8) Neurology, Auckland Hospital, New Zealand.

Danon's disease is known as an X-linked dominant disorder characterized by severe cardiomyopathy, mental retardation and mild myopathy in males, and cardiomyopathy in female carriers. All but one reported mutations are truncating and located in the exons shared by the three splice variants. In affected males all mutations lead to (almost) complete absence of LAMP-2 staining of muscle tissue. A family is presented with a variant of Danon's disease caused by a novel mutation in the LAMP2 gene. Three affected brothers and a maternal nephew showed normal intelligence, progressive myopathy, mild cardiac abnormalities, and retinopathy. Serum CK activity was slightly elevated, muscle biopsy showed autophagic vacuoles. LAMP-2 staining was apparently normal. One female carrier suddenly died at the age of 28 from a hitherto unrecognized cardiomyopathy. LAMP2 gene analysis revealed a new missense mutation (c.1150G>C) in splice variant B, leading to an amino-acid change (p.Gly384Arg).

1787/T

Auto-regulation of *GTF2IRD1* contradicts its proposed role in the causes of Williams syndrome. *S.J. Palmer[1], N. Santucci[1], E.S. Tay[1], J.W. Hook[2], F.A. Lemckert[2], P.W. Gunning[2,3], E.C. Hardeman[1].* 1) Muscle Development Unit, Children's Medical Research Institute, Sydney, NSW, Australia; 2) Oncology Research Unit, The Children's Hospital at Westmead, Sydney, NSW, Australia; 3) Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, NSW, Australia.

Williams-Beuren syndrome (WBS) is a complex disorder resulting from a hemizygous microdeletion within chromosome 7q11.23 involving 20 genes. Supravalvular aortic stenosis is a common feature and is caused by haploinsufficiency of elastin, but the remaining physical and neurological symptoms have no known specific genetic cause. Recent reports have associated many of the symptoms with two related DNA-binding proteins, GTF2IRD1 and GTF2I, and one report has linked GTF2IRD1 to the craniofacial abnormalities. We have generated a *Gtf2ird1* knockout mouse by deletion of the first coding exon and analysed expression levels by Q-RTPCR and northern blotting. We found that the mutant *Gtf2ird1* allele produces a deleted transcript and transcript levels are 2 to 3 times higher in mutant animals than in their normal siblings. On the assumption that direct auto-regulation may explain this finding, we conducted a phylogenetic footprinting analysis of *Gtf2ird1* and found a highly conserved region adjacent to the transcription start site, which contains a cluster of canonical Gtf2ird1 binding sites. We have shown by electrophoretic mobility shift assays that Gtf2ird1 binds to this region with high affinity. Interestingly, the binding reaction is dependent on the presence of multiple sites since reduction to a single site abrogates binding. To test for auto-regulation in humans, we obtained lymphoblastoid cell lines from six WBS patients and six controls and examined expression of *GTF2IRD1*, *CYLN2*, *GTF2I* and *GAPDH*. While *CYLN2* and *GTF2I* transcript levels in the WBS group are half the levels of the normal controls, *GTF2IRD1* levels are indistinguishable from normal. Therefore, although only one copy of *GTF2IRD1* remains in WBS patients, it escapes haploinsufficiency by up-regulation of its own promoter, thus redefining its potential role in the disease.

1788/T

High heritabilities of novel cardiovascular biomarkers in a large multigenerational family. *S.H. Shah, D. Thompson, H. Chen, T. Stabler, C. Haynes, B. Lambertson, S. Nelson, E. Dowdy, E.R. Hauser, W.E. Kraus, V.B. Kraus.* Dept Medicine, Duke Univ Med Ctr, Durham, NC.

Background: Cardiovascular disease (CVD) has a strong genetic component and is highly heritable. Furthermore, conventional biomarkers for CVD risk (i.e. lipids) are heritable. Recently, several novel biomarkers have been shown to be associated with risk of CVD, but the underlying genetic component of these intermediate biomarkers remains yet to be determined. Therefore, in a large US family, we evaluated the hypothesis that these biomarkers are heritable. **Methods:** A pedigree documenting approximately 3000 family members of a large, multi-generational, multiethnic family was created. Biological samples and clinical data were obtained on 365 of these family members. Commercially available assays were used to measure paraoxonase, d-dimer, high sensitivity C-reactive protein (hsCRP) and glycated albumin from frozen serum. Biomarker levels were log transformed prior to analysis. Sequential Oligogenic Linkage Analysis Routines (SOLAR) was used to estimate heritabilities; polygenic models were constructed and adjusted for age-at-sampling and sex. **Results:** We confirmed previous studies showing high heritability of hsCRP (h2r=0.59 [SD 0.15], p=0.00004). Paraoxonase showed the highest heritability (h2r=0.64 [SD 0.12], p=0.0000001). D-dimer showed moderate heritability (h2r=0.26 [SD 0.16], p=0.04), but glycated albumin was not heritable (h2r=0.12 [SD 0.21], p=0.3). **Conclusions:** We report for the first time heritability of novel CVD biomarkers in a large family with a burden of CVD reflective of the average United States population. Paraoxonase, with the highest heritability, is an esterase associated with HDL, whose activity has been shown to be inversely related to the risk of CVD; therefore, mutations in this enzyme may be important determinants of CVD risk. Further studies to identify the underlying genes for these heritabilites are pending.

1789/T

Analysis of four susceptibility SNPs on chromosome 9p21 between Italian MI patients and controls. *G.-Q. Shen[1], L. Lin[1], D. Girelli[2], O. Olivieri[2], R. Corrocher[2], Q.K. Wang[1].* 1) Cleveland Clinic, Cleveland, OH; 2) University of Verona, Italy.

Very recently, two independent genome-wide SNP association studies identified four SNP variants on chromosome 9p21 that were associate with coronary artery disease (CAD: rs10757274, rs2383206) and myocardial infarction (MI: rs2383207, rs10757278). However, it remains to be determined whether these SNPs are associated with CAD and MI in the Italian population. The purpose of this study was to investigate the association between the four SNPs and MI in an Italian Caucasian population. A total of 416 MI patients and 308 controls from Italy were carried out. SNP genotyping was performed using the 5' nuclease allelic discrimination assay with an ABI Prism 7900HT Sequence Detection System. Haplotypes were estimated using the Haploview software. The associations of SNPs or SNP haplotypes were evaluated using the Pearson's Chi-square test. Empirical P-values were calculated using 100,000 Monte Carlo simulations by the CLUMP program. Allelic frequencies of all four SNPs were significantly different between cases and controls (P-obs=0.014-0.037, OR=1.25-1.31). The association remained significant after adjusting for age, gender, smoking, total cholesterol, LDL and triglyceride (P-adj=0.005-0.021), and after permutation testing (P-emp=0.016-0.04). Association analysis of the SNPs using recessive, additive and dominant models were then carried out. All SNPs showed significant association with MI assuming an additive model (P= 0.016-0.038) or a recessive model (P-obs=0.004-0.034; P-emp=0.004-0.041). The GGGG haplotype was significantly associated with MI (P-obs=0.028, P-emp=0.028, OR=0.79). These results indicate that four SNPs on chromosome 9p21 also confer risk of MI in an Italian Caucasian population.

1790/T

CYP gene polymorphism is associated with essential hypertension in Koreans. *D. Shin, J. Han, Y. Bae, J. Ahn, S. Park, D. Choi, J. Ha, Y. Jang.* Cardiovascular Genome Ctr, Yonsei Col Medicine, Seoul, Korea.

The cytochrome P450 (CYP) enzyme pathway produces arachidonic acid metabolites that are vasoactive, that effects on renal sodium handling and water transport. Recent studies have been proposed to play a mechanistic role in essential hypertension (EH). We preformed a case-control study to evaluate the presently controversial question of whether CYP gene polymorphisms are associated with hypertension in Koreans. We studied a sample population of 515 Koreans, comprising of 300 controls and 215 cases with EH, which were recruited from Cardiovascular Genome Center in Korea. We analyzed 7 single nucleotide polymorphisms (SNPs) of CYP genes [CYP3A4 (rs2246709, rs4646437 and rs4646440), 2C9 (rs1057910 and rs4918758), 2D6 (rs16947), and 2J2 (rs2280274)]. All subjects were genotyped for these variants with by SNP-IT assays using the SNPstream 25K® System. The allele and genotype frequencies of CYP3A4 T16090C (rs2246709) polymorphism were significantly different between the hypertensive and the normotensive subjects (P = 0.0010, P = 0.0020). The CYP3A4 T16090C genotype, in comparison with the other CYP genotypes, was strongly associated with hypertension. In multiple regression analysis, the T allele was related to significant odds ratios for hypertension in a dominant model [0.645; 95% confidence interval (CI) = 0.453-0.919, P = 0.0150] and in a recessive model (0.365; 95% CI = 0.192-0.695, P = 0.0020), respectively. These findings suggest that the CYP3A4 T16090C polymorphism may be associated with regulation of blood pressure, and imply that this polymorphism is a protective factor for EH in Koreans. The functional role of CYP3A4 as a genetic contributor to hypertension susceptibility warrants further research.

1791/T

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Missense Variant is Reproducibly Associated with Early-Onset Myocardial Infarction in >1500 Cases and 1500 Controls. *A. Surti on behalf of The Myocardial Infarction Genetics Consortium.* The Broad Institute of Harvard and MIT, Cambridge, MA.

There are few, if any, replicable genetic associations for myocardial infarction (MI). We sought to replicate a set of single nucleotide polymorphisms (SNPs) reported to be associated with MI or related cardiovascular disease outcomes. We studied 27 SNPs at 20 loci (reported in the literature prior to December 2006) in the Myocardial Infarction Genetics Consortium (MIGen), consisting of 1544 cases of early-onset MI (men≤50y or women≤60y) and 1700 age- and gender-matched controls free of MI from five international sites: Spain, Finland, Sweden, Seattle, US, and Boston, US. Nearly all participants were of self-reported European ancestry. We studied SNPs in *ALOX5AP, CFH, ESR1, F5, F7, FGB, GATA2, KCNMB1, LGALS2, LTA, LTA4H, PCSK9, PLAT, PSMA6, PTGS2, SERPINE1, TNFSF4, USF1, VKORC1, and ZNF627.* Genotyping was performed using the Sequenom MassARRAY platform. Within each study site, Fisher's exact test was used to study association of SNPs with MI status. To summarize the statistical evidence across study sites, we performed a Cochran-Mantel-Haenszel (CMH) test stratified by study site. Mean age of MI cases was 45y among women and 48y among men. Of 1544 cases, 578 (37%) were women. Given our sample size, we had 90% power to detect a 1.25-fold effect size per allele at an alpha of 0.003 (alpha= 0.05/20 loci) and a risk allele frequency of 25%. Nonetheless, only a single SNP achieved a P<0.003, that being a *PCSK9* missense variant (rs11591147, R46L) recently identified by Cohen et al. (*N Engl J Med* 2006). In meta-analysis, the minor T allele (2.35% freq. in controls) was associated with a 60% lower odds of MI (OR 0.40, 95% CI 0.26 - 0.61, P=1.0x10-5). This DNA sequence variant represents among the first to be reproducibly related to MI in multiple populations. Genome-wide association involving >900,000 SNPs (Affymetrix Genome-Wide SNP Array 6.0®) is ongoing in the MIGen samples and may yield additional loci related to MI.

1792/T

A search for genetic variants attributing to the risk of formation of intracranial aneurysms. *K. Yasuno[1,2], A. Tajima[1], T. Cui[1], A. Narita[1,2], I. Inoue[1,2].* 1) Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan; 2) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, Saitama, Japan.

Rupture of an intracranial aneurysm (IA) causes subarachnoid hemorrhage (SAH), a catastrophic form of stroke. Familial aggregation of IA suggests existence of genetic risk factors attributing to formation of IAs. Thus far genome-wide genetic linkage studies were performed in various populations, however, consistent loci were not reported indicating existence of genetic heterogeneity underlying IAs. To identify genetic susceptibility to IAs, we adopted a two-stage genome-wide association study in Japanese IA patients and controls. We completed a first stage screening with genotyping over 318,000 SNPs on the Illumina HumanHap300 and/or HumanHap300-Duo BeadChips in 203 ruptured and 95 unruptured IA patients and 198 controls. 310,303 SNPs were designed and shown with good clustering on both chips. Among them, overall call rate was 0.998 and 273,382 SNPs with minor allele frequency > 0.02 and per-SNP call rate > 0.95 were directed to association study. We analyzed these data in various aspects to try to narrow down and identify the candidate genetic variants responsible for susceptibility to IA. The current analytical methods include standard single- and multiple-marker associations, analysis of gene-gene interactions, and identification of loss of heterozygosity regions and copy number variations shared among patients. For example, the single-marker allelic association test for each SNP resulted in 26 most significantly associated SNPs with p-value less than 0.0001, where all these SNPs were in Hardy-Weinberg equilibrium in cases, controls and the combined sample, respectively, at the significance level 0.01. The most updated results relating IA susceptibilities would be presented and discussed.

1793/T

Efficient Identification of Novel Developmental Cardiac Genes Through Transcriptional Profiling of Differentiating Mouse Embryonic Stem Cells. *R. Miller[1,2], N. Christoforou[2], J. Gearhart[2,4], A. McCallion[1,3].* 1) Institute of Genetic Medicine; 2) Institute for Cell Engineering; 3) Department of Comparative and Molecular Pathobiology; 4) Department of Obstetrics and Gynecology, Johns Hopkins University, Baltimore, MD, 21205.

The heart is the first organ to form and function in mammalian development. Congenital heart defects (CHD) are the most prevalent birth defects in the general population (~7 in 1000 live births), reflecting its complex and highly regulated genesis. To gain a better understanding of mechanisms involved in cardiac development, we set out to determine the transcriptional profile of mouse embryonic stem cells (mESCs) as they differentiate along a cardiac lineage. Using an Nkx2.5 cardiac specific promoter driving GFP, we marked and isolated differentiating cardiomyocytes (DCMs) at specific time points during the differentiation. By comparing the profile of DCMs with time-matched nonDCMs and undifferentiated mESCs we have identified genes whose expression is enriched in DCMs compared with non-DCM populations. Approximately 50% of these genes already have established roles in cardiac function and development. We will describe our efforts to evaluate the biological relevance of the remainder to cardiac development. To date we have completed RNA *in situ* hybridization of 25 novel candidates identified in this screen, determining the embryonic expression patterns at key points during cardiogenesis (E7.5, E8.5, E9.5). The majority (21/25) have expression in key cardiac structures, such as the cardiac crescent, heart tube, looping heart, inflow and outflow tract, and branchial arches. We will present this data as well as on-going experiments to expand expression analysis of our candidates and to evaluate their pathological relevance.

1794/T

Long QT syndrome patients with double heterozygous and compound heterozygous mutations. *J. Thistleton[1], K. Livesey[1], K. Thomson[1], H. Lord[1], E. Blair[2], A. Seller[1].* 1) Oxford Molecular Genetics Lab., Churchill Hospital, Oxford, United Kingdom; 2) Dept. of Clinical Genetics, Churchill Hospital, Oxford, United Kingdom.

To date we have screened over 250 probands, with either a suspected or definite diagnosis of Long QT syndrome, for mutations in the 4 cardiac K+ channels genes: KCNQ1, KCNH2, KCNE1 and KCNH2 and in the cardiac Na+ channel gene, SCN5A, using WAVE dHPLC technology and direct sequencing. Pathogenic variants have been identified in over 50% of our probands, thus enabling cascade screening and better clinical management of at risk relatives.

However, approximately 10% of our patient positive cohort show a complex molecular pattern, with a second mutation identified in either the same gene (compound heterozygosity) or in a different gene (double heterozygosity), which is much higher than has been described previously in the literature. This has raised issues for the interpretation of the clinical significance of each variant and for the provision of predictive testing to family members.

We present 2 cases of double heterozygosity for KCNQ1 and KCNH2, as well as KCNH2 and KCNE1, and a further 2 cases of compound heterozygosity for KCNQ1 and KCNH2. We describe the implications of these findings on molecular screening strategies and patient care, this data emphasising the importance of screening all relevant genes even when a pathogenic mutation is identified in one gene. We will also demonstrate the need for good liaison between clinical genetics and laboratory departments.

1795/T

A fast-throughput service for Familial Hypertrophic and Dilated Cardiomyopathy using High resolution melt curve analysis on the Lightscanner. *M. Wilson[1], J. Thistleton[1], K. Thomson[1], J. Livesey[1], J. McKinney[2], E. Blair[3], H. Watkins[4], A. Seller[1].* 1) Oxford Molecular Genetics Lab., Churchill Hospital, Oxford, United Kingdom; 2) Idaho Technology Inc., 390 Wakara Way, Salt Lake City, Utah 84108, USA; 3) Dept. of Clinical Genetics, Churchill Hospital, Oxford, United Kingdom; 4) Dept. of Cardiovascular Medicine, John Radcliffe Hospital, Oxford, United Kingdom.

High resolution melt curve analysis using the Lightscanner is a newly available technology that provides fast throughput mutation screening, with the capacity to produce results for a 96-well plate in less than 15 minutes. Using this technology there is the potential to reduce reporting times and decrease costs while maintaining the same high sensitivity and specificity as other screening technologies such as dHPLC and CSCE. Our service for Hypertrophic Cardiomyopathy (HCM) and Dilated Cardiomyopathy (DCM) provided an opportunity to test the utility of the Lightscanner in the diagnostic laboratory.

Mutations in the sarcomeric genes; β-myosin heavy chain gene (MYH7), myosin binding protein C gene (MYBPC3) and troponin T gene (TNNT2), are thought to account for up to 60% of familial HCM and approx. 10% of familial DCM. Since 2004 a service for HCM and DCM has been available in the Oxford Molecular Genetics Laboratory in conjunction with the Oxford Genetics Knowledge Park. In January 2007, analysis of the 84 amplicons of the sarcomeric genes was transferred from dHPLC to the Lightscanner, including additional analysis of the MYH7 rod domain and TNNI3.

Here we describe the validation carried out prior to the transfer of all service work and demonstrate both efficiency and cost savings as a result of introducing this technology into our diagnostic service.

1796/T

Genotype-by-diet interaction analysis of paraoxonase 1 reveals a QTL on chromosome 17. *V.P. Diego, H.H.H. Goring, D.L. Rainwater, S.A. Cole, T.D. Dyer, J.T. Williams, J.W. MacCluer, M.C. Mahaney, J. Blangero.* Dept Genetics, SW Foundation Biomed Res, San Antonio, TX.

It is increasingly appreciated that the macronutrient component of the diet has profound effects on the processes of oxidative stress and subclinical, chronic inflammatory stress. Experimental work has shown that saturated and unsaturated fatty acids have reciprocal effects on the gene expression of key players in oxidative stress and inflammation, such as the components of the toll-like receptor signaling pathway. We therefore sought to perform genotype-by-diet interaction (GDI) analyses of a biomarker of oxidative stress and inflammation under dichotomized saturated (SFAT) and unsaturated (UFAT) fatty acid intake environments in Mexican American families participating in the San Antonio Family Heart Study. For preliminary GDI analyses, we analyzed paraoxonase 1 (PON1), a novel biomarker of inflammation and oxidative stress. For the SFAT environment, we found a suggestive linkage signal (corrected (for degrees of freedom) LOD score = 2.76) on chromosome 12 at 17 cM. For the UFAT environment, we found one significant and one suggestive linkage signal on chromosome 17 at 64 cM and chromosome 12 at 22 cM (corrected LOD scores = 3.00 and 2.90, respectively). The only evidence of GDI at a QTL was found on chromosome 17 at 64 cM ($p = 0.00669$). Earlier analyses by our group have detected QTLs of significant effect at the structural location on chromosome 7q21-q22 and at chromosome 12 in the vicinity of current results. The current report, in addition, gives significant evidence of a QTL on chromosome 17 when taking into account the effects of unsaturated fatty acid intake. It is noteworthy that under standard linkage analysis (i.e., no interaction effects) there is only suggestive evidence of a QTL whereas the incorporation of dietary environment provides significant evidence of a QTL. Moreover, the GDI pattern exhibited at the chromosome 17 locus has a sensible biological interpretation. We conclude that GDI modeling can help to identify and localize QTLs, and aid in understanding underlying biological processes.

1797/T

Identification of major QTLs and positional candidate genes for gene by smoking interactions in hypertension and blood pressure traits. *M.E. Montasser, L.C. Shimmin, M.S. Leduc, C.L. Hanis, E. Boerwinkle, J.E. Hixson.* Human Genetics Center, School of Public Health, University of Texas at Houston.

Hypertension (HT) is mediated by the interaction of many genetic and environmental factors. Previous genome-wide linkage scans have localized numerous loci that show linkage to HT or blood pressure (BP), but results have proven difficult to replicate in part due to gene by environment interactions. Here we investigate the influences of gene by smoking (GxS) interaction on HT and BP in 4,764 sibships from the GENOA study (African Americans, Mexican Americans, European Americans). We used variance component methods for genome-wide linkage analysis of systolic BP (SBP), diastolic BP (DBP), and HT status separately for smokers, nonsmokers, and in the combined group for each race. We localized major QTLs for SBP only in nonsmokers on chromosome 15q26 (LOD=3.4), and only in smokers on chromosome 7q21 (LOD=1.4). The 15q26 and 7q21 QTLs show strong evidence for GxS interactions ($p = 0.0004$ and 0.009, respectively). We also found QTLs for SBP which do not show evidence for GxS interactions including chromosomes 17q24 (LOD=4.2), 20q12 (LOD = 3.5), and 6p22.2 (LOD=2.1). To follow-up linkage results, we have genotyped GENOA sibships for 167 SNPs on 25 positional candidate genes located in the linked regions on chromosomes 15q26 and 17q24. Using FBAT, we identified significant associations with SBP (only in smokers) in two genes in the 15q26 region including a nonsynonymous SNP in the gene for alanyl aminopeptidase (ANPEP), and an intronic SNP in the insulin-like growth factor 1 receptor (IGF1R) gene. We also found associations with DBP (only in nonsmokers) for an intronic SNP in the gene for neuronal nicotinic acetylcholine receptor alpha 5 subunit (CHRNA5) that previously has been associated with nicotine dependence. For the chromosome 17q24 region, we found significant associations with BP traits for two genes including cAMP-dependent protein kinase type I-alpha regulatory subunit (PRKAR1A) and apolipoprotein H (APOH). These results demonstrate the importance of considering gene by environment interactions in dissecting the genetic components of complex traits.

1798/T

Interaction between familial history of obesity and dietary fat intakes on obesity-related phenotypes. *AM. Paradis[1,2,5], G. Godin[3], L. Pérusse[1,4], MC. Vohl[1,2,5].* 1) Lipid Research Center, Québec, Canada; 2) Institute of Nutraceuticals and Functional Foods, Québec, Canada; 3) Faculty of nursing, Laval University, Québec, Canada; 4) Department of Social and Preventive Medicine, Laval University, Québec, Canada; 5) Department of Food Science and Nutrition, Laval University, Québec, Canada.

Obesity is under the influence of genetic and nutritional factors. The aim of this study was to evaluate whether familial history of obesity (FHO) interacts with dietary fat intake (DFI) to modulate indices of obesity. We recruited 664 participants aged between 18 to 55 years. A positive FHO (FHO+) was defined as having at least one obese first-degree relative and a negative FHO (FHO-) as no obese first-degree relative. Dietary intakes were collected from a food-frequency questionnaire. Body mass index (BMI), weight and waist girth were recorded using standard procedures. Fat mass and fat free mass were assessed by electrical bioimpedance. Individuals with FHO+ and FHO- had similar dietary intakes (energy, fat, protein, carbohydrate, cholesterol, and fibre). After adjustment for age and sex, individuals with FHO+ had higher BMI, weight, waist girth, fat mass and fat free mass ($p<0.001$) than individuals with FHO-. To test for the interaction between FHO and DFI, subjects were stratified on the basis of FHO and further on the basis of the percentage of energy derived from fat. The median value (33% of energy from fat) was used as a cutoff point. The effects of FHO, DFI and the interaction (FHO*DFI) were computed using the general linear model, controlling for age and sex as covariates. Significant interaction effects (FHO* DFI) were observed for BMI, weight, waist girth and fat mass (p interaction = 0.05, 0.04, 0.04, 0.02, respectively). Among individuals with FHO+, indices of obesity increased with increasing amount of DFI whereas these associations were not observed in individuals with FHO-. These results suggest a stronger relationship between DFI and obesity-related phenotypes in subjects with FHO+.

1799/T

Physical activity modifies the genetic effect of common variants in the FTO gene with body mass index (BMI). *E. Rampersaud, B.D. Mitchell, M. Fu, H. Shen, T.I. Pollin, J.L. Ducharme, A.R. Shuldiner, S. Snitker.* Department of Medicine, Univ Maryland, Baltimore, MD.

Recently, two groups reported associations with SNPs in intron 1 of the FTO gene on chromosome 16q12.2 with body mass index (BMI) and obesity in multiple large cohorts of Caucasian adults and children. We replicated this association in an Amish population and further assessed whether the genotype effects on BMI were modified by physical activity levels. Our sample included 627 Amish adults (mean BMI = 26.5±4.5 kg/m2) for whom physical activity was measured with Actical accelerometers worn on the hip for 7 consecutive days. Physical activity was expressed as counts, a raw measure of activity independent of body size. Nine SNPs (r2=0.50-1.0) between rs1861869 and rs9930506 in FTO, including the SNPs previously reported, were associated with sex and age adjusted BMI (P=0.04-0.0002) in an additive genetic model. In further analyses stratified by sex-specific median physical activity, our three most strongly associated SNPs (rs1861869, rs1861868, and rs147796) were associated with BMI in subjects with low ($p < 0.001$), but not high ($p > 0.20$) levels of physical activity (p-values for interaction: 0.015 - 0.012). Since Amish women have less physical activity and higher mean BMI compared with men (27.7±5.4 and 25.6±3.2 for BMI respectively, $P< 0.0001$), we evaluated whether sex differences influenced our results by examining the relationship of these SNPs with BMI in men and women separately. We observed significant associations in women with low physical activity levels ($p \leq 0.001$) and suggestive evidence in the same direction for men with low physical activity ($p = 0.05$). In conclusion, the FTO gene has been identified as a well replicated obesity-related gene, although no functional variant or known mechanism has yet been identified. Our results strongly suggest that the increased risk to obesity due to genetic susceptibility by FTO variants can be modified by physical activity. The efficacy of public health efforts to combat obesity may be increased by targeting genetically susceptible individuals such as those carrying FTO variants.

1800/T

Genome wide association analysis identifies SNPs near *MMP1* and *MMP3* as being strongly associated with matrix metalloproteinase-1 (MMP1) levels: The Amish Heredity and Phenotype Intervention (HAPI) Heart Study. *Y. Cheng[1], W.H.L. Kao[1], A.R. Shuldiner[2], B.D. Mitchell[2], P.F. McArdle[2], H. Shen[2], K. Ryan[2], T.I. Pollin[2].* 1) Johns Hopkins University, Baltimore, MD; 2) University of Maryland, Baltimore, MD.

Background MMP1 may play a role in cardiovascular disease (CVD) susceptibility by influencing plaque rupture via its ability to degrade extracellular collagens. **Methods** We performed a genome wide SNP scan of *ln*-transformed MMP1 levels using the Affymetrix GeneChip® Human Mapping 500K Array Set to identify genetic determinants of serum MMP1 levels in a cohort of 585 healthy Amish adults who were part of the HAPI Heart Study. SNPs with minor allele frequencies (MAF) ≥ 2%, Hardy-Weinberg Equilibrium p > 0.001 and genotype call rates ≥ 90% were included in the analysis (361,981 SNPs). Age- and sex-adjusted *ln*-MMP1 residuals were initially screened for association with SNPs using ANOVA as implemented in HelixTree v5.3. SNPs with p-values in the lowest 1% were re-analyzed assuming an additive genetic model using variance components as implemented in SOLAR to account for familial relationships. **Results** Median MMP1 level was 2.79ng/mL (inter-quartile range: 1.74 - 4.62ng/mL) with an estimated heritability of 84.7% (p<0.0001). Seventy-nine SNPs showed associations with MMP1 levels with genome wide significance ($p \leq 9.1x10^{-8}$), all residing in 11q22. The top 3 SNPs (rs603050, rs495366 and rs650108) each had $p \leq 1.4x10^{-19}$ for association with MMP1 levels; their MAF were 0.35-0.36 and they were in high linkage disequilibrium with each other (r^2>0.97) in the Amish. Rs603050 and rs495366 are in the intergenic region between *MMP1* and *MMP3* and rs650108 is within *MMP3*. Each SNP could explain 14.5-16.2% of the variation in MMP1 level. The difference in MMP1 levels between the two homozygous groups of rs603050 was 2.66ng/mL. **Conclusions** We provide strong evidence that serum MMP1 level is highly heritable and that SNPs near *MMP1* and *MMP3* explain a significant portion of the variation in MMP1 levels in the Amish. Identification of the functional SNP(s) that influence MMP1 levels may provide insights into genetic mechanisms of CVD.

1801/T

A genome-wide association study of brachial flow mediated dilation identifies novel candidate genes. *A. Parsa, E. Rampersaud, B.D. Mitchell, R. Horenstein, P.F. McArdle, H. Shen, J.R. O'Connell, R. Vogel, A.R. Shuldiner.* Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.

The vascular endothelium consists of the inner most layer of blood vessels and regulates many critical aspects of vascular function. Hyperemic-induced flow mediated dilation (FMD) is a non-invasive measure of endothelial function and has been associated with numerous disease states, such as hypertension, coronary heart and kidney disease. While studies have demonstrated moderate heritability, the specific gene variations influencing this trait are largely unknown. In this study, we have attempted to uncover FMD related genes by performing a high density genome-wide association study of FMD in the Old Order Amish. Methods: Brachial FMD percent change in diameter was measured under a precise protocol from 868 well characterized relatively healthy Old Order Amish participants of the HAPI Heart Study. The family structures included 633 siblings, 327 parent-offspring and 634 other related pairs. We estimated the heritability of FMD using variance components analysis, adjusting for age and sex. All subjects were genotyped using a 500K Affymetrix SNP array set. Regression analysis for each SNP with normally distributed FMD was performed. Results: The heritability of FMD was estimated at 0.29 (p < 0.001). SNPs mapping to five genes were very highly associated with FMD in our initial screening (by False Discovery Rate, p value range 10-9 to 10-6) and SNPs in another 17 genes were strongly associated (p< 10-6) with FMD. Of the 22 identified genes, 9 contained at least one intronic SNP and 13 were associated by SNPs from either up or downstream regions of the gene. Moreover, several of our candidate genes from chromosomally distinct loci demonstrate functional convergence. Conclusion: Our results confirm that baseline FMD is a significantly heritable trait, a finding which may have broader implications for individual genetic susceptibility to vascular disease. Furthermore, we have identified several candidate genes in addition to delineating a potential novel neural pathway related to endothelial function.

1802/T

Modifying effects of age, QTc interval, and androgen receptor gene variation in patients with hypertrophic cardiomyopathy. *J.M. Lind[1], C. Chiu[1,2], J. Ingles[1], N. Cochrane[1,2], S.E. Humphries[3], A.K. Heather[4], C. Semsarian[1,2,5].* 1) Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, Sydney, Australia; 2) Faculty of Medicine, University of Sydney, Australia; 3) Centre for Cardiovascular Genetics, Royal Free and University College Medical School, United Kingdom; 4) Gene Regulation Group, The Heart Research Institute, Sydney, Australia; 5) Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia.

Hypertrophic cardiomyopathy (HCM) is a clinically heterogenous disease, which suggests a number of factors exist which modify disease outcome. The magnitude of left ventricular hypertrophy is an important predictor of prognosis in patients with HCM. The aim of this study was to determine the contribution of a number of potential modifying factors, including age, blood pressure, QTc interval, presence of a sarcomere mutation, and genetic variation in sex hormone receptors, to the development of left ventricular hypertrophy in HCM. The study population included 174 unrelated individuals from an Australian HCM cohort. Clinical evaluation was performed, including clinical history, physical examination, ECG and 2D/M-mode echocardiography. Genetic analysis of repeat number variations within the androgen receptor (*AR*), estrogen receptor 1, estrogen receptor 2, and cytochrome P450 subfamily XIX genes, was performed in all patients. Younger age (P<0.001), a longer QTc interval (P<0.001), and fewer (CAG)n repeats within the *AR* gene (P=0.023) were significantly associated with higher maximal left ventricular wall thickness (LVWT) in males, in multivariate analysis. Younger age was the only significant predictor of higher maximal LVWT in females (P=0.014). A prolonged QTc interval (P=0.008) and age (P=0.036) were also significantly associated with presence of left ventricular out flow tract obstruction, adjusting for gender. We report three key factors, namely, age, QTc interval, and genetic variation in the *AR* gene, as potential modifiers of left ventricular hypertrophy in HCM. Understanding the impact of modifying factors will be helpful in the risk stratification and clinical management of patients with HCM.

1803/T

Genome-wide scan in affected sibling pairs identifies two novel susceptibility regions for venous thrombosis. *M.C.H. de Visser[1], R. van Minkelen[1], V. van Marion[1], J.C.J. Eikenboom[1], H.L. Vos[1], P.E. Slagboom[2], J.J. Houwing-Duistermaat[3], R.M. Bertina[1].* 1) Dept of Thrombosis and Haemostasis; 2) Dept of Molecular Epidemiology; 3) Dept of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands.

Venous thromboembolism (VTE) is a common disorder with an annual incidence of 1-3 per 1000 individuals. Both environmental and genetic risk factors are involved in the development of the disease (reported heritability about 50 to 60%). At present, few genetic risk factors are known (e.g., Factor V Leiden [FVL]). However, in most thrombophilic families these currently known genetic risk factors can not explain the familial clustering of thromboses, indicating that genetic risk factors are missing. The Genetics In Familial Thrombosis (GIFT) study aims at identifying novel thrombosis susceptibility alleles using an affected sibling pair approach. Via Dutch anticoagulation clinics 224 sibships with VTE at a young age (≤ 45 years) were recruited. Sibship size ranged from 2 to 4 affected siblings and in total 306 affected sibpairs were included. All affected sibs were typed for the 402 microsatellites of the ABI Prism linkage mapping set MD10 and for 5 SNPs (FVL, prothrombin 20210A and 3 ABO blood group SNPs). Multipoint linkage analysis (using the NPL S-all statistic) was performed with Merlin for the autosomes and with MINX for the X chromosome. The study population was enriched for the genetic risk factors FVL (35% in GIFT index patients vs. 20% in consecutive thrombosis patients) and ABO blood group non-O (83% vs. 57%), but not for prothrombin 20210A (6.7 % vs. 6.2%). Interestingly, linkage analyses did not show evidence for linkage at the FVL and ABO blood group loci. Because of suggestive linkage results at chromosome regions 7p and Xq these regions were followed up with extra markers, that were also typed in 375 parents and/or unaffected sibs to improve the information content. The linkage results support the presence of novel thrombosis susceptibility loci on 7p (maximum LOD score = 2.6, genome-wide p=0.0003) and on Xq (LOD score = 2.1, p=0.0009). This study was supported by grants NHS 2005T055 and NWO 912-02-036.

1804/T

Heterozygous mutations in the carnitine transporter gene SLC22A5 are not associated with cardiomyopathy. *C. Amat di San Filippo[1], M.R.G. Taylor[2], L. Mestroni[2], L.D. Botto[1], N. Longo[1,3].* 1) Medical Genetics/Pediatrics, Univ Utah, Salt Lake City, UT; 2) Dept Medicine, Univ Colorado, Denver CO; 3) Dept Pathology and ARUP Laboratories, Univ Utah, Salt Lake City, UT.

Carnitine is essential for the transfer of long-chain fatty acids across the mitochondrial membrane for subsequent beta-oxidation. Primary carnitine deficiency, a recessive disorder caused by defective OCTN2 carnitine transporters, can present with hypoketotic hypoglycemia and/or cardiomyopathy. Heterozygotes for this disease can have low plasma carnitine levels and can develop benign cardiac hypertrophy as adults. This study tested whether heterozygosity for primary carnitine deficiency was associated with cardiomyopathy. The frequency of mutations in the SLC22A5 gene encoding the OCTN2 carnitine transporter was determined in 324 patients with cardiomyopathy and compared to that described in the normal population. Missense variations identified in normal controls and patients with cardiomyopathy were expressed in Chinese Hamster Ovary cells to confirm a functional effect. Exons 2-10 of the SLC22A5 gene were amplified by PCR in the presence of LCGreen I™ and analyzed by dye-binding/high-resolution thermal denaturation followed by sequencing if needed. Exon 1 of the gene was sequenced in all patients. Heterozygosity for L144F, T264M, I312V, E317K and R488H was found in 6/324 patients with cardiomyopathy. Expression studies indicated that T264M decreased, E317K increased, while L144F, I312V, and R488H did not significantly affect carnitine transport. Expression studies of variants identified in normal controls indicated that L17F and Y449D had a functional effect, while F144L, V481I, V481F, M530V, and P549S did not change significantly carnitine transport. The frequency of variants affecting carnitine transport was 2/324 in patients with cardiomyopathy (0.61%) as compared to a reported frequency of 3/270 (1.11%) in the general population (odds ratio 0.6, 95% Confidence interval 0.1 to 3.3). Heterozygosity for primary carnitine deficiency is not more frequent in patients with unselected types of cardiomyopathy and is unlikely to be an important cause of cardiomyopathy in humans.

1805/T

Genotype-phenotype relationships in desminopathy. *H. Lee[1], A. Shatunov[1], M. Olivé[2], P. Vicart[3], A. Kaminska[4], K. Bushby[5], F. Muntoni[6], M. Dalakas[1], H. Goebel[7], L. Goldfarb[1].* 1) National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; 2) Hospitalet de Llobregat, Barcelona, Spain; 3) Univ. Paris, Paris 7 Denis Diderot, France; 4) Medical Univ. of Warsaw, Warsaw, Poland; 5) Inst. of Human Genetics, Newcastle upon Tyne, UK; 6) Imperial College, London, UK; 7) Mainz Univ. Med. Ctr, Mainz, Germany.

Desminopathy is one of the most common neuromuscular disorders, which is associated with mutations in desmin and alphaB-crystalline. These proteins are in close interactions in striated muscle Z-disc structure. Desminopathy patients may suffer from smooth muscle myopathy, neuropathy, respiratory dysfunction, facial paralysis, or cataracts. However skeletal myopathy and cardiomyopathy are two major clinical features, of which patients may suffer either one of the two, or both. With few exceptions, such phenotypic manifestations in members of the same family are concordant with respect to developing either skeletal or cardiac myopathy, which suggests a diverse underlying genetic cause of different desminopathy phenotypes. This gave us the impetus to investigate the possibility of genotype (type and location of forty-two reported desminopathy-causing mutations in the desmin gene) and phenotype (cardiac, respiratory and/or skeletal involvement) relationship among desminopathy patients. Our study includes clinically and pathologically characterized 91 desminopathy patients, all of whom have mutations in various domains of desmin. These patients were grouped into two-way contingency table according to: 1) the location of mutation in the desmin gene domain, and 2) the degree of cardiac and skeletal muscle involvement. Armitage trend test revealed that mutations in the 1B domain tend to cause predominantly cardiac myopathy when compared to mutations in 2B domain. Tail domain mutations also showed more cardiac involvement than skeletal myopathy when compared against 2B domain. We conclude that the location of desmin mutation exerts a significant influence on desminopathy phenotypes, with cardiomyopathy occurring more frequently with mutations in 1B and tail domains, while mutations in 2B domain are linked to the skeletal myopathy phenotype.

1806/T

Large genomic *FBN1* deletions detected by MLPA and SNP arrays provide evidence for true haploinsufficiency in Marfan syndrome. *G. Matyas[1], S. Alonso[1], A. Patrignani[2], M. Martl[2], E. Arnold[3], I. Magyar[1], C. Henggeler[1], T. Carrel[4], B. Steinmann[3], W. Berger[1].* 1) Medical Molecular Genetics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; 2) Functional Genomics Center Zurich, ETH and University of Zurich, Zurich, Switzerland; 3) Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland; 4) Clinic for Cardiovascular Surgery, University Hospital, Berne, Switzerland.

Purpose: Mutations in the *FBN1* gene cause Marfan syndrome (MFS), an autosomal dominant connective tissue disorder, which displays variable manifestations in the cardiovascular, ocular, and skeletal systems. Current molecular genetic testing of *FBN1*, although powerful, may miss mutations in the promoter region or in other noncoding sequences as well as partial or complete gene deletions and duplications. Methods: We successively applied multiplex ligation-dependent probe amplification (MLPA) and the Affymetrix Human Mapping 500K Array Set, which contains probes for ~500,000 single-nucleotide polymorphisms (SNPs) across the genome, to analyze copy number variation in genomic DNA samples of 101 unrelated patients with MFS or related phenotypes in whom standard molecular testing detected no mutation. Results: We identified *FBN1* deletions in two patients with MFS. Our high-resolution approach narrowed down the deletion breakpoints. Subsequent sequencing of the breakpoint junctions revealed the deletion sizes of 27 and 303 kb, respectively. Surprisingly, both deletions affect the putative regulatory and promoter region of the *FBN1* gene, strongly indicating that they abolish transcription of the deleted allele. This expectation of complete loss of function of one allele, i.e. true haploinsufficiency, was confirmed by transcript analyses.

Conclusions: Our findings emphasize the importance of screening for large genomic rearrangements in comprehensive genetic testing of *FBN1* and extend the molecular etiology of MFS by providing hitherto unreported evidence that true haploinsufficiency is sufficient to cause MFS.

1807/T

Reverse-hybridization-based genetic testing for the prediction of anticoagulant dose requirement. *H. Puehringer[1], C. Stoellberger[2], Q. Berisha[2], A. Dossenbach-Glaninger[3], W. Krugluger[3], C. Oberkanins[1].* 1) ViennaLab Diagnostics GmbH, Vienna, Austria; 2) Second Medical Department, Rudolfstiftung Hospital, Vienna, Austria; 3) Department of Clinical Chemistry, Rudolfstiftung Hospital, Vienna, Austria.

Coumarin derivatives (Warfarin, Phenprocoumon, Acenocoumarol) are the most widespread oral anticoagulant drugs for the prevention and treatment of arterial and venous thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide inter-individual variability in dose requirement. Despite adjustment for clinical variables, adverse events, such as delay in achieving a stable maintenance dose or bleeding complications, are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing cytochrome P450 isozyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin. We have developed a genetic test (PGX-Thrombo StripAssay) for the detection of -1639 G/A and 3730 G/A in the VKORC1 gene, and 430 C/T and 1075 A/C in the CYP2C9 gene. The assay is based on multiplex PCR, followed by reverse-hybridization of biotin-labeled amplification products to a parallel array of allele-specific oligonucleotides immobilized on membrane teststrips. Genotyping for VKORC1 polymorphisms and the functionally defective CYP2C9 variants *2 and *3 allowed us to classify our patients into distinct groups of high, intermediate and low dose responders to phenprocoumon (Marcumar), the most commonly used oral anticoagulant in Central and North European countries. Favourable properties, such as the rapid DNA extraction protocol, ready-to-use reagents and teststrips, as well as the potential for automation of the hybridization/detection step, make the StripAssay convenient and easy to perform within less than 6 hours. The results obtained in our study will assist clinicians to achieve a more individualized anticoagulant therapy. (oberkanins@viennalab.co.at).

1808/T

Truncating *BMPR2* mutation in a patient with pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia. *C.M. Rigelsky[1], R. Lehtonen[2], C. Eng[1], M.A. Aldred[1].* 1) Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 2) Department of Medical Genetics, University of Helsinki, Finland.

Pulmonary arterial hypertension (PAH) and hereditary hemorrhagic telangiectasia (HHT) are two distinct clinical entities that overlap in some individuals. PAH is characterized by elevated mean pulmonary arterial pressure. *BMPR2* is the only gene that has been associated with familial PAH; mutations are associated with autosomal dominant inheritance with reduced penetrance. HHT is an autosomal dominant condition characterized by epistaxis, telangiectasia and visceral lesions. HHT has been shown to be caused by mutations in *ACVRL1*, *ENG* or *SMAD4*. To date twenty-four families have been described with features of PAH and HHT. Eighteen of these were caused by mutations in *ACVRL1* and two by *ENG* mutations. We report here a 36-year-old woman diagnosed with PAH and HHT and a *BMPR2* mutation. She was initially diagnosed with PAH at the age of 24. At the age of 35, a computed tomographic scan of the chest revealed pulmonary AVMs, suggesting the possible diagnosis of HHT. Physical exam and review of her medical history revealed nasal telangiectasia and spontaneous nighttime epistaxis. She met HHT diagnostic criteria based on the presence of pulmonary AVMs, epistaxis and nasal telangiectasia. She was adopted, so no family history was available. In this patient, mutation analysis of *ACVRL1*, *ENG* and *SMAD4* were all normal. However, analysis of *BMPR2* revealed a germline nonsense mutation in exon 10, designated c.1297C>T (p.Q433X). This is the first known report of a patient with a clinical diagnosis of PAH and HHT who has a germline mutation in *BMPR2*. It is currently not clear how a *BMPR2* mutation could result in an HHT phenotype. *BMPR2, ACVRL1*, *ENG* and *SMAD4* are all members of the TGF-beta/BMP superfamily; however, crosstalk between the TGF-beta and BMP signaling pathways is not fully understood. While HHT is not common among individuals with *BMPR2* mutations, this case highlights that analysis of the *BMPR2* gene is indicated in patients affected with both HHT and PAH who do not harbor *ACVRL1*, *ENG* or *SMAD4* mutations.

1809/F

Molecular dissection of NRG1-ERBB4 signaling implicates PTPRZ1 as a potential schizophrenia susceptibility gene. *T. Sakurai[1, 2], L. Georgieva[3], N. Takahashi[1], S. Harroch[4], V. Moskvina[3], N. Norton[3], M.J. Owen[3], M.C. O'Donovan[3], J.D. Buxbaum[1, 5, 6].* 1) Psychiatry, Mount Sinai Sch Medicine, New York, NY; 2) Pharmacology, Mount Sinai Sch Medicine, New York, NY; 3) Psychological Medicine, Sch of Medicine, Cardiff Univ, Cardiff, UK; 4) Neuroscience, Inst Pasteur, Paris, France; 5) Genetics and Genomics, Mount Sinai Sch Medicine, New York, NY; 6) Neuroscience, Mount Sinai Sch Medicine, New York, NY.

Neuregulin and the neuregulin receptor ERBB4 have been genetically and functionally implicated in schizophrenia. We used the yeast two-hybrid system to identify proteins that interact with ERBB4, in order to identify genes and pathways that might contribute to schizophrenia susceptibility. We identified the MAGI scaffolding proteins as ERBB4-binding proteins. After validating the interaction of MAGI proteins with ERBB4 in mammalian cells, we demonstrated that ERBB4 expression, alone or in combination with ERBB2 or ERBB3, led to the tyrosine phosphorylation of MAGI proteins, and that this could be further enhanced with receptor activation by neuregulin. As MAGI proteins were previously shown to interact with receptor phosphotyrosine phosphatase β/ζ (RPTPβ), we postulated that simultaneous binding of MAGI proteins to RPTPβ and ERBB4 forms a phosphotyrosine kinase/phosphotyrosine phosphatase complex. Studies in cultured cells confirmed both a spatial and functional association between ERBB4, MAGI, and RPTPβ. Given the evidence for this functional association, we examined the genes coding for MAGI and RPTPβ for genetic association with schizophrenia in a Caucasian United Kingdom case-control cohort (n = ~1,400). PTPRZ1, which codes for RPTPβ, showed significant, gene-wide and hypothesis-wide association with schizophrenia in our study (best individual SNP allelic P = 0.0003; gene-wide P = 0.0064; hypothesis-wide P = 0.026). The data provide evidence for a role for PTPRZ1, and for RPTPβ signalling abnormalities, in the etiology of schizophrenia. Furthermore, the data indicate a role for RPTPβ in the modulation of ERBB4 signalling which may in turn provide further support for an important role for neuregulin/ERBB4 signalling in the molecular basis of schizophrenia.

1810/F

Identification of differentially expressed proteins in the plasma of heroin abusers. *H. Zhou[1,2], H. Zhu[3], J. Liu[4], J. Xu[5], J. Li-Ling[2], M. Li[6], S. Jia[1].* 1) Peking University Shenzhen Hospital, Shenzhen 518036, China; 2) Department of Medical Genetics, China Medical University, Shenyang 110001, China; 3) Chronic Disease Prevention and Cure Station of Bao'an, ShenZhen 518133, China; 4) Center for Disease Prevention and Control, Shenzhen 518020, China; 5) Shenzhen Detoxification Center, Shenzhen 518019, China; 6) Proteomics Laboratory, Sun Yat-sen University, Guangzhou 510060, China.

Aim: To identify differentially expressed proteins in the plasma that may be used as biomarkers for heroin addiction through a two-dimensional (2-D) gel electrophoresis/mass spectrometry approach. Method: Following removal of albumin and IgG, plasma from 5 abusers and 5 normal controls were separated by 2-D gel electrophoresis using pH 4~7 drystrip and PAGE. Gel images were analyzed using ImageMaster Elit 5.0. Differential proteins were selected and analyzed through tandem mass spectrometry. Results: Average spot number for samples was 563±23. Five spots that differed by more than 1.5 fold between the two groups were obtained through image analysis. Through tandem mass spectrophotometric fingerprinting, above spots were identified as fibrinogen gamma (increased by 5 fold), human α-1-B-glycoprotein (decreased by 1.8 fold), uncleaved alpha 1-antitrypsin (increased by 2.5 fold), chain D of transthyretin (decreased by 2.0 fold) and ceruloplasmin (increased by 6.6 fold). Conclusion: Difference between heroin abusers and normal controls was identified as a component of blood plasma proteome. Some of these proteins may have a role in the damage to central nervous system through heroin abuse. Such proteins may provide novel biomarkers for diagnosis and therapeutic targeting, as well as clues for understanding the mechanism of heroin abuse.

1811/F

Repetitive Behaviors in Children with Autism and Maternal Prenatal Problems. *R.K. Abramson[1], A.V. Hall[2], S.A. Ravan[2], M.L. Cuccaro[3], J. Gilbert[3], M. Pericak-Vance[3], H.H. Wright[1].* 1) Dept Neuropsychiatry Univ South Carolina Sch. Med, Columbia, SC; 2) Dept Communication Sciences, Univ. South Carolina Sch. Public Health, Columbia, SC; 3) Univ Miami Inst Hum Gen, Miami, FL.

Leonard, 2006, in a population study identified common prenatal problems that increase risk for Autistic Disorder(AD). Little is known how specific prenatal factors affect expression of AD. This study will examine whether maternal prenatal problems are associated with specific behaviors in the child with AD. Subjects (n=149) were from the Duke/USC molecular study of AD. The ADI-R, Pregnancy Assessment Monitoring System (PRAMS), the Aberrant Behavior Checklist (ABC), and the Repetitive Behavior Scale-Revised (RBS) were completed for each child. Four prenatal factors were identified: infectious illness, F1; bleeding/early loss, F2; hypertension-edema- preeclampsia, F3; and diabetes, F4. One-way multivariate ANOVA revealed no significant results for the dependent variables of the ADI-R Q19 -Speech, the Insistence on Sameness Factor (Shao, 2003), or the ABC scores. There were significant results for F1 and RBS subscales Compulsive Behavior -CB, F(1,149)=9.143, p=.003; Ritualistic Behavior- RB, F(1,149)=4.465, p=.036; and Sameness Behavior- SAB, F(1,149)=13.584, p=.000. There were significant results for F3 and RBS subscales Stereotyped Behavior- SB, F(1,148)=11.09, p=.001 and Restricted Behavior- REB, F(1,148)=8.707, p=.004, and for F4 and SB, F(1,148)=26.757, p=.000; CB, F(1,148)=8.703, p=.004; and REB, F(1,148)=21.414, p=.000. There was a significant interaction between F3 and F4 for SB, F(1,148)=19.358, p=.000 and REB, F(1,148)=9.951, p=.002. Pearson correlations were significant between F1 and CB, r=.197, p=.016; RB, r=.206, p=.012; and SAB, r=.273, p=0.00l; between F4 and SB, r=.270, p=.00l; CB, r=.202, p=.014; and REB, r=.277, p=.00l; between F1 and ABC-irritability, r=.149, p=.035; and ABC-hyperactivity, r=.148, p=.036 and between F2 and ABC hyperactivity, r=.141, p=.045. Thus, there may be a relationship between certain prenatal problems and repetitive behaviors and prenatal infectious illness, an environmental factor, may affect the expression of repetitive behaviors in AD.

1812/F

Genome-wide scan in a large French-Canadian Restless Legs Syndrome kindred: fine-mapping towards a novel candidate locus for linkage. *A. Levchenko[1], S. Provost[4], L. Xiong[1], M.P. Dubé[4, 5], J. St-Onge[1], P. Thibodeau[1], A. Desautels[2, 3], G. Turecki[2], J. Montplaisir[3], G.A. Rouleau[1, 5].* 1) CHUM Research Center, Notre Dame Hospital, Montreal, Quebec, Canada; 2) Research Center, Douglas Hospital, McGill University, Québec, Canada; 3) Centre d'étude du sommeil, Hôpital du Sacré-Cœur de Montréal and Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada; 4) Montreal Heart Institute Research Center, Montreal, Quebec, Canada; 5) Department of Medicine, University of Montreal, Montreal, Quebec, Canada.

Restless Legs Syndrome (RLS) is a sensory-motor disorder affecting approximately 10% of the general population, with reported tendency to be more prevalent in populations of European origin. More than 60% of cases show segregation in families. We analyzed a large RLS kindred of French-Canadian (FC) origin using microsatellite 10 centi-Morgan genome-wide scan. Preliminary genome-wide two-point and multipoint linkage analysis, using MLINK and SIMWALK2 software, allowed to pinpoint several genomic regions harboring multipoint LOD scores greater than 1, 2 and 3, under a dominant model. The regions are on chromosomes 1p, 1q, 2q, 4p, 11q, 16p, 18q, and 22q. The fine-mapping, in order to establish a region significantly linked to RLS in this family, which will allow undertaking RLS gene cloning, is ongoing. To estimate the power of the family, single point power analysis was carried out using SLINK, UNKNOWN and MSIM, in which five alleles with frequencies set to 0.1, 0.1, 0.2, 0.2 and 0.4 were simulated. The disease was set at a distance of 1.25 cM which is the average distance between a gene and a marker in a 5 cM genome scan, showing that the family is able to reach LOD scores of 3.9. Previously, linkage analysis and large FC pedigrees allowed our team to report novel RLS loci on chromosomes 12q and 20p13.

1813/F

Analysis of gene expression in familial mesial temporal lobe epilepsy associated with hippocampal atrophy. *C.V. Maurer-Morelli[1], C.S. Rocha[1], R. Secolin[1], R.R. Domingues[1], F. Cendes[2], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, FCM/UNICAMP - Brazil; 2) Department of Neurology, FCM/UNICAMP - Brazil.

Rationale: Hippocampal atrophy (HA) is the most prominent pathological substrate in patients with intractable mesial temporal lobe epilepsy (MTLE) and until recently, this finding was exclusively associated with predisposing environmental factors. However, we identified the first *locus* for familial MTLE associated with HA, which strongly suggests that HA may also have a genetic predisposition. Surgical specimens of patients with intractable MTLE, offer a unique opportunity to address questions related to the pathophysiology of HA in the context of MTLE. The aim of this study was to perform gene expression studies in tissue samples from familial MTLE patients who underwent surgery for medically intractable seizures. **Methods:** This study was performed using Human Genome U133 Plus 2.0 array (Affymetrix). High-quality total RNA from one control hippocampus (from autopsy) and three surgical specimens (from pharmacoresistant epilepsy patients) were isolated by TRIzol (Invitrogen-Life Technologies). We used 6 μg of starting material in the one-cycle target labeling protocol (Affymetrix). Data was acquired by GeneChip Scanner 3000 (Affymetrix) and analyzed using MAS5.0 expression measure (Affymetrix). **Results:**Comparison between control and disease hippocampi identified 2300 genes which were differently expressed and they are related to many cell functional classes, such as transcription factors, enzymes, signaling and structural function. Interesting, among these we identified five genes which were present in disease hippocampi, but not present in control specimen and are localized within the candidate region for familial MTLE identified on chromosome 18p: *ADCYAP1, TYMS, DLGAP1, PTPRM* and *YES1*. **Conclusions:** Our study brings functional information related to gene expression profile into the current efforts to unravel the molecular mechanism responsible for familial MTLE associated with HA. Additional studies are underway in order to compare gene expression profile of familial and non-familial MTLE hippocampi. Supported by FAPESP and CNPq.

1814/F

A *Neurexin 1* deletion implicates a synaptic defect in the pathophysiology of autism. *K.J. Meyer[1], L.K. Davis[1], A.L. Librant[1], D.S. Rudd[1], E.M. Berg[4], C.M. Taylor[2], J. Piven[5], E.M. Stone[2,4], V.C. Sheffield[3,4], T.H. Wassink[1], Autism Genome Project Consortium.* 1) Department of Psychiatry, University of Iowa, Iowa City, IA; 2) Department of Opthalmology and Visual Sciences, University of Iowa, Iowa City, IA; 3) Department of Pediatrics, University of Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 5) Neurodevelopmental Disorders Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Autism is a pervasive developmental disorder (PDD) characterized by impairments in communication and social interaction as well as restricted interests and repetitive behaviors. Family and twin studies have demonstrated a substantial genetic component in the development of autism. Considerable efforts have been made to identify genes that confer susceptibility to autism through linkage analysis and association studies. However, the mode of inheritance for autism is complex and this effort has met with limited success. Therefore we utilized a new approach, screening the genomes of multiplex autism spectrum disorder (ASD) families for copy number variants (CNVs) in collaboration with the Autism Genome Project (AGP) with the Affymetrix 10K SNP microarray. We identified two affected female siblings, both harboring an identical hemizygous 355kb CNV loss that deleted coding exons from the gene *NRXN1*. Microsatellite mapping indicated no transmission of paternal alleles across the deletion interval, indicating paternal germline mosaicism. Studies have shown that neurexins are located on the presynaptic terminus of both excitatory and inhibitory synapses and that they function as cell adhesion molecules, binding to neuroligins on the postsynaptic terminus. The neurexin/neuroligin complex has previously been implicated in autism and is hypothesized to have several functions including regulation of the ratio of excitatory to inhibitory synapses. Based on these data, we have further evaluated the role of *NRXN1* in the development of autism by screening a large sample of individuals with autism for *NRXN1* mutations and testing *NRXN1* SNPs for association.

1815/F

Sydenham's Chorea: a study of the Ser9Gly polymorphism in the *DRD3gene.* *D.M. Miranda[1], L.A. De Marco[2], H. Correa[1], A.L. Teixeira[3], F. Cardoso[3], W. Boson[2], M.A. Romano-Silva[1].* 1) Mental Heathy, UFMG, Belo Horizonte, Minas Gerais, Brazil; 2) Pharmacology Department, UFMG, Belo Horizonte, Minas Gerais, Brazil; 3) Internal Medicine Department, UFMG, Belo Horizonte, Minas Gerais, Brazil.

Sydenham's Chorea is a neuropsychiatric presentation of rheumatic fever, it is characterized by weakness, uncoordinated movements and emotional lability. Rheumatic fever usually occurs in children between 5 and 15 years old and follows an infection by Streptococcus beta-haemolyticus, but a familiar susceptibility seems to determine who will or will not develop Rheumatic Fever and Sydenham's Chorea. Few studies have investigated some polymorphisms and genes associated with Sydenham's Chorea. Actually Sydenham Chorea is a self-limited disorder but, when necessary a pharmacological treatment with good results is the use of dopaminergic antagonists. The pharmacological response and findings of association between the Ser9Gly polymorphism of DRD3 gene and tardive diskinesia - another movement disorder - motivated our study. It consisted on clinical evaluation and study of Ser9Gly polymorphism in DRD3 gene of 45 patients from the Rheumatic Fever Clinic of Universidade Federal de Minas Gerais and 46 healthy controls. The genotyping was performed as described by Segman et al. (1999). The genotype findings were statistically analyzed by the Chi-square test. We did not find association between Ser9Gly polymorphism and Sydenham's Chorea. This was a pilot study with a small number of patients investigated, however, even considering the limitations Ser9Gly does not seem to be a polymorphism associated with Sydenham's Chorea. References: Segman R, Neeman T, Heresco-Levy U, Finkel B, Karagichev L, Schlafman M, Dorevitch A, Yakir A, Lerner A, Shelevoy A, Lerer B. Genotypic association between the dopamine D3 receptor and tardive dyskinesia in chronic schizophrenia. Mol Psychiatry. 1999;4(3):247-53.

1816/F

Identification of sequence variants in miRNA target sites of NTRK3 associated to anxiety disorders. *M. Muinos-Gimeno[1], M. Guidi[1], B. Kagerbauer[1], M. Gratacòs[1], R. Martín-Santos[2], M. Torrens[2], M.P. Alonso[3], J.M. Menchón[3], X. Estivill[1, 4], Y. Espinosa-Parrilla[1].* 1) Genes and Disease Program, CeGen and CIBERESP (CRG-UPF); 2) Pharmacology Research Unit and Psychiatry Department, IMIM-Hospital del Mar; 3) Psychiatry Department, Hospital Bellvitge; 4) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Neurotrophins and their receptors have been implicated in the pathophysiology of anxiety disorders. Genetic and functional data point to the neurotrophin-3 receptor gene (*NTRK3*) as a candidate for psychiatric disease. Post-transcriptional regulation by miRNAs has been recently shown to contribute to the differential regulation of *NTRK3* isoforms. The requirement of a perfect base pairing between the miRNA seed region and its target mRNA, indicates that allelic variants in target sites could contribute to susceptibility to disease. We suggest that functional allelic variants at 3'UTRs of *NTRK3* may be predisposing to anxiety disorders. Interrogation of the 3'UTRs of two different *NTRK3* isoforms for miRNA target sites using TargetScan, miRanda and PicTar prediction programs resulted on 65 miRNAs as candidate regulators of *NTRK3*. Re-sequencing of 5 kb corresponding to two different 3'UTRs of *NTRK3* isoforms was performed in DNA samples of 60 patients with panic disorder and 60 patients with obsessive-compulsive disorder (OCD). Twelve new allelic variants and 2 known SNPs were identified, 5 in predicted target sites for 6 different miRNAs on the truncated isoform. Three of these allelic variants are located within the seed region and are thought to disrupt the repression of the *NTRK3* gene by the predicted miRNA. Functional validation of these 6 putative miRNA target sites was performed by dual luciferase assays in HeLa cells. Case-control studies, in 450 patients with different types of anxiety disorder and 350 controls, revealed that one of the variants was only present in a patient with panic disorder and none of the controls. Moreover, association of another *NTRK3* miRNA variant to OCD hoarding type was shown. Functional mutagenesis studies are being performed. Supported by Spanish Government (FI05/00061,R&C program) and Generalitat de Catalunya.

1817/F

Genes regulated by MECP2 as candidate genes for autism. *N. Nakashima, T. Yamagata, Z. Yu, K. Suwa, M. Mori, M.Y. Momoi.* Pediatrics, Jichi Medical University, Shimotsuke, Tochigi, Japan.

Several genes were identified to be regulated by MECP2, a gene for Rett syndrome (RTT). Some downstream genes of MECP2 may contribute for autistic phenotype of RTT, and such genes are candidate genes for autistic disorder (AD). The alternation of imprinting status or the defect of some transcriptional mechanisms on these genes, or gene substitutions on them may relate to AD. To detect the responsible genes for AD, we analyzed the expression level of the genes regulated by MECP2 on lymphoblasts of AD, RTT and controls. And, these genes were analyzed for mutations on AD patients. Among the genes regulated by MECP2, some were imprinted only in the brain. But several genes were confirmed to be imprinted in lymphocytes addition to the brain. Therefore, it is reasonable to analyze the expression level of such genes on lymphoblasts. Patients diagnosed as AD according to the criteria of DSM-IV were enrolled in this study after the informed consents by their parents. The genes studied included DLX5, PEG10, BDNF, SGK, IGFBP3 and IGF2. And also, DLX6 that belonged to the same family with DLX5 and localized next to DLX5 was analyzed. Expression level of each gene was detected in 12 AD patients, three RTT patients and eight control individuals by Real time PCR method, respectively. For mutation analysis, up to one hundred Japanese AD patients were enrolled. All exons and introns nearby of these genes were amplified by PCR, the fragment was analyzed by DHPLC, and finally confirmed by direct sequencing. The expression level of DLX5 and SGK were high in RTT patients and some of AD patients. Instead, the expression level of other genes were not different among the lymphoblasts analyzed. No causative mutation was detected in the genes regulated by MECP2. One missense mutation was detected in DLX6 on one siblings with AD. Although no causative mutation was detected in DLX5 and DLX6 on AD patients in the previous reports, DLX6 might relate for AD. From our results, it is considered that AD and RTT share common pathway after MECP2 through some of the genes. It is required to analyze another genes regulated by MECP2 further to clarify the contribution to autism.

1818/F

Serotonin transporter polymorphism in Japanese patients with bipolar disorder. *N. Oribe, H. Kawasaki, H. Mitsuyasu, Y. Kobayashi, L. Gotoh, A. Takata, S. Kanba.* Dept.Neuropsychiatry, Kyushu University, Fukuoka, Japan.

Since serotonin transporter (SERT) is one of the sites of action of antidepressants, this gene has been indicated to be related to the pathophysiological mechanisms of mood and anxiety disorders. Differences in SERT expression and function produced by gene polymorphisms are associated with several psychiatric diseases. Two polymorphic regions of SERT gene, a 44-base-pair (bp) insertion / deletion polymorphism in the promoter region (SERTPR) and variable number of tandem repeats (VNTR) in second intron (SERT-in2), have been characterized. SERT-in2 is reported to be associated with unipolar depression, bipolar depression, schizophrenia, and anxiety disorders. SERTPR is also shown to be associated with unipolar and bipolar depression. However, the results from various sources are inconsistent. In this study we investigated the frequency distribution of polymorphic variants of short (S, s) and long (L, l) alleles, genotypes and haplotypes of SERTPR, and SERTin2, in patients with bipolar disorder (BD) and compared them with those obtained from the Japanese healthy population. Twenty bipolar disorder patients diagnosed using the Structured Clinical Interview for DSM-IV (SCID) and nineteen healthy volunteers were included in this study. The SERTin2 and SERTPR are amplified by polymerase chain reaction (PCR). Allele sizes were determined by electrophoresis. Association analysis of each polymorphism was performed between bipolar disorder patients and normal individuals. SERTPR indicated statistically significant difference between two populations (chi square = 8.114, d.f. = 2, P = 0.0173) ,whereas SERTin2 shows no differences (chi square = 1.767, d.f. = 2, P = 0.4133). We are now carring out further analysis with more samples. All subjects were given informed consent before blood collection strictly based on the ethical regulations of Kyushu University.

1819/F

COMT 'tag' SNPs associated with quantitative cognitive variables in multiplex, multigenerational schizophrenia sample. *K. Prasad[1], L. Almasy[2], R. Gur[3], R. Gur[3], M. Pogue-Geile[1], M. Talkowski[1], K. Chowdari[1], V. Nimgaonkar[1].* 1) Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 2) Southwest Foundn Biomed Res, San Antonio, Tx; 3) Univ Pennsylvania Sch Med, Philadelphia, PA.

Background: Catechol-O-methyl transferase (COMT) gene variations have been associated with cognitive performance in both schizophrenia (SCZ) and healthy subjects. We comprehensively examined the association of COMT polymorphisms with variability in cognitive functions in a series of multiplex, multigenerational (MM) Caucasian families with SCZ. Methods: Consenting participants in 56 MM families were administered the Computerized Neurocognitive Battery (CNB). The CNB evaluates speed (response time) and accuracy of performance on abstraction and mental flexibility, attention, verbal, spatial and face memory, and spatial ability. We selected the 'tag' SNPs from common SNPs from the HapMap, Seattle SNP database and published literature (r2 cut off <0.8). These SNPs were genotyped among 561 members of our MM SCZ families using the SNPlex assay (ABI Biosystems, Inc). Measured genotype analyses accounting for family relationships were performed in SOLAR. Results: Accuracy of attention (rs4646315), verbal memory (rs9332377, rs4646316) and spatial processing (rs165815) were significantly different across these alleles ($p \leq 0.05$). Speed of language processing was associated with rs933271. Suggestive associations were also observed for spatial memory. Discussion: These results suggest that variations in COMT are associated with variability in distinct cognitive domains in MM families with SCZ. Our group had previously reported that these cognitive measures were heritable and distinguish the patients from their relatives and healthy controls. Such observations in MM families suggest that these estimates may not be substantially inflated by environmental factors and that specific gene variations could account for such variability. On the same sample, RGS4 variations were associated with face and verbal memory. Taken together, these observations lend initial clues to the possibility that variations in different susceptibility genes may be associated with variability in distinct cognitive domains.

1820/F

Large-scale screening of X-linked synaptic genes in Tourette Syndrome cases. *J-B. Rivière[1], A. Piton[1], J. Gauthier[1], R. Joober[2], Y. Dion[3], G. Tellier[4], P. Lespérance[1], S. Chouinard[1,4], F. Richer[1], G.A. Rouleau[1,4].* 1) Centre for the Study of Brain Disease, CHUM Research Centre, Montreal, Quebec, Canada; 2) Douglas Hospital Research Centre, Montreal, Quebec, Canada; 3) McGill University Health Centre, Montreal, Quebec, Canada; 4) Sainte Justine Hospital, Montreal, Quebec, Canada.

Tourette Syndrome (TS) is a complex neurodevelopmental disorder with a strong genetic component often associated with various behavioral abnormalities such as obsessive-compulsive disorder (OCD) and attention deficit-hyperactivity disorder (ADHD). Decades of classical genetic studies in TS and other complex neurological disorders have had limited success in the identification of susceptibility genes, highlighting the necessity of new genetic approaches. Various evidence support an implication of X-linked genes in TS, and mutations in synaptic genes may account for the genetic predisposition in a significant proportion of TS cases. We therefore decided to directly sequence X-linked genes coding for synaptic proteins in 96 French-Canadian TS patients selected according to their familial history (including only families with a possible X-linked inheritance). Based on various methods and databases, we catalogued all the known genes potentially coding for synaptic proteins on the X chromosome. We selected more than 100 genes that are currently being screened according to several criteria including expression in brain tissues, involvement in synaptogenesis, animal models showing neurobehavioral abnormalities, association with related diseases, etc. We expect to identify several potentially functional variants that will be further investigated. Genetic validation will include functional prediction, screening of additional TS cases and unaffected controls, cosegregation analysis in TS families as well as association studies with potential sub-phenotypes (OCD, ADHD, anxiety, sleep disturbances, etc.). By the end of this study, we expect to identify several susceptibility genes for TS and its related comorbidities.

1821/F

Association study of five human genes involved in melatonin signaling pathway and photoentrainment (AANAT, MTNR1A, MTNR1B, OPN3, OPN4) in mood disorders. *V. Soria[1], M. Gratacos[2], J.R. Gonzalez[2], J. Valero[3], E. Martinez-Amoros[1], M. Bayes[2], A. Gutierrez[3], R. de Cid[2], J.M. Crespo[1,4], L. Martorell[3], E. Vilella[3], A. Labad[3], J.M. Menchon[1], J. Vallejo[1], X. Estivill[2,5], M. Urretavizcaya[1].* 1) Mood Disorders Research, Hospital Universitary de Bellvitge, Hospialet, Barcelona, Catalonia, Spain; 2) Genes and Disease Program, CeGen and CIBERESP, Center for Genomic Regulation (CRG-UPF), Barcelona; 3) Grup d'Investigació en Psiquiatria. Hospital Universitari Institut Pere Mata, Rovira i Virgili University. Reus. Tarragona, Catalonia, Spain; 4) Department of Psychiatry, Barcelona University. Barcelona, Catalonia, Spain; 5) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Disruption of circadian rhythms including abnormalities of circadian phase position and melatonin secretion, have been described in mood disorders (MD). We performed a genetic case-control study for the following candidates genes: Arylalkylamine N-acetyltransferase (AANAT), opsin 3 (OPN3), opsin 4 (OPN4), melatonin receptor 1A (MTNR1A) and melatonin receptor 1B (MTNR1B). The sample consisted of 365 unrelated patients (218 Unipolar Major Depressive Disorder, 147 Bipolar Disorder) diagnosed according to DSM-IV criteria and 419 screened control subjects. We genotyped a set of 29 TagSNPs representative of the patterns of common variation identified in European population selected from HapMap project dataset covering the entire genomic region of OPN3, OPN4, MTNR1A, MTNR1B and validated variants in AANAT. The SNPlex Genotyping System was used. Single SNP case-control association analysis considering the MD phenotype identified a positive association in MTNR1B which did not remain significant after Bonferroni correction, and a significant association in a SNP located in the promoter region of AANAT which remained significant after multiple testing correction. Subjects carrying the AANAT rare allele had almost two-fold probabilities of suffering from MD (OR = 1.84; CI95% = 1.32-2.56; p = 0.0003). Our results support the hypothesis that the circadian clock mechanisms could contribute to the pathophysiology of MD.

1822/F

Genetic Association Between Alzheimer's disease and Neuroglobin, a positional and functional candidate gene. *M.M. Szymanski[1], R.H. Wang[2], M.D. Fallin[3], S.S. Bassett[2], D. Avramopoulos[1,2].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Psychiatry, Johns Hopkins University, Baltimore, MD; 3) Bloomberg School of Public Health, Johns Hopkins University, Baltimore MD.

Neuroglobin (NGB) is a member of the vertebrate globin family involved in cellular oxygen homeostasis, increasing oxygen availability to the brain tissue and providing protection under hypoxic or ischemic conditions, potentially limiting brain damage. It is located on chromosome 14, in a region that we have previously shown significant linkage for late onset Alzheimer's disease (AD) when using the presence of psychotic symptoms as a covariate. In a follow up study of our linkage results we have explored NGB as well as four additional positional candidate genes for association with the risk for developing AD, with or without psychotic symptoms. We found that both psychotic AD and non-psychotic AD patients showed association with different but overlapping NGB single nucleotide polymorphisms (SNPs). We further investigated this association by genotyping a total of 37 SNPs within NGB. We found 2 SNPs showing strong association with AD (p=0.0012 and 0.009). In an unrelated family based sample, the same trend was observed, although not statistically significant, possibly due to the low power of that sample. NGB transcript level analysis in 31 AD brains and 29 control brains showed that NGB is downregulated with increasing age, it is higher in males and it is upregulated in AD patients. However the associated SNPs did not show effects on expression. We conclude that NGB is an interesting functional and positional candidate for AD, however further study is needed to replicate the observed association and point to possible mechanisms of involvement.

1823/F

Identification of novel heterozygous nonsynonymous variations of (*ANG*), *VEGF* and *ALS2* in sporadic ALS (SALS) patients and its implication in the genetic risks of SALS. *Y. Takahashi, J. Goto, S. Tsuji.* Department of Neurology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

[Background] Although the molecular basis of SALS has been mostly unknown, recent molecular genetic researches have indicated that rare variations of disease-related genes such as angiogenin (*ANG*) are associated with genetic risks of SALS. We have conducted the comprehensive analysis of disease-related genes in SALS patients to seek for such variations potentially associated with SALS. [Methods] Genomic DNA samples from 33 sporadic ALS patients in whom mutations of *SOD1* or *DCTN1* were excluded were used in this study, consisting of 18 definite ALS, 9 probable ALS, 3 possible ALS, and 3 ALS-plus patients based on the El Escorial and the revised Airlie House diagnostic criteria. DNA samples from 238 controls were also used. We have screened all the exonic and flanking intronic sequences of *ANG, VEGF* and *ALS2* using a DNA microarray-based resequencing system and direct nucleotide sequence analysis method. The screening of controls was conducted using denatured high-performance liquid chromatography (DHPLC). [Results] In 33 SALS patients, 7 novel heterozygous nonsynonymous variations including 1 variation in *ANG*, 1 in *VEGF* and 5 in *ALS2* were identified. Three of the 7 variations, 1 nonsynonymous variation in *ANG* (N49S) and 2 nonsynonymous variations in *ALS2* (Q435L and P1016T), were not found in 476 control chromosomes. [Conclusion] This study revealed 3 novel nonsynonymous variations in *ANG*) and *ALS2* which were only found in SALS patients. Further large-scale case-control studies or functional studies of these variations are necessary to clarify their relevance to the pathogenesis of SALS. This study suggested that the comprehensive resequencing is a promising approach to identify rare variations potentially associated with the genetic risks of SALS.

1824/F

Gene dysregulation in FXTAS. *F. Tassone[1,2], D. Garcia-Arocena[1], C. Iwahashi[1], R. Hagerman[2,3], E. Berry-Kravis[4], P. Hagerman[1,2].* 1) Department of Biochemistry and Molecular Medicine, UC Davis, CA, USA; 2) M.I.N.D. Institute, UC Davis, Medical Center, Sacramento, CA, USA; 3) Department of Pediatrics, UC Davis Medical Center, Sacramento, CA, USA; 4) Departments of Pediatrics, Neurology, and Biochemistry, RUSH University Medical Center; Chicago, IL,USA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder characterized by action tremor, gait ataxia and other features including autonomic dysfunction,parkinsonism, cognitive decline and peripheral neuropathy. Neuropathological studies of postmortem FXTAS brains have demonstrated significant cerebrum white matter disease, Purkinje cell loss in the cerebellum, and the presence of intranuclear inclusions in neurons and astrocytes throughout brain. Immunocytochemical studies and mass spectrometric analysis have revealed the presence within the inclusions of a number of different proteins, including ubiquitin, lamin A/C, the RNA binding protein hnRNP A2, Hsp70; and two other small heat shock proteins, aB-crystallin and Hsp27. In addition, FMR1 mRNA was found within the inclusion, consistent with the proposed RNA-toxicity model for FXTAS, wherein the expanded RNA itself triggers the pathogenic process. A striking feature of CNS cellular dysfunction in FXTAS, is the disorganization of the lamin A/C nuclear architecture in brain tissue and neural cells from FXTAS cases, in addition to a stress response involving induction of aB crystallin. To determine if the features of the CNS cellular phenotype in FXTAS also extend to transcriptional dysregulation, we studied the pattern of expression of a number of genes, implicated in FXTAS pathogenesis, in FXTAS tissues, including brain and primary fibroblasts, and compared to age matched controls. We identified several genes whose expression is dysregulated in premutation tissues. Indeed, measurements of mRNA levels of LMNA, GLT1 as well as of stress proteins (including aBCry, Hsp27 and Hsp70) indicate significant differences in samples derived FXTAS patients compared to age-matched controls. These observations indicate that abnormal expression of the expanded CGG-repeat FMR1 mRNA leads to gene and cellular dysregulation in FXTAS.

1825/F

Identification and characterization of a new locus responsible for recessive late-onset cerebellar ataxia (LOCA). *I. Thiffault[1,2], M. Tetreault[1,2], J. Allyson[1,2], I. Gosselin[1,2], L. Loiselle[1], J. Mathieu[3], JP. Bouchard[4], J. Lessage[5], B. Brais[1,2,3].* 1) Laboratoire de Neurogenetique et de la Mobilite, Research Centre CHUM-Notre-Dame, Montreal, PQ, Canada; 2) Centre for the study of brain diseases (CEMC), Research Centre CHUM-Notre-Dame, Montreal, QC, Canada; 3) Carrefour de la Sante de Jonquière, Saguenay, QC, Canada; 4) Department of Neurology, CHUL, Quebec City, QC, Canada; 5) Department of Radiology, CHUM-Notre-Dame, Montreal, QC, Canada.

Recessive ataxias are a heterogeneous group of neurodegenerative diseases. Late-onset ataxias have largely been considered as either milder forms of dominant ataxias or sporadic diseases seemingly not caused by genetic factors. Their prevalence is unknown, but together they are poorly recognized cause of decreased mobility in aging. The unique population structure of the aging Quebec population, with its numerous large elderly living sib ships, provides an exceptional setting to explore the recessive bases of late-onset neurodegenerative diseases that interfere with mobility. We have uncovered two French-Canadian regional clusters of recessive cases of late-onset cerebellar ataxia. One cluster of three families originating within a 20km region of the Eastern Townships and one of 5 families from the Saguenay-Lac-Saint-Jean, a region well known for its founder effects. Recruitment to date has led to our present 34 families cohort which includes 58 affected cases and their 125 sibs. The major clinical feature is the onset during the six and seventh decades (mean: 61.3) of gait ataxia. In all cases the ataxia progresses and may lead to the loss of walking. In some cases further evolution leads to pyramidal, extrapyramidal and autonomic manifestations suggesting a progression in a multiple system atrophy cerebellar subtype (MSA-C). All MRI show some degree of cerebellar atrophy. A genome-wide scan uncovered linkage to a region not previously associated with an ataxia locus or any other neurodegenerative disorders (LOD 5.18). Two major haplotypes explained 55% and 22% of the carrier chromosomes suggesting that they are more than two common mutations.

1826/F

Pinpointing candidate genes for non-syndromic core autism by high resolution SNP array based segmental aneuploidy screening. *B. van der Zwaag[1], W.G. Staal[2], M. Poot[3], R. Hochstenbach[3], N. Verbeek[3], H.A. Spierenburg[1], R. van 't Slot[4], M.V. de Jonge[2], M.R. Nelen[3], E. van Daalen[2], H.K. Ploos van Amstel[3], H. van Engeland[2], J.P.H. Burbach[1].* 1) Rudolf Maguns Institute of Neuroscience, Dept. of Pharmacology and Anatomy, UMC Utrecht, Utrecht, Netherlands; 2) Dept. of Child and Adolescent Psychiatry, UMC Utrecht, Utrecht, Netherlands; 3) Dept. of Medical Genetics, UMC Utrecht, Utrecht, Netherlands; 4) Dept. of Biomedical Genetics, UMC Utrecht, Utrecht, Netherlands.

Autism spectrum disorders (ASD) are common neurodevelopmental disorders characterized by impaired reciprocal social interaction, communicative deficits, and restricted and repetitive interests and patterns of behavior. The risk for ASD is highly determined by genetic factors, but only a few genes have been unambiguously linked to the disorder (e.g. MeCP2, FMR1, TSC1/2, and SHANK3), and the pathways involved have remained elusive. Whole genome copy number analysis has shown that inherited and de novo copy number variations (CNVs) contribute significantly to ASD etiology. However, most of these CNVs contain many genes, in part explaining the presence of diverse and often severe clinical features in these patients in addition to ASD. Based on the hypothesis that autism patients with only a limited clinical co-morbidity are likely to carry relatively small pathogenic chromosomal aberrations, we have screened 54 patients with core autism without additional clinical features for segmental aneuploidies on the Infinium HumanHap300 SNP platform. In total we identified 81 regions with copy number changes that did not occur in healthy controls (n=266), ranging in size from 4.3 Kb to 6.21 Mb. There was little to no overlap between individual patients, suggesting significant heterogeneity in ASD. Nineteen of the aberrant regions overlapped with CNVs previously reported in ASD (Vorstman, Mol.Psych. 2006), and 11 contained one or more genes that could contribute to ASD. This has allowed us to pinpoint several candidate genes for ASD, including SHANK3. Selected candidate genes, which have been implicated in synaptogenesis and actin dynamics, were all expressed in the developing murine brain cortex.

1827/F

No association of *SORL1* SNPs with Alzheimer's disease. *R.L. Minster[1], S.T. DeKosky[2], M.I. Kamboh[1].* 1) Department of Human Genetics; 2) Department of Neurology, University of Pittsburgh, Pittsburgh, PA.

A recent study has reported significant association of multiple variants within the SORL1 (sortilin-related receptor, also known as SORLA or LR11) gene and Alzheimer's disease (AD) in several population samples (Nat Genet 2007;39:168-77). However, no individual single-nucleotide polymorphism (SNP) or haplotype was associated with AD risk in all samples, raising the possibility that the associations are spurious. We hypothesized that if these associations are real then they should be replicated in a large and independent case-control cohort. We examined four SNPs in the gene that showed significant associations in the reported discovery and replication North European family and case-control samples: *rs668387* (c.939+163C>T), *rs689021* (c.939+3362G>A), *rs2070045* (c.3561T>G) and *rs3824968* (c.4752T>A). The four SNPs were genotyped in up to 1,023 Caucasian Americans with late-onset Alzheimer's disease (LOAD) and up to 876 age-matched healthy Caucasian Americans. All four variants were genotyped using fluorogenic 5' nuclease assays. We observed no statistically significant association of these SNPs with the risk of AD either individually or in combination (haplotype) or stratified by *APOE*. Although *SORL1* is an excellent biological candidate gene because of its role in the amyloid precursor protein (APP) recycling pathways, our data suggest that the role of *SORL1* variation in relation to AD risk, if any, is modest at best.

1828/F

HapMap-based analysis of the schizophrenia candidate gene *NRG1* in the German population. *T.W. Mühleisen[1], M. Mattheisen[1], S. Herms[1], R. Fürst[1], A. Georgi[3], I. Nenadic[4], R. Abou Jamra[2], J. Schumacher[2], P. Propping[2], T.G. Schulze[3], M. Rietschel[3], M.M. Nöthen[1], S. Cichon[1].* 1) Dept Genomics, Life and Brain Ctr, Univ Bonn, Bonn, Germany; 2) Inst Hum Genet, Univ Bonn, Bonn, Germany; 3) CIMH, Mannheim, Germany; 4) Dept Psychiatry, Univ Jena, Jena, Germany.

Schizophrenia (SCZ) is a genetically complex psychiatric disorder. *Neuregulin 1 (NRG1)* is a strong positional candidate, and several independent studies have recently reported association between SCZ and this locus. In the present study, we systematically tested *NRG1* for association with SCZ in the German population. We selected 348 haplotype tagging SNPs from HapMap covering the entire *NRG1* and flanking sequences and capturing all haplotypes with a frequency > 1% in the European population. The study sample consisted of 2,304 individuals comprising a sample of 210 trios and an independent sample of 820 cases and 854 population-based controls, all of German origin. In the trios, we found a cluster of haplotypes spanning a 40 kbp region associated with SCZ. Interestingly, this region overlaps with the previously reported risk-haplotype HapICE. In the case/control sample, two regions showed association with SCZ. The 20 kbp region, located 120 kbp upstream of HapICE, represents a cluster of associated haplotypes with P=0.00331 as the most significant P value. The 37 kbp region is located in intron 1 of the *GGF2* isoform. In addtition to the analysis of the total sample, we separately analyzed 8 phenotypic subgroups that possibly represent a more homogeneous etiology of SCZ. The greatest effect was seen in the gender-specific groups. In the 'female' group (338 cases, 368 controls), signals concentrated in a 260 kbp region, covering the previously reported risk-haplotypes HapIRE, HapD and parts of HapICE. In contrast, the 'male' subgroup (482 cases, 486 controls), did not show any significant association in that region but in a region farther downstream. Our study provides supportive evidence that *NRG1* is involved in the etiology of SCZ, and that different risk-variants contribute to disease susceptibility in females and males.

1829/F

Dosage changes in alpha-synuclein are rare in familial PD, yet promoter variation is associated with disease. *N. Pankratz[1], W.C. Nichols[2,3], V.E. Elsaesser[2], M.W. Pauciulo[2], D.K. Marek[2], C.A. Halter[1], A. Rudolph[4], T. Foroud[1], Parkinson Study Group - PROGENI Investigators.* 1) Medical & Molec Genetics, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) University of Cincinnati College of Medicine, Cincinnati, OH; 4) University of Rochester, Rochester, NY.

Mutations in five genes result in both autosomal dominant and autosomal recessive forms of Parkinson disease (PD). These mutations, however, are responsible for PD in fewer than 5% of patients with disease. One of these genes, alpha-synuclein (SNCA) has been reported to act as both a causative and a susceptibility gene for PD. Missense mutations, as well as whole gene duplications and triplications, have been found to segregate with disease. Variation in the promoter region has been shown to alter protein expression in vitro, providing biological plausibility that such variation can increase susceptibility. We performed a detailed study of SNCA in a sample of 517 families consisting of 873 individuals meeting strict diagnostic criteria for PD. Using data from a previous genome screen, one affected individual from each of the 92 families showing the greatest evidence of linkage to the region of chromosome 4 near SNCA was screened for dosage alterations in this gene using MLPA; none were found. The full sample was then genotyped for the Rep1 polymorphism in the promoter region of SNCA that was previously reported to be associated with PD susceptibility. Disease models were evaluated using logistic regression employing only one individual per family. Similar to a recent meta-analysis, cases had a 3% higher frequency of the 263 allele compared to controls (OR= 1.44; p=0.04). Unlike the meta-analysis, there was an inverse linear relationship between the number of 263 alleles and age of onset (p=0.04). While this replication is modest, it further strengthens the evidence that the Rep1 polymorphism in the SNCA promoter conveys a moderate increase in the risk for PD.

1830/F

Dopamine Related Genes in Autism. *N. Schnetz-Boutaud[1], B.M. Anderson[1], M.L. Summar[1], J. Bartlett[1], M. Cuccaro[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetic Research, Vanderbilt Univ, Nashville, TN; 2) Miami Institute for Human Genomics, Miami, FL.

Introduction: Autism is a severe neurodevelopmental disorder with a strong genetic component. Despite numerous genome screens and individual candidate gene studies, the underlying genetic etiology remains largely unknown. Increasing evidence suggests that autism is more genetically complex than previously thought, and that single gene approaches toward dissecting autism genetics may not be informative. We are taking the alternative approach of testing for interactive effects of multiple genes within the dopamine pathway. Methods: We tested 60 SNPs within 14 different genes related to dopamine including DRD1, DRD2, DRD3, DRD4, DRD5, DBH, YWHAB, and COMT. SNPs were chosen to represent the linkage disequilibrium patterns across each gene, and included when possible common coding variants. The dataset consists of over 345 multiplex families and 292 parent-child trios collected at two centers in the southeast United States. Initial analyses included single locus family-based association tests, considering both parental and proband gender. Subsequent analyses examined explicitly for gene-gene interactions using multifactor dimensionality reduction (MDR). Results: Single locus analyses generated marginally significant results for YWHAB and DBH. However, these did not survive correction for multiple comparisons. Preliminary two-way interaction analysis with MDR did not identify any significant interactive effects; higher-order interaction analyses are ongoing. Conclusions: As expected, none of the tested genes generated significant results when considered individually. The lack of a strong two-locus interactive effect suggests that either interactions among these genes do not exert a strong effect on autism, or the effect requires a higher order interaction.

1831/F

Association study between 5HTTLPR polymorphisms of the serotonin transporter *(SLC6A4)* gene and Thai patients with autism. *W. Suwannarat[1], N. Ruangdaraganon[2], T. Hansakunachai[3], R. Sothanayongkul[2], T. Somboontham[2], T. Sripo[1], W. Maisrikhaw[1], V. Praphanpoj[4], P. Limprasert[1].* 1) Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand; 2) Department of Pediatrics, Ramathibodi Hospital, Faculty of Medicine, Mahidol University, Bangkok, 10400, Thailand; 3) Department of Pediatrics, Faculty of Medicine, Thammasat University, Pathumthani, 10120, Thailand; 4) Rajanukul Institute, Bangkok, 10400, Thailand.

Autism is a form of pervasive developmental disorder (PDD) manifested by qualitative impairment in social interactions, language and communication, and restricted interest and repetitive behaviors. Although a large genetic contribution is strongly suspected in autism, the specific underlying genetic variants remain unidentified. Hyperserotoninemia has been reported in some autistic patients, and several studies have demonstrated an association between 5HTTLPR polymorphisms in the serotonin transporter gene *(SLC6A4)* and autism, indicating a possible involvement of the serotonin system in the etiology of autism. To explore this situation further, we did a case-control association study between 5HTTLPR polymorphisms and Thai autistic patients. One-hundred-and-twenty patients fulfilling the DSM-IV criteria for autistic disorder (87 individuals) or PDD-NOS (33 individuals) were recruited from two university hospitals in Bangkok. One hundred and fifty-two normal controls were collected from the same ethnic backgrounds. 5HTTLPR polymorphisms were genotyped and named as long (L) or short (S) alleles. Genotypes were compared between patients and normal controls using chi-square statistics. The L/L genotype was more common in patients than in controls (13.3% vs 3.9%, P = 0.0188). When we analyzed either male patients alone (106 individuals) or only patients with autism (87 individuals), the associations were still statistically significant with P = 0.0076 and P = 0.0199, respectively. Our findings support previous reports suggesting an association between the 5HTTLPR polymorphism of *SLC6A4* and patients with autism.

1832/F

Identification and characterization of a new locus responsible for a recessive congenital muscular dystrophy. *M. Tetreault[1], J. Allyson[1], I. Thiffault[1], L. Loisel[1], JP. Bouchard[2], B. Brais[1,3,4].* 1) Laboratoire de neurogenetique et de la mobilite, Center for the Study of Brain Diseases, Centre de recherche du CHUM-Notre-Dame, Montreal, QC, Canada; 2) Service de neurologie, Hopital de l'Enfant-Jesus, Universite Laval, QC, Canada; 3) Clinique des maladies neuromusculaires, Centre de readaptation Marie-Enfant, Hopital Ste-Justine, Montreal, QC, Canada; 4) Clinique des maladies neuromosculaires, Carrefour de Sante de Jonquiere, Saguenay, QC, Canada.

Congenital muscular dystrophies are a heterogeneous group of disorders characterized by hypotonia and muscle weakness and divided in five different groups. We have recruited a cohort of five affected individuals and their non-affected family members. All the affected individuals from five different families from the same little village on the Madeleine Islands in the province of Quebec are known to be related. The small population of Madeleine Island (13 thousands) from an Acadian ancestry make this region of Quebec a suitable population for linkage analysis on recessive diseases. We have sent the five affected individuals and a parent for a SNP Genome Wide Scan (GWS) using the Illumina HumanHap300 chip. The analysis of the GWS results by homozygosity mapping with the hypothesis that all affected individuals shared a common ancestor chromosome uncovered the locus for this new CMD. There is no neuromuscular phenotype described to date on the locus we have identified. The affected individuals share a 0.9Mb candidate interval. With this study, we have shown that we can uncover new recessive disease by using regional cluster such as the Madeleine Island.

1833/F

Further support for involvement of *Reelin* gene variation in working memory performance. *J. Wedenoja[1,3], A. Tuulio-Henriksson[2], T. Paunio[1,2,4], J. Suvisaari[2], A. Loukola[1], J. Ekelund[1,2,4], T. Partonen[2], J. Lönnqvist[2,3,4], H. Stefansson[5], L. Peltonen[1,3,6].* 1) Dept of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Dept of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland; 3) Dept of Medical Genetics, University of Helsinki, Finland; 4) Dept of Psychiatry, Helsinki University Central Hospital, Finland; 5) deCODE genetics, Reykjavik, Iceland; 6) Broad Institute of Harvard and MIT, Cambridge, MA, USA.

Shortage of true positive results in gene identification for mental disorders has increased interest towards quantitative traits, which provide more power in analysis and may thus help in the susceptibility gene search.

We have demonstrated replication of schizophrenia (SZ) linkage to chromosome 7q21-32 in 352 Finnish families (n=1626). A regional *Reelin* (*RELN*) gene on 7q22, encoding glycoprotein involved in neuronal migration regulation during brain development, and contributing to synapse remodelling, crucial for cognitive abilities, showed robust association with an intragenic microsatellite marker in a subsample of 186 neuropsychologically tested families (n=618) to traits measuring visual (p=.003) and verbal (p=.000006) working memory, memory (p=.002), and executive functioning (p=.002). Also animal studies have supported the role of *RELN* variation in cognitive processes.

We utilized an independent Finnish sample of neuropsychologically tested 67 SZ patients and 121 healthy controls, genotyped with Illumina 317K SNP array as part of the SGENE consortium, and analyzed 105 *RELN* intragenic SNPs. Among patients, multiple SNPs associated to verbal attention (p=.006) and working memory (p=.005), learning (p=.001), and memory (p=.006). Among controls, multiple SNPs associated to verbal attention (p=.002), visual attention (p=.00003) and working memory (p=.000003), memory (p=.002), and processing speed (p=.0007). The strongest signals emerged from the high LD region where the previously associated microsatellite is located. Our results provide further evidence for involvement of *RELN* variation in cognitive functions.

1834/F

MicroRNA genes on the X-chromosome of patients with neuropsychiatric disorders. *J. Yan[1], J. Feng[1], K. Noltner[1], W. Li[1], C. Schwartz[2], S. Sommer[1].* 1) Dept Molecular Genetics, City of Hope, Duarte, CA; 2) J.C. Self Research Institute, The Greenwood Genetic Center, SC, USA.

Individual microRNAs (miRNAs) moderately down regulate many genes, typically by two- to four-fold. Disruption of a miRNA binding site in the SLITRK1 gene has been associated with Tourette's syndrome. To explore the role of miRNAs in neuropsychiatric disease, six microRNA genes on the X chromosome (let-7f-2, mir-384, mir-223, mir-224, mir-325 and mir-361) were analyzed in 96 males with schizophrenia, 90 patients with autism (67 males and 23 females) and 70 males with mental retardation and 192 male controls and 190 female controls (716.5 kb of total sequence). A transition in the let-7f-2 mature miRNA sequence was found in a patient with schizophrenia. The variant occurs at a nucleotide conserved through C. elegans. The variant was not found in 10,000 control chromosomes without schizophrenia. mir-384 was deleted in one patient with mental retardation and not in 556 male controls. In addition, single base substitutions were found in mir-224 in a patient with autism and in mir-325 in a patient with psychosis. These variants were not found in 10,000 control X-chromosomes. In summary, three different ultra rare single base substitutions were found on sequence analysis of 256 (272 chromosomes) neuropsychiatric cases and zero ultra rare variants in 572 control chromosomes. In addition, the whole miRNA deletion was found in one male patient with mental retardation and zero of 556 male controls.

1835/F

The role of opioid receptor genes in heroin-induced subjective responses. *D. Zhang[1], L. Jin[1,2].* 1) Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, China; 2) CAS-MPG Partner Institute for Computational Biology, Shanghai, China.

We reported earlier that *OPRM1* was associated with self-reported positive responses on first use of heroin. To further evaluate the role of opioid receptor genes (μ, δ and κ receptor gene: *OPRM1*, *OPRD1*, *OPRK1*, respectively) and their interaction underlying heroin-induced subjective responses, we conducted association analyses of tagging SNPs (tSNPs) in *OPRD1* and *OPRK1* with subjective responses, respectively. Multinomial non-conditional logistic analysis revealed that the genotype CT at rs12404612 (located within *OPRD1*) and the C allele of rs1691808 (in *OPRK1*) were associated with heroin-induced subjective responses, respectively, but the association vanished after adjusting for multiple testing. However, strong interactions were detected between *OPRM1-OPRD1* and between *OPRM1-OPRK1* in subjective responses on first heroin use. The findings suggest that *OPRM1* play the most important role in self-reported positive responses on first use of heroin among three opioid receptor genes.

1836/F

Familial interstitial deletion of Xp11.22 in two brothers with autistic spectrum disorder. *Y. Qiao[1, 2, 6, 7], X. Liu[3, 6, 7], C. Harvard[2, 6], MJ. Hildebrand[1, 6], JJA. Holden[3, 4, 5, 6], E. Rajcan-Separovic[2, 6], MES. Lewis[1, 6].* 1) Dept Medical Genetics, UBC, Vancouver, Canada; 2) Pathology, UBC; 3) Psychiatry, Queen's Univ, Kingston, Canada; 4) Physiology, Queen's Univ, Kingston; 5) Autism Research Program, Kingston; 6) ASD-Canadian American Research Consortium (www.autismresearch.com); 7) The first two authors contributed equally to this work.

Xp11 is an unstable genomic region that contains numerous candidate genes for X-linked mental retardation (MR). Using array CGH (1 Mb whole genome array), we identified an interstitial deletion of Xp11.22 in a male proband with an autism spectrum disorder (ASD). The deletion, confirmed to be maternal in origin, was also detected in an autistic male sibling by real-time (RT) qPCR, but was not detected in an unaffected female sibling. The affected siblings have identical behavioral and physical phenotypes including ASD, moderate MR, normal growth parameters, facial asymmetry, low-set ears with thickened helices, broad and high nasal root, cleft lip, and coarse facies. The brothers have both had normal routine karyotyping and Fragile X findings as well as negative 22q11 and subtelomeric FISH. The unaffected mother has strongly skewed (>90%) X-inactivation, further suggesting this inherited submicroscopic change is causative of the phenotypes seen in the two siblings. RT qPCR characterization of the Xp11.22 del region (53,887,400bp~ 54,359,100bp) shows a 470 kb deletion fully encompassing the PHF8 (OMIM: 300560; midline craniofacial formation and cognitive function) and partially WNK3 (OMIM: 300358; promotes cell survival via procaspase-3 activation) genes; both reported in X-linked MR. PHF8 is also implicated in cleft lip/palate, seen in both brothers. A duplication in this region (~950 kb distal to our deletion), has also been reported in another male with ASD further suggesting that this genomic region is prone to rearrangement and is closely associated with ASD. Further screening of 400 ASD individuals has not identified additional cases carrying this deletion. To the best of our knowledge, this is the first report of an Xp11.22 deletion in ASD and implicates genes PHF8 and WNK3 in the pathogenesis of autistic disorder.

1837/F

Linkage studies in a large German family with restless legs syndrome. *K. Lohmann[1], Y. Lu[1, 2, 3], S. Winkler[1], A. Kleensang[4], T. Lohnau[1], A. Rakovic[1], H. Muhle[2], I.R. König[4], P.L. Kramer[5], U. Stephani[2], A. Ziegler[4], C. Klein[1].* 1) Neurology, University Lübeck, Lübeck, Germany; 2) Neuropediatrics, University of Kiel, Germany; 3) Geriatrics, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; 4) Medical Biometry and Statistics, University of Lübeck, Germany; 5) Neurology, OHSU, Portland, OR, USA.

Restless legs syndrome (RLS) is a common sensory-motor disorder characterized by paresthesias and an intense urge to move the legs. It exhibits a considerable familial aggregation. To date, no gene mutation has been found, although five gene loci have been mapped in primary RLS to chromosomes 12q, 14q, 9p, 2q, and 20p (RLS1-5). We identified a four-generational German RLS family with 37 family members including 15 affected cases. Mode of inheritance follows an autosomal dominant pattern. Disease onset was mainly in childhood or adolescence. We performed a detailed linkage analysis using microsatellite markers. In a first step, we screened the five known loci and excluded linkage to RLS1, 2, 4, and 5. However, we identified a likely new RLS gene locus (RLS3*) on chromosome 9p. A haplotype centromeric to RLS3, flanked by D9S974 and D9S1118, was shared by all twelve investigated patients and generated a maximum LOD score of 3.60 by model-based multipoint linkage analysis. Eleven of these patients carried a common haplotype extending telomeric to D9S2189 that is located within RLS3. Only one unaffected child carried this disease-associated haplotype. In a second step, we carried out a genome-wide scan and found hints for linkage to an 8.2Mb region on chromosome 10 where all but one affected shared a haplotype. Four unaffected relatives, including three children also carried this haplotype. We are currently sequencing candidate genes in the linked regions but have not yet identified any mutation in the first ten tested genes on chromosome 9p. The high frequency and the broad phenotypic spectrum of RLS probably hamper the identification of a causative gene. Our family with a relatively homogeneous phenotype and very early disease onset represents a unique opportunity to further elucidate the genetic causes of the frequent RLS.

1838/F

Genetic Susceptibility in Autism. *S.E. Owens[1], M.L. Summar[2], J.L. Haines[2], M. Aschner[1].* 1) Dept Pediatrics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Ctr for Human Genetics Research, Vanderbilt Univ Medical Ctr, Nashville, TN.

Autism is a common neurodevelopmental disorder with both genetic and environmental components. Genetic susceptibility to mercury (Hg) toxicity has been advanced as a plausible explanation for autism in a subset of children. Potentially, even "safe" Hg levels could be implicated in the etiology of autism due to genetic susceptibility that alters metabolism or intracellular compartmentalization. To identify genetic polymorphisms associated with autism that influence the extent of individual susceptibility to Hg neurotoxicity, we are conducting a thorough search for polymorphisms in four genes (MT1a, DMT1, LAT1 and MTF1) involved in Hg transport and clearance in the general population and in autistic individuals and assessing their frequency and association to the disorder. LAT1 and DMT1 have been invoked in Hg transport and MTF1 and MT1a are inducible by Hg exposure. Using a sample pool of 48 unrelated individuals from both the general and autistic populations we employed Single Strand Conformation Polymorphism analysis to screen all of the exons, 5` and 3` untranslated regions, 1kb upstream of the transcript start, and the exonic bordering regions for genetic variations. We have identified and characterized a number of polymorphisms in MT1a, DMT1, MTF1 and LAT1. Seven of the eleven polymorphisms identified in MTF1 are novel. Two of the seven MT1a polymorphisms identified are nonsynonymous (Thr27Asn, and Lys51Arg). Eight DMT1 polymorphisms identified to date have been previously reported. Thirteen LAT1 variants are currently being characterized. Polymorphisms were evaluated for differences in allele frequencies using Fisher's exact test ($p<0.05$). Preliminary findings failed to show association with autism for any of these variants. Further analysis with the TaqMan genotyping system in a larger dataset of 224 autistic samples and 224 controls is underway. This work was supported by grants T32 ES007028 and NIEHS R01 07331.

1839/F

***PDE11A* Global Haplotype Is Associated With Major Depression.** *H.R. Luo, L. Ribeiro, J. Licinio, M.L. Wong.* Center for Pharmacogenomics, Psychiatry & Behavioral Sci, Univ Miami Miller Sch Med, Miami, FL.

Cyclic nucleotide phosphodiesterases (PDEs) hydrolyze the intracellular second messengers cAMP and cGMP to their corresponding monophosphates and play an important role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. Previously, we showed that one individual haplotype, which includes five SNPs in the *PDE11A* gene, is associated with major depressed disorder (MDD) based on block-by-block analysis. There are three PDE genes, namely *PDE11A*, *PDE1A*, and *PDE6D*, located in chromosome 2q31-q35. In this study, we have further explored whether the whole region 2q31-q35 contribute to MDD, we have analyzed the global haplotype by examining 23 SNPs in the *PDE11A*, *PDE1A* and *PDE6D* genes using PHASE software. Only one haplotype has been found to be associated with MDD. This haplotype is different from the most common haplotype at SNPs in *PDE11A* and *PDE1A*. There is no difference between the depressed and control groups when analyzing the global haplotype including six SNPs in the *PDE1A* gene. When including 16 SNPs across 440kb in the *PDE11A* gene, 18 common haplotypes (with frequency higher than 1.4%) have been found in the studied population. The results from both linkage disequilibrium analysis and phylogenetic network for the 16 SNPs showed that several historic recombinations have happened in the *PDE11A* gene. Combined with the genotype distribution between the two groups, the frequencies of three and seven haplotypes are significantly higher and lower in the depressed group than that of the controls, respectively. The frequency of one haplotype is significantly lower in the remitter than that of nonremitter group for the depressed participants treated with either desipramine or fluoxetine. Thus, our data indicate that the *PDE11A* global haplotype is associated with both MDD and antidepressant treatment response. Further experiment such as deep sequencing the whole *PDE11A* gene in the case-control populations is needed to confirm our findings.

1840/F

The Extension and Replication of Prior Nicotine Dependence Associations in the Iowa Adoption Studies. *R. Philibert[1], T. Gunter[1], P. Madden[2], A. Heath[2], S. Orzack[3], A. Todorov[2].* 1) Dept Psychiatry, Univ Iowa, Iowa City, IA; 2) Dept Psychiatry, Washington University, St. Louis, MO; 3) Fresh Pond, Inc., Cambridge, MA.

The Iowa Adoption Studies (IAS) is the largest longitudinal case and control adoption study of complex behavioral disorders in the United States. Because genetic and environmental effects are independent in this randomized adoption paradigm, the IAS cohorts are an ideal setting in which to delineate the role of genetic and gene-environment interactions in the initiation and maintenance of substance use disorders, especially nicotine dependence. In 2007, a consortium of NIDA investigators announced the results of their genome wide and candidate gene SNP analyses of nicotine dependence. As part of the NIDA Genetics Consortium, we have attempted to confirm and extend all the 150 most significant genome wide and candidate gene SNP findings from their studies using the genetic and clinical resources of the IAS. We report the results of our analyses with respect to the most promising candidate SNPs from their initial reports. We conclude that nicotine dependence results from the interactions of a large number of small effect loci with the exact effect size of each variant being dependent on its epistatic, environmental and gene-environmental interaction profile.

1841/F

Beyond major locus: looking for modifiers in RLS. *I. Pichler[1], F. Marroni[1], C. Beu Volpato[1], S. Pedrotti[1], D. Grazio[1], A. De Grandi[1], C. Klein[2,3], P.P. Pramstaller[1,2,4].* 1) Institute of Genetic Medicine, European Academy, Bolzano, Italy; 2) Department of Neurology, University of Lübeck, Germany; 3) Department of Human Genetics, University of Lübeck, Germany; 4) Department of Neurology, General Regional Hospital, Bolzano, Italy.

Restless legs syndrome (RLS [MIM 102300]) is a common, yet under-diagnosed, and frequently inadequately treated neurological disorder. Estimated prevalence rates vary widely from 2% to 15% of the general population. Epidemiologic and linkage studies demonstrate that genetic factors contribute consistently to RLS. We systematically assessed three population microisolates (n=1,167) in the western Alps of South Tyrol (Italy) for the presence of RLS and a novel locus on chromosome 2q (RLS-4) was identified. Since a co-occurence of RLS and Parkin mutations has been reported recently, 126 RLS patients were tested for Parkin mutations by gene dosage studies. In a total of 10 individuals with RLS a Parkin mutation (heterozygous deletion of exon 7) was detected. It occurred with RLS in six out of 16 patients (37.5%) of one family, which is linked to the chromosome 2q locus. The mutation was absent in a group of 145 healthy controls. Based on recent studies suggesting that heterozygous mutation carrier status in familial Parkinson Disease influences age at onset, the effect of the Parkin-mutation and the haplotype on chromosome 2q on the age at onset was investigated. An association test showed a significant effect for the chromosome 2q haplotype (p=0.009) and the effect of the Parkin-genotype resulted in a p-value of 0.07. A deeper investigation of the role of this mutation in RLS is underway. The discovery of major and modifier genes for RLS will not only provide new insights in the pathophysiology of this disorder, but will presumably also increase our understanding of other movement disorders and how they interact.

1842/F

BRI2 (ITM2B) shows Genetic Association with Late onset Alzheimer's Disease. *F. Zou[1], J. Kim[1], V.M. Miller[1], Y. Levites[1], K.J. West[1], C.W Zwizinski[1], B.D. Moore[1], L. Ma[1], D. Cangemi[1], G.D. Bisceglio[1], S. Younkin[1], V.S. Pankratz[2], R.C. Petersen[3], N. Graff-Radford[4], D. Dickson[1], T. Rosenberry[1], T.E. Golde[1], S.G. Younkin[1].* 1) Dept Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Dept Biostatistics, Mayo Clinic, Rochester, MN; 3) Dept Neurology, Mayo Clinic, Rochester, MN; 4) Dept Neurology, Mayo Clinic, Jacksonville, FL.

The biologic effects of mutations in the BRI2 (ITM2B) and APP genes support the hypothesis that cerebral accumulation of amyloidogenic peptides in familial British and familial Danish dementias and Alzheimer's disease (AD) is associated with neurodegeneration. Our recent findings show that wild type BRI2 has a robust inhibitory effect on Aβ aggregation both in vitro and in transgenic mouse models. To evaluate the pathophysiologic significance of these findings, we analyzed 6 SNPs in the ITM2B gene for association with late onset AD. These 6 SNPs formed 8 haplotypes that showed significant global association (p=0.045) in the large series of American Caucasians that we examined (1693 AD, 1891 Control). In subjects with an age at diagnosis/entry of 60-80 years (1050 AD, 1059 Controls), the significance of haplotypic association improved considerably despite the reduced number of subjects (global p = 0.006). In this age group, there were 14 ITM2B genotypes that occurred at least 10 times, each formed by a single haplotype pair with probability over 99%. The H1/H5 (p=0.039), H2/H2 (p=0.05), H1/H6 (p=0.051), and H2/H5 (p=0.14) genotypes showed significant or suggestive association with risky ORs of 2.1, 1.5, 2.7, and 3.0 respectively. To determine if this set of genotypes is associated with altered expression of the ITM2B gene, ITM2B mRNA was analyzed in the cerebellum of 141 AD brains. Consistent with our results in transgenic mouse models, ITM2B mRNA levels were significantly increased by 32% in the 116 subjects with low risk genotypes as compared to the 25 subjects with high risk genotypes (p=0.02 by two sided Mann Whitney test). These results identify BRI2 as a novel factor that influences risk for AD by modulating Aβ aggregation and deposition.

1843/F

Genomic Characterization of Schizophrenia Candidate Gene Regions. *A.Q. Nato, X. Kong, F. Chen, C. He, C. Chiu, L.M. Brzustowicz, T.C. Matise.* Department of Genetics, Rutgers University, Piscataway, NJ 08854.

Schizophrenia (SZ) affects 1% of the worldwide population and is considered the most devastating mental disorder. Family, twin and adoption studies have revealed that SZ has a complex genetic aetiology. At present, a handful of genetic factors are strongly implicated in SZ but it is likely that many more remain to be identified. In this study we analyzed data from 43 genome-wide scans and 2 meta-analyses for linkage to SZ and partitioned the genome into eleven genomic regions (designated as SCRs) that show either significant evidence of linkage in 1 or more scans or suggestive evidence in at least 4 scans where the peak lod scores lie within 25 cM of each other. Detailed descriptive web-pages for each of the SCRs are provided on our website. We characterize each SCR by identifying the known and predicted genes within these regions and categorize them based on whether they are linked to phenotypes, GO terms, diseases, or pathways, and on whether they are linked or associated with SZ in published linkage scans, meta-analyses, microarray studies, and association studies. Additionally, within each SCR, we identify copy-number variants, segmental duplications, defined regulatory regions, putative miRNA binding sites, rearrangement hotspots, and evolutionarily conserved sequence motifs that are candidate regulatory elements. We provide object-specific web links to existing large databases, thereby facilitating access to relevant subsets of data. We also compare the sequences of our SCRs with each other to identify homologous stretches of DNA that may include important regulatory elements. The total SCR coverage is 236 cM with SCR sizes ranging from 15.88 cM to 29.81 cM. As new genome scans are published, our SCRs are re-evaluated and refined. Our approach provides a novel method to identify and prioritize SZ susceptibility regions and genomic elements that could be applied to other complex diseases.

1844/F

Systematic screening of synaptic X chromosome genes as candidates for autism and schizophrenia. *A. Piton[1], J. Gauthier[1], F. Hamdan[2], Y. Yang[1], D. Spiegelman[1], E. Henrion[1], O. Diallo[1], S. Laurent[1], L. Destroismaisons[1], J. Duguay[1], L. Karemera[1], F. Kuku[1], M. Cote[1], J. Roussel[1], K. Lachapelle[1], P. Drapeau[3], G. Rouleau[1].* 1) Centre for the Study of Brain , CHUM Research Centre Notre-Dame Hospital, Montréal, Québec, Canada; 2) Division of Medical Genetics, Hopital Sainte-Justine, Montreal, QC; 3) Universite de Montreal, Department of Pathology and Cellular Biology, Montreal, QC.

Autism (AUT) and schizophrenia (SCZ) are two common neurodevelopmental disorders, which result from the combination of genetic and environmental factors. Linkage studies on the whole genome and association studies with candidate genes have failed to clearly identify the genes involved in the pathogenesis of these two diseases. We hypothesize that several different rare variants in numerous genes, including de novo variants, could lead to these diseases. Such variants cannot be identified by classical genetic methods. Therefore, we decided to directly sequence, in 288 AUT and SCZ patients, genes coding for proteins involved in the synapse, as defects in synaptic processes can lead to impairment in cognitive function. We decided to focus on the X chromosome, as evidence supports its implication in the predisposition to AUT especially, but also to SCZ. Using various methods and sources, we established a complete list of 183 synaptic and potentially synaptic genes located on this chromosome and we ranked them according to their relevance for the diseases (role in synapse formation, expression in brain tissues or impairment in learning in human or in animal models). We selected in this way 104 X-linked candidate genes that are currently being sequenced in our cohort of 288 patients. We expect to identify more than 200 variants that will be further analyzed genetically. The most interesting ones will be validated functionally using animal models (zebrafish, fruitfly, worm or mice neurons). By the end of this study, we expect to identify and validate several causative variants for AUT or SCZ in different synaptic genes, that will allow a better understanding of mechanisms underlying the development of these two common neurodevelopmental diseases.

1845/F

Evidence for autosomal dominant inheritance of absolute pitch modulated by musical training. *E. Theusch[1,2], B. Levinson[2,3], E.A. Athos[2,3], J. Gitschier[1,2,3].* 1) Program in Biomedical Sciences; 2) Institute for Human Genetics; 3) Departments of Medicine and Pediatrics, University of California San Francisco, San Francisco, CA.

The etiology of absolute pitch, the rare ability to instantaneously recognize and label tones with their musical note names without using a reference note for comparison, has been debated for over a century. Familial aggregation and other data have indicated that absolute pitch has a genetic basis, but environmental factors, such as musical training, are also important for the development of absolute pitch. In order to confirm and expand upon these observations, we examined the collection of data from our web-based absolute pitch study on over 3,000 individuals to assemble twin data, to conduct pedigree analysis, and to investigate the associations of musical training initiation age, gender, and absolute pitch possession. In our study, 3 of 4 monozygotic twin pairs exhibited concordance for absolute pitch possession, while one individual in the remaining monozygotic twin pair had no musical training and therefore could not demonstrate any absolute pitch abilities. In comparison, 7 of 13 dizygotic twin pairs were confirmed or reported concordant for absolute pitch possession, while the remaining 6 were discordant. Our collection of pedigree and other family data was consistent with an autosomal-dominant-with-incomplete-penetrance mode of inheritance, and the majority of the instances where absolute pitch possession skipped generations involved individuals who did not receive musical training before the age of 10. Interestingly, the males in our study started musical training later than females on average, though we observed no significant differences in absolute pitch prevalence between genders. Overall, our data indicate that an individual's genetic makeup and musical training history are both important in determining whether or not the individual possesses absolute pitch.

1846/F

SNP Fine Mapping of Chromosome 8p21 in Schizophrenia. *V.K. Lasseter[1], D. Avramopoulos[1], M.D. Fallin[2], J.A. McGrath[1], P.S. Wolyniec[1], G. Nestadt[1], K.Y. Liang[3], Y. Liu[4], P.-L. Chen[4], D. Valle[4], A.E. Pulver[1].* 1) Department of Psychiatry & Behavioral Sciences, The Johns Hopkins School of Medicine, Baltimore, MD; 2) Department of Epidemiology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Biostatistics, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) Institute of Genetic Medicine, The Johns Hopkins School of Medicine, Baltimore, MD.

Previously, we reported a schizophrenia susceptibility locus (SSL) on 8p22-p21 with a maximum NPL of 3.64 (p=.0001) at D8S1771 (at ~25.5 Mb) in a linkage scan of 54 European Caucasian (EUC) families (Blouin et al. 1998). Further microsatellite genotyping for these families supported a dominant model (LOD=4.10) peaking at D8S1048 (26.9 Mb)(Pulver, unpublished data) with an estimated 61% of families linked to this SSL. Linkage to an 8p SSL has been replicated in independent samples (Straub et al. 2002; Suarez et al. 2006) and in meta-analyses (Lewis et al. 2003). In a collaborative genome-wide SNP-based linkage scan (Levinson et al., 2006) of 737 EUC pedigrees (including the 54 original and 73 new families from Johns Hopkins), an 8p SSL was confirmed with a peak Zmean of 3.22, p=.0004, at rs9797 (26.6 Mb). We conducted SNP studies in a 4.4 Mb region around rs9797 (1536 SNPs) using a subset of 103 Johns Hopkins 8p-linked families. Linkage analyses with 70 pairwise uncorrelated tag SNPs (r2<.05) found the maximum NPL of 6.95 ($p=2.95 \times 10^{-10}$) at rs11994515 (26.9 Mb). Family-based association analyses (FBAT, additive model) revealed nominal p-values <.001 for two SNPs 5' prime upstream of the adrenergic alpha-1 receptor gene, ADRA1A, at 26.8 Mb, in a relative gene desert. Seven other SNPs were associated at a nominal p<.01 level: 6 SNPs in EBF2 (early B-cell factor 2) and one SNP 14k downstream from the 3' end of EPHX2(epoxide hydrolase 2, cytoplasmic). Finally, modest support (p<.05) was seen for SNPs within 13 of the 32 genes in the region, with a concentration of 5 SNPs in DPYSL2, a gene previously indicated in our studies in the Ashkenazim (Fallin et al., 2005). These analyses help prioritize further genetic studies to characterize a chromosome 8p21 SSL.

1847/F

Interaction and Association Analysis of μ-, δ- and κ-opioid Receptor Genes in Substance Dependence Using A Pattern Discovery-based Method. *Z. Li[1,2], H. Zhang[3,4], H.R. Kranzler[7], X. Luo[3,4], J. Gelernter[3,4,5,6].* 1) Department of Computational Genetics, High Throughput Biology, Inc, Livingston, NJ; 2) Center for Computational Biology and Bioinformatics, Columbia University, New York, NY; 3) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 4) Department of Genetics, Yale University School of Medicine, New Haven, CT; 5) Department of Neurobiology, Yale University School of Medicine, New Haven, CT; 6) VA Connecticut Healthcare System, West Haven Campus, CT; 7) Department of Psychiatry, University of Connecticut School of Medicine, Farmington, CT.

Polymorphisms in the μ-, δ- and κ-opioid receptor genes (OPRM1, OPRD1 and OPRK1, respectively) have been reported to be associated with substance dependence (SD). In the present study, we assessed the joint effect of all three receptor genes on alcohol, cocaine, and opioid dependence (AD, CD and OD, respectively) using a pattern discovery-based association test. Genotype data for 13 OPRM1 single nucleotide polymorphisms (SNPs), 11 OPRD1 SNPs and 7 OPRK1 SNPs were obtained from 382 European Americans (EAs) affected with SD (among them, 318 with AD, 171 with CD, and 91 with OD) and 338 EA control subjects. Specific marker and haplotype patterns (consisting of marker alleles of the three receptor genes) were found to be significantly more frequent in cases than in controls. Additionally, both marker- and haplotype-based gene-gene interaction analyses demonstrated an interactive effect of OPRM1 SNPs (located in haplotype block 1) and OPRD1 SNPs on AD and CD, and an interactive effect of OPRM1 SNPs (located in haplotype block 2) and OPRK1 SNPs on AD and OD. Taken together, findings from this study support previous biological findings that the interaction of the three opioid receptors can modulate the action of opioid and non-opioid drugs and alcohol. Future study is needed to investigate the joint effect of the three receptor genes on the individual response to specific pharmacotherapies for SD.

1848/F

Genomic Convergence of Candidate Genes in Late-Onset Alzheimer Disease. *M. Pericak-Vance[1], G. Beecham[1], E. Martin[1], M. Slifer[1], Y.-J. Li[2], J. Gilbert[1], J. Haines[3].* 1) University of Miami, Miller School of Medicine, Miami FL; 2) Duke University, Durham NC; 3) Vanderbilt University, Nashville TN.

Late-onset Alzheimer disease (LOAD) has a strong genetic component. Yet to date only the apolipoprotein E (APOE) gene has been consistently associated with the disease. Linkage studies, which are often replicated, do not have the locational detail required to implicate a single gene. Candidate gene association studies have the ability to associate a single gene with LOAD, but have thus far suffered from a lack of replicability. One solution to this problem is genomic convergence, combining the information from these previous linkage and association studies with information from other genetic studies such as new linkage and association studies and expression data. We have utilized genomic convergence by combining information from previous genetic studies with new data from ~5,400 SNPs in 350 previously tested candidate genes. Association testing was performed on 518 cases and 531 controls, with all cases meeting NINDS-ADRDA criteria for AD and all controls testing cognitively normal on MMSE exams. Standard quality control measures were performed and samples were tested for population substructure. Using Armitage's Trend test, 60/350 (17%) of the genes had at least one SNP with p-values < 0.025 (uncorrected), including APP, BACE2, CTNNA3, and HFE. Interestingly, these genes have positive results in each type of analysis (linkage, association, and SAGE studies). This substantially exceeds the expected percentage of significant results and suggests that a number of these previously identified genes have true (if modest) effects in LOAD.

1849/F

Association and Copy Number Variation analysis of *SHANK3* as a candidate gene for autism. *N.H. Sykes[1], I. Sousa[1], C. Allan[1], A. Jefferson[1], N. Alsamhouri[1], A. Morris[1], A. Pagnamenta[1], J. Lamb[2], A.J. Bailey[3], A.P. Monaco[1], IMGSAC[4].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Centre for Integrated Genomic Medical Research, The University of Manchester, Manchester, UK; 3) University Department of Psychiatry, Park Hospital for Children, Oxford, UK; 4) http://www.well.ox.ac.uk/~maestrin/iat.html.

Autism is a severe neurodevelopmental disorder that usually occurs due to a complex genetic predisposition. It is characterized by impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and activities. *SHANK3* is a scaffolding protein present on 22q13 that is found in excitatory synapses opposite to the pre-synaptic active zone. It is a binding partner of the neuroligin proteins, some of whose genes have been found to contain mutations in a small subset of individuals with autism. A number of recent studies have found *SHANK3* to be disrupted by deletions ranging from hundreds of kilobases to megabases in several individuals with autism. To further analyse this gene's involvement in autism, 12 haplotype tagging SNPs were chosen across *SHANK3* using Tagger in Haploview v4.0beta12 ($r^2 > 0.8$, MAF > 0.05) and data from the HapMap phase II (release 21). These SNPs were then genotyped in 338 affected sibling pairs from the IMGSAC sample. The extent of homozygosity across these SNPs was examined as a potential indicator of hemizygosity. 54 individuals out of the 1603 typed were found to be homozygous across all 12 SNPs, 23 of which were affected probands. 6 FOSMID clones are currently being used as FISH probes across a region of ~ 150kb covering the *SHANK3* gene to look for deletions in these samples. Association analysis was carried out using the Transmission Disequilibrium Test (TDT), but no significant association was found. Further association results and FISH analysis will be presented.

1850/F

Genome-wide linkage analysis in 152 sib-pair families with schizophrenia from Indonesia. *D.B. Wildenauer[1], Irmansyah[2], Herianí[2], M. Knapp[3], S.G. Schwab[4].* 1) CCRN, University of Western Australia, Claremont, WA, Australia; 2) Department of Psychiatry, University of Indonesia, Jakarta; 3) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany; 4) WAIMR, University of Western Australia, Nedlands, WA, Australia.

Schizophrenia has a milder outcome and course in developing countries as compared to developed countries. In addition to social and familial factors, there may be a contribution of genetic factors. We have collected a sample with 152 families with two or three affected siblings and parents with schizophrenia for linkage- and family based association studies in Indonesia. Clinical diagnosis of schizophrenia was made using DSM and ICD criteria. Genomic DNA was isolated from blood cells. A genome-scan was performed by Marshfield Research Organisation, using the panel with 402 Short tandem repeat markers. Linkage analysis was performed with GENEHUNTER and MERLIN using 140 independent sib-pairs. A maximum MLS score of 3.5 (P = 0.00006) was obtained on chromosome 3p24.1. Additional minor linkage peak were detected at chromosome 1q23.1 (P=0.0057), 5q33-34 (P=0.012), and 10q26-qter (P=0.018). The locus at Chromosome 3p is supported by findings of a meta analysis. Loci on 1p, 5q, and 10q are reported by a number of linkage studies and are therefore interesting for follow-up studies . These findings will be the starting point for fine mapping as well as for studies of association/linkage disequilibrium with schizophrenia.

1851/F

Genetic correlation between autistic trait and general intelligence in a proband- based twin sample. *T. Nishiyama[1,2], H. Tanii[3], T. Miyachi[4], K. Ozaki[5], S. Sumi[6].* 1) Dept of Biological Information, Nagoya City Univ, Nagoya, Japan; 2) Master of Public Health Program by Biostatistics, National Institute of Public Health, Wako, Japan; 3) Nagoya Child Welfare Center, Nagoya, Japan; 4) Osaka-Hamamatsu Joint Research Center for Child Mental Development, Hamamatsu, Japan; 5) Japan Science and Technology Agency, Tokyo, Japan; 6) Nagoya Western Rehabilitation Center for Children with Disabilities, Nagoya, Japan.

Background: Mental retardation are the most associated conditions in autistic spectrum disorders (ASDs), although the relation between MR and ASDs remain unresolved. The purpose of this study is to examine genetic and environmental sources of covariation between an autistic trait and intelligence quotient (IQ) in a twin sample.**Methods:**Subjects in the present study ware a cohort of twins born between 1993 and 2001 who were ascertained through at least one proband having an ASD in the catchment area (Sumi 2006). The Childhood Autism Rating Scale (CARS; Schopler 1980) was used to assess a severity of autistic traits among ASDs as a unitary dimension.45 twin pairs with demonstrated diagnostic reliability were subjected to a sex-limited bivariate Cholesky models, incorporating sex differences of each causal influence and correction for ascertainment bias of an autistic trait, by using the Mx software (Neale and Cardon, 1992). **Results:**The model fitting showed significant a genetic correlation between an autistic trait and IQ and the additive genetic contributions to both traits was entirely shared in common. Althogh a significant non-shared environmental correlations between both traits was also found, there was evidence of trait-specific, shared environmental, and non-shared environmental contributions to IQ and trait-specific, non-shared environmental contributions in an autistic trait. The estimated genetic correlations between both traits were -0.96 /-0.95 for boys/ girls, respectively. **Conclusions:**There is substantial overlap between the genetic factors that influence both an autistic trait and IQ to the same extent in both sexes. These findings reinforce the validity of the autistic uni-dimensional view including cognitive abilities.

1852/F

***LRRK2* Screening in a Canadian Parkinson's Disease Cohort.** *L. Racacho[1,2], D.A. Grimes[2,3], F. Han[1,2], M. Panisset[4], D.E. Bulman[1,2,3].* 1) Department of Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, Canada; 2) Centre for Neuromuscular Disease, Ottawa Health Research Institute - University of Ottawa, Ottawa, Canada; 3) Department of Medicine, Division of Neurology, The Ottawa Hospital, Ottawa, Canada; 4) Unité des Troubles du Mouvement André Barbeau, CHUM Hôtel-Dieu, Montréal, Canada.

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene have become the most common known cause for developing Parkinson's disease. The frequency of mutations described in the literature varies widely depending on the population studied with most reports focusing only on screening for the most common p.Gly2019Ser mutation. In this study seven exons (19, 24, 25, 31, 35, 38, and 41) in *LRRK2* where mutations have been reported were screened in 230 unselected Parkinson's disease patients using denaturing high-performance liquid chromatography. The sequencing of samples with heteroduplex profiles revealed five novel and two known intronic sequence variants. In our cohort, we were unable to detect any of the known mutations in these exons or identify novel mutations within *LRRK2.* Therefore, despite the availability of diagnostic *LRRK2* genetic testing it is unlikely to yield a positive result in this population.

1853/F

Comprehensive Copy Number Variant (CNV) analysis of neuronal pathways genes in psychiatric disorders. *E. Saus[1], A. Brunet[1], M. Gratacos[1], J.R. Gonzalez[1], L. Armengol[1], X. Estivill[1,2] on behalf of the Psychiatric Genetics Consortium.* 1) Genes and Disease Program, CeGen and CIBERESP, Center for Genomic Regulation (CRG-UPF), Barcelona, Catalonia, Spain; 2) Pompeu Fabra University, Barcelona, Catalonia, Spain.

A Copy Number Variation (CNV) is defined a DNA segment that is one kilobase or larger and present at variable copy number in comparison with a reference genome. Because CNVs can confer risk to complex disease traits, their study in psychiatric disorders is of great interest. The aim of this work was to perform a comprehensive screening of CNVs in different groups of psychiatric patients. The sample analyzed consisted of 170 patients of each group: affective disorders, eating disorder, anxiety disorders and schizophrenia, as well as 170 control individuals. Based on the Central Nervous System transmitter systems, we selected 364 genes involved in neuronal pathways, including metabolyzing enzymes, receptors, transporters, proteins interacting with the transmitter receptors and proteins involved in its signal transduction. We used the Database of Genomic Variants to identify genes predicted to be in CNVs. We designed four Multiple Ligation Probe Amplification assays to detect variations in their copy number between patients and controls. The results were analyzed with MLPAstats, a new package from R software, developed by our group. Seventy-five genes were included in the analysis. We did not find significant differences between cases and controls when single genes were analyzed. When comparing the total number of gains and losses in psychiatric patients versus control subjects, we found that controls tend to carry a higher number of CNVs. These initial results, although needing replication in larger samples, may indicate the involvement of neuronal pathways genes contained in CNVs in psychiatric disorders.

1854/F

Positive selection within the schizophrenia-associated $GABA_A$ receptor β_2 gene. *W.S. Lo[1], Z. Xu[1], Z. Yu[2], F.W. Pun[1], S.K. Ng[3], J. Chen[1], K.L. Tong[1], C. Zhao[3], X. Xu[3], S.Y. Tsang[1], M. Harano[4], G. Stöber[5], V.L. Nimgaonkar[6], H. xue[1].* 1) Department of Biochemistry, Applied Genomics Laboratory and HKH Bioinformatics Center; 2) Graduate program of Atmospheric, Marine, and Coastal Environment; 3) and Graduate Program of Bioengineering, Hong Kong University of Science and Technology, Hong Kong China; 4) Department of Neuropsychiatry, Kurume University School of Medicine, Fukuka, Japan; 5) Department of Psychiatry and Psychotherapy, University of Würzburg, Würzburg, Germany; 6) Departments of Psychiatry and Human Genetics, University of Pittsburgh School of Medicine, and Graduate School of Public Health, Pittsburgh.

The $GABA_A$ receptor plays a major role in inhibitory neurotransmissions. SNPs and haplotypes in *GABRB2*, the gene for $GABA_A$ receptor β_2 subunit are associated with schizophrenia, and correlated with the expression of two alternatively spliced β_2 isoforms. In this study, using chimpanzee as an ancestral reference, high frequencies were observed for the derived (D) alleles of the four schizophrenia-associated SNPs in *GABRB2*, suggesting the occurrence of positive selection for these derived alleles. Coalescence-based simulation showed that the population frequency spectra and the frequencies of H56, the haplotype having all four D alleles, significantly deviated from neutral-evolution expectation in various demographic models. The variations in DD-genotype frequencies in five human populations suggested that the positive selections of the D alleles are recent and likely ongoing. The divergence between the DD-genotype profiles of schizophrenic and control samples pointed to the schizophrenia-relevance of positive selections, with the schizophrenic samples showing weakened selections compared to the controls. These DD-genotypes were previously found to increase the expression of β_2, especially its long isoform. Electrophysiological analysis showed that this long β_2 isoform favored by the positive selections is more sensitive than the short isoform to the inhibition of the receptor function by energy depletion. These findings represent the first demonstration of positive selection in a schizophrenia-associated gene.

1855/F

Pharmacogenetic markers for cholinergic effects on smoking cessation. *J. Sarginson[1], J. Killen[2], S. Fortmann[2], L. Lazzeroni[1], A. Schatzberg[1], G. Murphy[1].* 1) Psychiatry and Behavioral Scieces, Stanford University School of Medicine, Stanford, CA; 2) Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA.

Breaking the cycle of nicotine addiction is difficult even with treatment. Many patients prescribed pharmacologic treatments for smoking cessation experience side effects that result in treatment discontinuation, or start smoking again soon after completing treatment. We are performing a pharmacogenetic study to identify markers for the efficacy and tolerability of bupropion and transdermal nicotine (TN), two treatments for smoking cessation. We are utilizing clinical data and DNA obtained from two smoking cessation studies. In the first, 276 smokers received bupropion and TN for 8 weeks. In the second, 301 smokers received bupropion plus TN for 11 weeks, followed by 14 weeks of placebo or bupropion. Our genetic analysis focuses on two regions, 15q24 and 8p11.2, which were recently implicated in nicotine dependence in a large scale candidate gene study (Saccone et al., Am J Hum Genet 80:856-66, 2007), and contain a total of 5 nicotinic acetylcholine receptor subunits (alpha-5, alpha-3, beta-4, beta-3, alpha-6) between them. Nicotinic cholinergic receptors are activated directly by TN, and are present on neurons that are affected by bupropion. SNPs were selected for a genetic screen of the two regions if they met one of the following criteria 1) they have demonstrated or predicted functional consequences or 2) they are tagging polymorphisms for haplotypic bins or 3) they have been implicated in the genetics of nicotine addiction based on a literature review. The primary clinical outcome measures for this study are point-prevalence abstinence and time to relapse, but craving, withdrawal symptoms and adverse events due to the study drugs, including changes in weight and mood are also considered. These results will provide a comprehensive analysis of genomic regions linked to smoking that contain a number of excellent candidate genes important in the actions of nicotine and bupropion. (Supported by the NIH and the California Tobacco-Related Disease Research Program).

1856/F

Association and gene-gene interaction of SLC6A4 and ITGB3 in autism. *D.Q. Ma[1], H. Mei[3], E.R. Martin[1], R. Rabionet[2], J. Jaworski[1], I. Konidari[1], H.H. Wright[4], R.K. Abramson[4], J.H. Haines[5], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Univ. of Miami,MIHG,Miami,FL; 2) Univ. Pompeu Fabra,Barcelona,Spain; 3) North Carolina State Univ.,Raleigh, NC; 4) Univ. of South Carolina, Columbia, SC; 5) Vanderbilt Univ.,Nashville, TN.

Autism is a heritable neurodevelopmental disorder with substantial genetic heterogeneity across subphenotypes and subpopulations. One of the most consistent findings, platelet hyperserotonemia, implicates the serotonin pathway. SLC6A4, a serotonin transporter gene; and ITGB3, which encodes for glycoprotein IIIa (GPIIIa) have been implicated. However, the association of SLC6A4 with autism remains inconsistent. Recent studies indicate a sex-specific genetic structure on serotonin levels for both genes and a genetic and expression interaction between them. In addition, a de novo copy number mutation study suggested a different genetic background for sporadic families.This study was conducted in an independent dataset taking into account potential gender and family history as well as gene-gene effect. Family-based association analysis was performed using the functional polymorphisms (5HTTLPR and RS5918) and three additional SNPs within each gene. Gene-gene joint effect was tested by extended multifactor dimensionality reduction (EMDR) and MDR-phenomics using gender of affecteds and family history (Fam+) as covariates. Nominal significant associations were found within SLC6A4 (RS2066713: p=0.0384) and ITGB3 (RS3809865: p=0.0314) with the signal from the Fam+ families, but not from the entire dataset or in families stratified by gender of affecteds [male only (MO) vs.> one female (F1)]. EMDR identified a 2-locus joint effect (RS1042173 and RS3809865) in F1 families and a marginally significant 2-locus joint effect (17P6713SLC6A4 and RS5918) in Fam+ families. MDR-phenomics confirmed these effects while using gender of affecteds (p=0.023) and family-history as covariant (p=0.014). In conclusion, SLC6A4 and ITGB3 might play a role in the etiology of autism independently or interactively. The effect may be modified by the type of families in terms of gender of affected and/or family history.

1857/F

Combined Family Based and Case-Control association studies in four European Populations shows that several neurotrophin genes are involved the susceptibility to eating disorders. *J.M. Mercader[1], E. Saus[1], M. Gratacos[1], R. de CId[1], A. Carreras[1], A. Puig[1], J.R. Gonzalez[1], M. Bayes[1], F. Fernandez-Aranda[2], E. Cellini[3], B. Nacmias[3], J. Hebebrand[4], A. Hinney[4], C. Boni[5], P. Gorwood[5], X. Estivill[1,6].* 1) Genes and Disease Program, CeGen and CIBERESP, Center for Genomic Regulation (CRG-UPF), Barcelona, Barcelona, Catalonia, Spain; 2) Psychiatric Service, Ciutat Sanitaria Bellvitge, L'Hospitalet, Catalonia, Spain; 3) University of Florence, Florence, Italy; 4) University of Duisburg-Essen, Essen, Germany; 5) Hospital Louis Mourier, Paris, France; 6) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Animal models and association studies propose BDNF and its high affinity receptor, NTRK2, as a key regulator of eating behaviour. To study the involvement of other neurotrophins as susceptibility factors for eating disorders, we have performed a family based and population based association study for Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophic Tyrosine Kinase Receptor type 1 (NTRK1), Neurotrophic Growth Factor Receptor (NGFR), Neurotrophin 5 (NT4/5), Tyrosine Kinase Receptor type 3 (NTRK2), Neurotrophin 3 (NTF3), Neurotrophic Tyrosine Kinase Receptor type 3 (NTRK3), Ciliary Neurotrophic Factor (CNTF), and Ciliary Neurotrophic Factor Receptor (CNTFR). The clinical samples included 420 trios, 408 index cases and 385 controls from four European countries: Spain, Italy, France and Germany. Tag SNPs were selected from the CEU HapMap project dataset, and all SNPs were genotyped using SNPlex technology. We performed Family Based Association Studies using the FBAT software and SNPassoc software to analyze the effect of SNPs on minimum body mass index and to perform the case-control studies. When taking eating disorders as a whole group, 14 nominal associated SNPs were found, in NGF, NTF3, NTRK3, P75, CNTFR and NTRK2. Interestingly, there were 6 nominal associated SNPs in NTRK3, one of which was significantly associated after Bonferroni Correction (p = 9.6 E-5). These results suggest the involvement of other neurotrophin genes apart from BDNF and NTRK2 in the susceptibility of eating disorders.

1858/F

Clock Genes may Influence Bipolar Disorder Susceptibility and Dysfunctional Circadian Rhythm. *J. Shi[1], J.K. Wittke-Thompson[1], J.A. Badner[1], E. Hattori[2], E.S. Gershon[1], C. IIU[1].* 1) Dept Psychiatry, Univ Chicago, Chicago, IL; 2) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute (BSI), Wako, Saitama 351-0198, Japan.

Several previous studies suggest that dysfunction of circadian rhythms may increase liability to bipolar disorder (BP). We conducted a two-phase association study in BP families at 15 circadian genes, including ARNTL, ARNTL2, BHLHB2, BHLHB3, CLOCK, CRY1-2, CSNK1D, CSNK1E, DBP, NR1D1, PER1-3,and TIMELESS. The Sibling-Transmission Disequilibrium Test (sib-tdt) analysis showed nominally significant association of BP with 3 SNPs within or near the CLOCK gene (rs534654, p = 0.0097; rs6850524, p = 0.012; rs4340844, p = 0.015). These 3 SNPs and rs2279665 at TIMELESS, also showed nominally significant association with several circadian phenotypes identified in BP patients, including early insomnia, middle insomnia, late insomnia, insomnia with mania, and rapid cycling. Several haplotypes in the CLOCK gene region were nominally associated with both disease and BP with late insomnia ($p < 0.05$). However, none of these associations reached gene-wide or experiment-wide significance after correction for multiple-testing. We detected a significant multi-locus interaction between rs6442925 in the 5' upstream of BHLHB2, rs1534891 in CSNK1E, and rs534654 near 3' of CLOCK gene in the samples in the second phase of the study (p = 0.00000172), which remained significant after correcting for multiple tests using the false discovery rate method. Our results suggest an interaction between three circadian genes in susceptibility to bipolar disorder and weak effects of several circadian genes on dysfunctional rhythms in patients with bipolar.

1859/W

Analysis of five late-onset Alzheimer's disease (LOAD) candidate genes. *M. Allen[1], N. Ertekin-Taner[1,2], C. Cox[1], F. Zou[1], S. Younkin[1], M. Carrasquillo[1], L. Younkin[1], D. Dickson[1], N. Graff-Radford[2], R. Petersen[3], S.G. Younkin[1].* 1) Department of Neuroscience, Mayo Clinic Jacksonville, Jacksonville, FL; 2) Department of Neurology, Mayo Clinic Jacksonville, Jacksonville, FL; 3) Department of Neurology, Mayo Clinic, Rochester, MN.

We present follow up studies for five previously reported LOAD candidate genes. Glutathione-S-Transferase 1 and 2 (GSTO1 and GSTO2) are located on chromosome 10 which has been repeatedly demonstrated to show linkage with LOAD and its intermediate phenotypes. Others have shown significant association between GSTO variants and age at onset of AD, as well as differential expression levels, implicating these genes in AD etiology. We genotyped four variants within GSTO1 and 2, two of which were previously reported. Three of the four SNPS analyzed in our combined case control data set (n>3700) revealed suggestive association (p=0.045-0.21) with LOAD. These four SNPs form five common haplotypes and nine multilocus genotypes (MLG). All of the haplotypes and four of the MLGs show suggestive association in the combined dataset that is strongest in subjects with an age at diagnosis above 80 (p=0.05-0.23). Glyceraldehyde-3-phosphate dehydrogenase (GAPD) and related genes GAPDS and pGAPD (GAPD pseudogene) have been implicated in LOAD. We genotyped three previously reported SNPs in these genes. Others reported MLGs with replicable significant association in three LOAD series. Though we were unable to replicate the MLG result, analysis of the independent variants supported a role for GAPD in disease etiology (rs1136666, OR 0.867, p=0.009) for our combined case control series (n>3700). Expression studies and analysis of additional variants in these genes are underway.

1860/W

Transformation Efficiency of B Cells by Epstein Bar Virus in the NINDS Human Genetics DNA and Cell Line Repository. *J.L. Andrews*[1], C.M. Ziccardi*[1], M.A. Keller[1], C.M. Beiswanger[1], K.A. Gwinn[2], R.A. Corriveau[1].* 1) Coriell Institute for Medical Research, Camden, NJ 08103; 2) NINDS-NIH NINDS/NIH Bethesda, MD 20817.

The NINDS Human Genetics DNA and Cell Line Repository, established in 2002, has banked over 16,000 unique samples from subjects representing cerebrovascular disease, epilepsy, motor neuron disease, Parkinsonism, and controls. The goal of the Repository is to accelerate research in the genetics of nervous system disorders. An important part of this endeavor is to use Epstein-Barr virus (EBV) to create cell lines by transforming B cells from peripheral blood. These cell lines serve as a renewable resource of genomic DNA from banked samples. Here we evaluate the efficiency of transformation of B cells from peripheral blood by EBV. Variables include the age of subject, volume of blood submitted, time between blood collection and processing, and neurological disorder. Preliminary analysis shows that blood with volumes less than 4 ml and in transit more than eight days have a decreased incidence of transformation. Additionally, samples submitted from younger subjects are more likely to transform successfully than from an older population, with the exception of the very oldest subjects (over 90 years old) that display transformation efficiencies comparable to subjects aged 46 to 50 years (~94%). Lastly, samples from subjects with the neurological diseases banked by the NINDS Repository do not appear to differ in their transformation efficiency as compared to control subjects. Further studies will consider what factors may contribute to the overall decrease in transformation efficiency associated with increased age of the donor.

1861/W

Brain MRI voxel based morphometry in 1q23 linked familial schizophrenia. *A. Bassett[1], A. Ho[1], N. Costain[1], A.P. Crawley[2], D.J. Mikulis[2], E.W.C. Chow[1].* 1) Dept Psychiatry, Univ Toronto, CAMH, QS, Toronto, ON, Canada; 2) Dept Medical Imaging, TWH, Univ Toronto, Toronto, ON, Canada.

Familial schizophrenia (FS) is a hereditary form of schizophrenia involving families with several individuals affected with the illness and where the illness appears to be transmitted. Region-of-interest (ROI) and voxel based morphometry (VBM) studies on schizophrenia have reported decreased grey matter in the anterior cingulate gyrus. This study examined VBM findings from MRI brain scans of families with FS previously shown to be linked to 1q23 and the NOS1AP gene. We scanned 12 subjects with FS, 18 unaffected siblings (UA1) and 7 second degree relatives (UA2) from 6 families from one site and 7 subjects with FS and 7 UA1 subjects from 4 families from a second site. An ANCOVA on grey matter covaried with age, IQ, gender and intracranial volume revealed differences in the 3 groups from the first site in the left post-central gyrus, right anterior cingulate gyrus, right superior temporal gyrus, and the cerebellum. Post-hoc t-tests between FS patients and UA1 subjects showed no significant difference in voxel clusters. Results from T-tests with mapwise correction show that UA1 subjects have significantly decreased volume at the right anterior cingulate (p = 0.001), the left postcentral (p = 0.022) and the left superior temporal (p =0.048) gyri compared to UA2 subjects. In addition, FS patients showed decreased volume after mapwise correction at the right anterior cingulate gyrus (p = 0.001), the right angular gyrus (p = 0.008), and the cerebellum (p = 0.001) compared to UA2 subjects. Similar findings were found in subjects from the other site. Results suggest that UA1 subjects may be more similar neuroanatomically to FS patients than to UA2 subjects in these 1q23-linked FS families. Moreover, the right anterior cingulate gyrus may represent an endophenotype for 1q23-linked FS. These findings need to be replicated in other forms of FS.

1862/W

Identification of genes regulated by the Extracellular Signal-Regulated Kinase (ERK1/2) in primary mouse astrocytes. *L.S. Correa-Cerro[1], D. Heffron[1], Y. Zhang[2], G. Vande-Woude[2], J.W. Mandell[1].* 1) Dept of Pathology, Univ Virginia, Charlottesville, VA; 2) Department of Molecular Oncology, Van Andel Research Institute, Grand Rapids, MI.

Astrogliosis is defined by cellular hypertrophy and process extension, increased glial filament production, and some degree of proliferation. Astrogliosis has been identified in Parkinson's and Alzheimer's diseases as well as in several others human brain injuries. Increasing evidence points to neuroprotective roles of astrogliosis in the setting of brain injury. We used Affymetrix Mouse 430 2.0 Arrays to test the hypothesis that FGF2 acting via the MEK-ERK intracellular signaling pathway, leads to pathway-specific gene expression changes in astrocytes which promote the morphological plasticity of reactive astrocytes. Astrocyte cultures were prepared from 1-day-old mouse brains and treated with human recombinant FGF2 and the specific MEK inhibitor U0126 or DMSO (vehicle control) for 24h. Array data identified 89 up-regulated and 179 down-regulated genes (>2-fold change). Six of the upregulated genes were confirmed by real time PCR: Tgfbi, Rasgrf1, Rgs16, Esm1, Errfi1, and Spp1. Eight known negative feedback signaling genes were induced by FGF2: Rgs16, Dusp6, Dusp4, Spry4, Spred1, Spred2, Spred3, and Errfi1. Errfi1 (ERBB receptor feedback inhibitor 1), gene encodes a cytoplasmic protein known to be upregulated with cell growth and cell stress. To test the role of ERRFI1 in regulation of astrogliosis we performed histological and immunohistochemical studies of brain tissue from adult Errf1Δ/Δ, and control wild types mice. We found no evidence of brain malformation in four Errf1Δ/Δ mice studied. Phosphorylated ERK levels were not qualitatively changed as determined by immunohistochemistry. Astrogliosis markers, including GFAP, Vimentin, and PCNA did not differ in knockout mice compared to controls. Although baseline astroglial activation appears unaffected by loss of Errfi1 the possibility that injury or stress-induced astroglial activation will be exaggerated in Errf1Δ/Δ mice remains to be tested.

1863/W

Glucocerebrosidase gene mutations are associated with Parkinson's disease in a population from Southern Italy. *E.V. De Marco[1], G. Annesi[1], P. Tarantino[1], F.E. Rocca[1], G. Provenzano[1], D. Civitelli[1], I.C. Cirò Candiano[1], F. Annesi[1], S. Carrideo[1], F. Condino[1], G. Nicoletti[1,2], D. Messina[1], F. Novellino[2], M. Morelli[2], A. Quattrone[1,2].* 1) Institute of Neurological Sciences, National Research Council, Mangone Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Recent studies have reported clinical, neuropathological and genetic associations between Parkinson's disease (PD) and Gaucher's disease (GD). Screenings for GBA mutations in PD subjects belonging to different populations have suggested that heterozygosity may be a susceptibility factor predisposing to PD. Until now, no data exist in the Italian population. We screened 395 PD patients coming from Calabria for the N370S and the L444P mutations. A control group consisting of 483 subjects coming from the same geographical area of the patients was used to determine the mutation frequency in the general population. Genotyping was performed by using PCR amplification followed by restriction enzyme digestion with XhoI for the N370S substitution and NciI for the L444P substitution. Mutation frequencies in cases and controls were compared using Fisher's exact test. We found 11 patients (2.7%) carrying a heterozygous mutant GBA allele: three of them had the N370S mutation and eight had the L444P mutation. In the control group 1 subject (0.2%) carried a heterozygous L444P mutation. These distributions were significantly different between patients and controls (p=0.0018). Carriers of a GBA mutations had an increased risk of developing PD (OR = 13.6; 95% CI, 1.8 to 105.8; p = 0.001). Clinical characteristics were similar in patients with or without GBA mutations. The present study demonstrates a significant association of some GBA mutations with PD in a sample from Southern Italy. Differing from previous reports, the most common mutation detected in our study was the L444P (eight patients and one control), while the N370S substitution (three PD patients) was never found in 483 control subjects. Our results support evidence that altered GBA may represent a risk factor for developing PD in our population.

1864/W

MRI brain differences in children with 22q11.2 deletion syndrome and siblings. *A. Ho[1], A.P. Crawley[3,4], D.J. Mikulis[3,4], A.S. Bassett[1,2], E.W.C. Chow[1,2].* 1) Clinical Genetics Research Program, Center for Addiction and Mental Health, Toronto, Ontario, Canada; 2) University of Toronto, Department of Psychiatry; 3) University of Toronto, Department of Medical Imaging; 4) Toronto Western Hospital, Department of Medical Imaging.

22q11.2 deletion syndrome (22qDS) is a genetic syndrome associated with a microdeletion at the 22q11.2 region. Individuals with 22qDS are associated with many physical and cognitive features and are at an increased risk for the development of psychiatric disorders. Past imaging studies on children with 22qDS have reported reduction of overall brain volume, the superior temporal gyrus, the hippocampus, and the cerebellum. This study obtained MRI brain scans from 13 children (5 males, 8 females) with 22qDS and 4 siblings (2 males, 2 females) between the ages of 8 - 12 using voxel based morphometry (VBM). A t-test revealed a significant difference in IQ between the 22qDS children and their siblings (mean = 65.6, S.D. = 13.5; mean = 104.3, S.D = 14.9 respectively, p = 0.001), No significant difference for total intracranial volume (ICV) was found between the two groups (22qDS group mean = 1449 mL, S.D. = 141 mL; Sibling group mean = 1501 mL, S.D. = 164 mL). Results from a t-test on grey matter with mapwise correction and ICV as a covariate revealed significant volume reduction in the cerebellum (p = 0.001), and the cingulate gyrus (p = 0.004). In 22qDS, however, results were not insignificant when IQ was included as a covariate. Findings on the cerebellum from this study are consistent with past 22qDS literature that suggest reduced cerebellar volumes may be a core feature of 22qDS. However, the results were possibly attenuated by a small sample size, and the inclusion of IQ as a covariate. Replication with a larger sample may help to further reinforce the findings in this study.

1865/W

Prevalence of autism spectrum disorders in patients with Möbius sequence. *E. Kluczinik[1], C.A. Kim[3], C.H. Gonzalez[2], J.A. Paz[2], M.J. Marques-Dias[2].* 1) Psychiatry, Hospital das Clínicas da FMUSP, São Paulo, SP, Brazil; 2) Neurology, Instituto da Criança, São Paulo, SP, Brazil; 3) Genetics Unit, Instituto da Criança, São Paulo, SP, Brazil.

Introduction: Autism spectrum disorders (ASD) are neurodevelopment disorders without an established single etiology but with important contributions from genetics, neuropsychiatry, functional and neuroimage investigations. Möbius sequence (MS) is a rare congenital disorder characterized by complete or partial facial and abducens nerve palsy (hypomimia and strabismus). Other cranial nerves involvement, orofacial malformations and limb defects are frequently associated. MS may have a genetic or environmental origin and, in Brazil, it has been related to misoprostol use in abortion attempts. Even if the exact time of developmental insult for each of these conditions cannot be identified, there are evidences suggesting that it may occur as early as 4 to 6 weeks of embryogenesis. Many studies suggest that association of cranial nerve palsies, a variety of limb and systemic malformations, both with genetic and environmental origin, may be associated with autism spectrum disorders, statistically more frequent than in normal population. Here we studied a cohort of MS patients to detect ASD. Methods: 36 patients (19 girls and 17 boys), 52.7% with misoprostol exposition in uterus, preliminarily diagnosed with MS in a multidisciplinary basis have been prospectively submitted to a psychiatric evaluation utilizing CARS and Vineland adaptative behavior scale. Results: eleven patients (30.5%) have been diagnosed as having ASD, five of them with a history of misoprostol exposition. Conclusions: The high prevalence of ASD in this series is similar to that detected in a Swedish study of thalidomide exposed patients and in another Brazilian study of the effects of the use of misoprostol in pregnancy. These findings suggest that early insults in embryogenesis could be associated with ASD.

1866/W

Glia specific changes associated with motor neuron vulnerability in ALS and FTD: DNA and tissue microarray study. *L.C. Kudo[1], J. Pomakian[2], L.V. Parfenova[1], H. Vinters[2], M. Wiedau-Pazos[1], S.L. Karsten[1,3].* 1) Dept Neurology; 2) Dept. of Pathology and Lab. Med., UCLA, Los Angeles, CA 90095; 3) Los Angeles Biomedical Institute, Harbor-UCLA Medical Center, Torrance 90509.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that selectively affects motor neurons in the central nervous system. Recently, it was shown that glia is one of the key factors contributing to specific motor neuron vulnerability. To complement our study performed on motor neurons (Kudo et al, ASHG 2006) and to identify specific glial factors that may contribute to motor neuron degeneration we examined glial expression profiles in two mouse models of motor neuron degeneration: familial ALS linked to SOD1-G93A and frontotemporal dementia with ALS linked to TAU-P301L. Dissected frozen lumbar spinal cords from 3 months old female transgenic mice and their non-transgenic littermates were axially cryosectioned. Glia surrounding motor neurons from the anterior horn of the spinal cord was laser-capture microdissected according to previously established protocol (Kudo et al, 2006). RNA extracted from these cells was used for microarray experiments using Aglilent's Mouse Whole Genome Oligonucleotide Microarray. Identified gene expression changes indicated that SOD1-and TAU- induced neurodegeneration might have partially common mechanism. We further investigated the relevance of our findings in mouse models to human disease by performing protein expression analysis on post-mortem ALS samples using tissue microarray technology (TMA). We constructed ALS TMA with CNS tissues from ALS patients, ALS with frontotemoporal dementia (FTD) patients, and age and sex matched controls. Each TMA block includes ALS, ALS with FTD, and control samples of the most affected regions, such as the cervical and lumbar spinal cord, and less vulnerable regions, such as the cortical and subcortical areas of the brain. Of the 8 motor neuron specific genes altered in both SOD1 and TAU mouse models, 5 had commercially available antibodies and were tested on our TMA. MWP and SLK co-directed this project.

1867/W

Ion channel genes associated with migraine with aura. *K.S. LaForge[1,2], D.R. Nyholt[3], M. Kallela[1,2], P. Tikka-Kleemola[1,2], M.A. Kaunisto[1,2], P. Lahermo[1], K.R.J. Vanmolkot[4], G.M. Terwindt[4], S. Purcell[5], M.J. Daly[5], C. Kubisch[6], M. Dichgans[7], D.R. Cox[8], J. Kaprio[2], A.M.J.M. van den Maagdenberg[4], L. Peltonen[2,5], M. Wessman[1,2], A. Palotie[1,2,5].* 1) Finnish Genome Center, Univ. of Helsinki, Helsinki, Finland; 2) Research Program for Molecular Medicine, Univ. of Helsinki, Helsinki, Finland; 3) Genetic Epidemiology Laboratory, Queensland Institute of Medical Research Brisbane, QLD, Australia; 4) Dept. of Human Genetics, Leiden Univ. Medical Centre, Leiden, the Netherlands; 5) The Broad Institute of MIT and Harvard, Cambridge, MA; 6) Institute of Human Genetics, University of Cologne, Cologne, Germany; 7) Dept. of Neurology, Klinikum Grosshadern, Munich, Germany; 8) Perlegen Sciences Inc., Mountain View, CA.

Twin studies have firmly established the heritability of common forms of migraine. Mutations in three ion-transporting genes cause a rare Mendelian form, familial hemiplegic migraine, yet identification of genes involved in non-Mendelian migraine has proved elusive. We hypothesize that common variants of ion channel genes may also underlie common forms of migraine with aura (MA). We performed a saturation candidate gene study of 155 ion channel genes in a Finnish migraine study sample. Tagging SNPs with MAF≥0.10 and LD of r^2≥0.80 were selected for each gene. Allelic association tests were performed for 5269 markers in 841 cases and 884 controls. Twenty-four SNPs distributed in 15 genes had (uncorrected) significance values of p<0.005. We selected SNPs in these genes for further validation in two population-based cohorts and two clinic-based cohorts. In a population-based Australian sample of 1127 migraine cases and 1129 controls, evidence for allelic association was detected with the intracellular chloride channel gene *CLIC5*. A second population sample of 800 cases and 946 controls from the Netherlands did not yield any significant allelic associations when all cases were analyzed; however, when MA cases (n=258) were compared to controls, we found suggestive evidence for association with the potassium channel gene *KCNB2*. These findings are currently being followed up in clinic-based cohorts from Cologne and Munich.

1868/W

Runs of homozygosity reveal highly penetrant recessive loci in schizophrenia. *T. Lencz[1,2,3], C. Lambert[4], P. DeRosse[1], K.E. Burdick[1,2,3], T.V. Morgan[5], J.M. Kane[1,2,3], R. Kucherlapati[5,6], A.K. Malhotra[1,2,3].* 1) Dept. of Psychiatry Research, Zucker Hillside Hosp, Glen Oaks, NY; 2) Dept of Psychiatry, Albert Einstein College of Medicine, Bronx, NY; 3) Feinstein Institute for Medical Research, Manhasset, NY; 4) Golden Helix, Inc., Bozeman, MT; 5) Harvard Partners Center for Genetics and Genomics, Cambridge, MA; 6) Dept. of Genetics, Harvard Medical School, Boston, MA.

Evolutionarily significant selective sweeps may result in long stretches of homozygous polymorphisms in individuals from outbred populations. We developed whole genome homozygosity association (WGHA) methodology to exploit this phenomenon and identify genetic risk loci for schizophrenia (SCZ). Applying WGHA to 178 SCZ cases and 144 healthy controls genotyped at 500,000 markers, we found that runs of homozygosity (ROHs), ranging in size from 200kb to 15MB, were common in unrelated Caucasians. ROHs did not appear to reflect regions of copy number variation, segmental duplication, or low recombination. ROHs appear to reflect regions undergoing positive selection; frequency of each ROH in healthy subjects was significantly correlated with other measures of positive selection, such as iHS ($P<5*10^{-10}$) and Tajima's D ($P<5*10^{-8}$).

ROHs were significantly more common in SCZ, and a set of nine ROHs significantly differentiated cases from controls. Each of these 9 "risk ROHs" included genes relevant to post-synaptic structure and/or neuronal survival, and four contained or neighbored genes previously associated with SCZ. Using logistic regression, total number of risk ROHs significantly predicted group status (χ^2=62.6, df=1, $P=2.5*10^{-15}$; permuted P=0.00095), with each additional risk ROH imparting an odds ratio of 2.83 (95%CI=2.10-3.81). Results suggest that recessive effects of relatively high penetrance at CNS-relevant loci may explain a proportion of the genetic liability for SCZ.

1869/W

The MMP-2 gene contributes to functional outcome after stroke but not to stroke susceptibility. *H. Manso[1,2], T. Krug[1], B. Nunes[2], I. Albergaria[2], G. Gaspar[2], L. Gouveia[3], I. Matos[4], M.V. Baptista[5], G. Lopes[6], R. Taipa[6], J.P. Gabriel[7], M.R. Silva[8], C. Dias[2], F. Gonçalves[9], M. Correia[6], J.M. Ferro[3], S. Oliveira[1], A.M. Vicente[1,2].* 1) Instituto Gulbenkian de Ciência, Portugal; 2) Instituto Nacional Saúde Dr. Ricardo Jorge, Portugal; 3) H. Sta. Maria, Portugal; 4) H. Distrital Mirandela; 5) H. Garcia de Orta; 6) H. Geral Sto. António, Portugal; 7) H. S. Pedro; 8) H. Fernando Fonseca; 9) H. Universidade Coimbra, Portugal.

Given the increased life expectancy of populations, finding ways of preventing stroke and adequate treatment is a priority, requiring the characterization of risk factors for disease and functional outcome. In this study we analyzed the role of two specific matrix metalloproteinase (MMP) genes, MMP-2 and MMP-9, in stroke susceptibility and recovery. MMPs are zinc-endopeptidases that contribute to brain damage in stroke, promoting edema and hemorrhage and triggering cell death. Recent studies in rats, however, suggest that MMPs may mediate repair in later stages after stroke, through their role as regulators of neurogenesis, axon regeneration and processing of biologically active growth factors. 11 tag SNPs in the MMP-2 gene and 3 tag SNPs in the MMP-9 gene were tested in a population sample of 533 stroke patients and 507 controls in the same age range. No association with stroke risk was found for single markers or haplotypes at either gene, indicating that these do not significantly contribute to disease susceptibility. We further analyzed the role of these genes in functional outcome after stroke in 403 patients under 65, assessed three months after a stroke episode using the modified Rankin Scale (mRS). Using the Kruskal-Wallis non-parametric test, we found a significant association of one MMP-2 SNP with mRS scores (rs2241145: χ^2=6.729, df= 2, P=0.035), with several specific haplotypes including this SNP associated (0.0032<P<0.0046) with favorable (mRS≤1) or bad (mRS≥2) recovery. None of the markers at MMP-9 were associated with stroke susceptibility or recovery. The results suggest that genetic variation in MMP-2 may be an important mediator of functional outcome after stroke.

1870/W

Deletions in GCH1 are a frequent cause of Dopa-responsive dystonia. *U. Müller[1], D. Steinberger[2], C. Troidl[1], K. Brockmann[3], M. von der Hagen[4], C. Feiner[5], J. Henke[6], B. Zirn[1].* 1) Institut für Humangenetik, Justus-Liebig-Universität, Giessen, Germany; 2) Bioscientia, Ingelheim, Germany; 3) Pädiatrie II, Universität Göttingen, Germany; 4) Neuropädiatrie, Universität Dresden, Germany; 5) Neurologische Praxis, Tuttlingen, Germany; 6) Institut für Blutgruppenforschung, Köln, Germany.

Molecular diagnosis of Dopa-responisve dystonia (DRD) is usually done by sequencing the six exons of the gene GCH1. This method does not detect heterozygous deletions which have been reported in DRD. We therefore assessed the frequency of deletions and point mutations in GCH1 in a large cohort of patients with Dopa-responsive dystonia (DRD). A total of 136 dystonia patients were studied. These were divided into two groups according to clinical criteria. Group 1 included dystonia patients with a dramatic therapeutic response to L-Dopa plus/or circadian fluctuation of symptoms (definite or probable DRD). Group 2 included those dystonia patients in whom clinical data were incomplete and L-Dopa response was not striking or not tested (possible DRD). 57 patients were assigned to group 1 and 79 to group 2. We found a GCH1 point mutation in 27 patients of group 1 (47.4%) and in 4 of group 2 (5.1%). Of these, nine single and one double mutation have not been recognized before. GCH1 deletions were detected by qPCR in four patients of group 1 (7.0%) and in one patient of group 2 (1.3%). The entire GCH1 gene (exons 1 to 6) was deleted in four patients, and a partial deletion (exons 3 to 6) was found in one. Three of the four complete GCH1 deletions were familial and one had occurred de novo. The partial deletion was familial. The high frequency of deletions of GCH1 demonstrates that deletion analysis should be included in the routine molecular diagnosis of DRD.

1871/W

A multistage genome-wide association study with follow-up study provides strong evidence for 4 susceptibility loci in schizophrenia. *M.C. O'Donovan[1], N. Craddock[1], G. Kirov[1], I. Nikolov[1], N. Norton[1], H. Williams[1], T. Peirce[1], V. Moskvina[1], L. Carroll[1], L. Georgieva[1], M. Hamshere[1], P. Holmans[1], N. Williams[1], I. Giegling[2], H. Jürgen Möller[2], D. Morris[3], A. Corvin[3], M. Gill[3], D. Rujescu[2], M. Owen[1].* 1) Psychological Medicine, Cardiff University, Cardiff, United Kingdom; 2) University of Munich, Munich, Germany; 3) Trinity College, Dublin, Ireland.

Introduction: Several candidate susceptibility genes for schizophrenia have been reported but most of the genetic risk for schizophrenia remains to be attributed to specific genes. With the aim of identifying novel risk genes, we have undertaken a multi-stage association study based upon a total of ~ 9000 subjects.

Methods: Collaborating with the Wellcome Trust Case Control Consortium (WTCCC), we conducted a GWA study on a discovery sample of 476 UK schizophrenic cases and 3000 UK controls (Stage 1a) and supplemented the findings for our top hits ($p<10^{-5}$)in an additional UK 170 cases (Stage 1b). Associations that remained at a threshold $p<10^{-5}$ were genotyped in an additional 1600 cases and 3700 controls (Stage 2)

Results: Six regions after stage 1b contained SNPs at p< 10-5, of which 4 had p<5x10-7. For the most significant SNP at 4 of the 6 loci, we replicated the association to the risk allele in stage 2, an observation unlikely by chance ($p=1.5x10^{-5}$). Three are entirely novel, the other has been previously proposed by others. Our study identifies and replicates associations at 4 schizophrenia loci.

Additional Authors: Jonathan Marchini (Uni of Oxford), Chris Spencer (Uni of Oxford), Hin-Tak (Uni of Cambridge), Annette M Hartmann, Emma Quinn.

Acknowledgement: We would like to thank the WTCCC which undertook much of the pre and post genotyping QC, sampling handling, and who scored all the genotypes in this study.

1872/W

Genome-Wide Association Study for Schizophrenia in the Quebec Founder Population. *N. Paquin[1], P. Van Eerdewegh[1], J. Raelson[1], P. Croteau[1], J. Segal[1], M. Lapalme[1], A. Monette[2], A. Langlais[3], E. Stip[4], H. Fournier[1], B. Paquin[1], J. Hooper[1], A. Belouchi[1], T. Keith[1].* 1) Genizon BioSciences, St-Laurent, QC, Canada; 2) CSSS du Suroît, Valleyfield, QC, Canada; 3) Clinique médicale de l'est, Sherbrooke, QC, Canada; 4) Hôpital Louis-H. Lafontaine, Montreal, QC, Canada.

To identify susceptibility genes for schizophrenia, we performed a GWAS using the Quebec founder population (QFP). 516 cases and 516 matched controls were individually genotyped for 374,187 SNPs on the Infinium assay (Illumina). The marker map consisted of the HAP300 chip supplemented by 56,683 SNPs based on LD structure in the QFP. 352,728 SNPs and 343,297,218 genotypes with a call rate of 99% and a minor allele frequency > 4% were used in genetic analyses. Genome-wide single-marker and haplotype case-control association analyses were performed, with haplotypes of 1, 3, 5, 7 and 9 markers defined by a sliding window. Based on permutation studies, regions with P values that met the criteria for genome-wide significance were identified for both haplotype and single marker association tests, demonstrating that a well-powered GWAS with the QFP was achieved with a relatively small sample size. Among significant signals, haplotype analyses yielded 3 regions with $p < 10^{-7}$, including 1 region with $p < 10^{-8}$, whereas single-marker association identified 6 regions with $p < 10^{-5}$ including 2 with $p < 10^{-6}$. Examples of top candidate loci will be described, including information on the length of the regions and the relevance of the encoded genes in relation to schizophrenia. Additional regions identified from gender-specific and paranoid sub-phenotype analyses will also be presented. Evidence for gene-gene interactions from genome-wide conditional analyses identified both epistatic and independent risk factors within and between biological pathways. Candidate regions are well resolved with many of them containing only one or two genes. The identified genes have been used to infer a GeneMap, consisting of networks of interacting disease genes and their biological pathways. The emerging GeneMap includes both novel and known pathways in neurological development, synaptic plasticity, learning, memory and other neurological disorders.

1873/W

Identification of a possible new locus on chromosome 17p13.2 as a novel candidate for autism, through array-based comparative genomic hybridisation. *S. Raskin[1, 2], A. Stachon[3], C.R. Marshall[4], S.W. Scherer[4].* 1) Department of Genetics, Pontificia Universidade Catolica, Curitiba, Parana, Brazil; 2) Laboratorio Genetika, Curitiba, Parana, Brazil; 3) Department of Psychiatry, University of Toronto, Centre of Addiction and Mental Health, Clinical Genetics Research Program, Toronto, Ontario,Canada; 4) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario, Canada.

Autism spectrum disorders (ASD) refer to a broader group of neurobiological conditions, pervasive developmental disorders.. Despite several arguments for a strong genetic contribution, the molecular basis of a most cases remains unexplained. A patient presenting with non-syndromic ASD was investigated using a DNA microarray constructed from large insert clones designed to target clinically significant areas of the genome, in addition to all the telomere and pericentromeric regions, for a total of 622 discrete loci. Also, the DNA sample was assessed for the copy number variation (CNV) content using signal intensities obtained from Affymetrix 500K SNP arrays and multiple CNV calling algorithms. The CNVs detected were compared against the Database of Genomic Variants as well as a set of 1500 unpublished controls to determine the significance of results. Validation experiments typically included performing quantitative PCR and/or FISH The method was able to identify a 17p13.2 deletion. The distal extent of the deletion could be 160 Kb away. The two clones found to be deleted in this patient are approximately 4Mb distal from the NLGN2 gene. These results show that array comparative genomic hybridisation should be considered to be an essential aspect of the genetic analysis of patients with nn-syndromic ASD. Moreover, besides their importance for diagnosis and genetic counselling, they may allow the delineation of new contiguous gene syndromes associated with ASD. Finally, the detailed molecular analysis of the rearranged regions may pave the way for the identification of a new ASD gene.

1874/W

Distribution and immuno-localization of the NPC protein p62 in human and mouse brains. *N. Shoshani[1,2], L. Basel-Vanagaite[1,2], S. Liraz-Zaltsman[3,4], A. Biegon[3,5], G. Rechavi[2,6], N. Amariglio[2,6], A.J. Simon[6], M. Shohat[1,2].* 1) Department of Medical Genetics, Rabin Medical Center, Petah Tikva, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Israel; 3) Joseph Sagol Neuroscience Center, Sheba Medical Center, Israel; 4) Department of Pharmacology, Hebrew University, Jerusalem, Israel; 5) Brookhaven National Laboratory, NY; 6) Institute of Hematology, Cancer Research Center, Sheba Medical Center, Israel.

p62 protein, encoded by the nup62 gene, is an important component of the nuclear pore complex (NPC). Mutated nup62 causes autosomal recessive Infantile Bilateral Striatal Necrosis (IBSN), characterized by symmetrical degeneration of the caudate nucleus, putamen, and occasionally the globus pallidus, with little involvement of the rest of the brain. The aims of our study were to examine postmortem stability and distribution of p62 in the human brain, specifically in basal ganglia, and its cellular immuno-localization in normal human postmortem and mouse brains. For human brain, cortical and basal ganglia regions were dissected from a coronal slice from a neuropathology-free female aged 59 years at death, with a postmortem delay (time from death to freezing) of 38 hours. For mouse brain, total brain was dissected from a coronal slice without postmortem delay. Series of consecutive cryostat sections from both brains were prepared. p62 expression and immuno-localization studies were performed using Western blot and immuno-fluorescent analyses, using specific antibodies against p62, GFAP and NeuN. p62 was detected in all human brain regions examined by Western blot. Its immuno-localization in the putamen of both brains was confined to the nuclear envelope. p62 was expressed in neurons, as detected by its co-expression with NeuN, but its expression in astrocytes, when co-expressed with GFAP, was weaker than in non-astrocyte neighboring cells. The results indicate that the NPC in human brain frozen up to 38 hours postmortem is preserved, and p62 is expressed in human and mouse basal ganglia with cellular localization to the nuclear envelope. The neuronal expression of p62 in mouse brain suggests that mutated p62 in IBSN affects primarily the neurons of basal ganglia.

1875/W

Parkinsonian spectrum associated with glucocerebrosidase mutations. *E. Sidransky[1], G. Lopez[2], M. Hallett[2], O. Goker-Alpan[1].* 1) MGB/NHGRI/NIH, Bethesda, MD; 2) NIA/NIH, Bethesda, MD.

Alterations in the gene encoding for the lysosomal enzyme glucocerebrosidase (GBA) result in Gaucher disease (GD). Clinical, pathologic and genetic studies suggest that mutant glucocerebrosidase is associated with a phenotype characterized by parkinsonism and progressive neurologic deterioration. To define the neurologic spectrum among subjects with parkinsonism carrying GBA mutations, nine subjects (6M:3F), were followed up to 36 months in a prospective study. Cognitive function, oculomotor and motor deficits were tested by the same team. Olfactory evaluation was done using University of Pennsylvania Smell Identification Test. Genotypes were confirmed by DNA sequencing. The N370S mutation was the most common GD allele. Others included L444P, c.84insG and a recombinant allele. The mean age of onset of parkinsonian manifestations was 50 (40 -65) and disease duration was 7.4 years (1.2 -16). At presentation, four subjects had tremor, 5 had symptoms related to bradykinesia and rigidity, and one also had apraxia. Six were diagnosed with classical PD, three with the akinetic-rigid type. Three subjects were considered to have "parkinson plus" syndrome because of early cognitive changes and hallucinations. All, but one were L-Dopa responsive. Other atypical manifestation included myoclonus, EEG abnormalities and clinical seizures. Autonomic dysfunction was observed in three, and five of 6 subjects tested had olfactory loss. In half, cognitive changes were reported later in the disease course, often accompanied by depression. Glucocerebrosidase mutations are associated with a spectrum of parkinsonian phenotypes, frequently with loss of olfaction. This spectrum ranges from classic PD, mostly the akinetic type, to a less common phenotype characteristic of Lewy Body Dementia.

1876/W

Depression as a genetic sub-phenotype of Alzheimers disease. *M. Slifer[1], B. Plassman[3], D. Steffens[3], J. Gilbert[2], J. Haines[4], M. Pericak-Vance[2].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Miami Institute for Human Genetics Miami, FL; 3) Duke University Medical Center, Durham, NC; 4) Vanderbilt University Medical Center, Nashville, TN.

In 2002 the American Psychiatric Association published diagnostic criteria for the depression of Alzheimer disease (DAD) recognizing the phenomenological distinctiveness and clinical importance of a disorder affecting up to half of those suffering from Alzheimer disease (AD). However, little is known regarding the genetic properties of DAD. In this study, two independent datasets of AD patients (from the Collaborative Alzheimer Project and Duke Twins Study of Memory in Aging) are used to demonstrate a genetic component to DAD. Among non-twin full siblings concordant for AD, we observe significantly elevated sibling recurrence risk for DAD (n=96; Odds Ratio=8.3; p<0.001). The DAD recurrence risk effect is even more pronounced among monozygotic twins concordant for AD (n=38; Odds Ratio=25; p=0.006). Furthermore, including individuals with depression who had a non-AD etiology for their dementia decreased sibling risk, suggesting that DAD is specific to AD and not simply the consequence of cognitive disorders more generally. Additionally, including individuals with AD who had depressive disorders that were temporally unrelated to their AD also decreased sibling risk, illustrating that DAD does not appear to be the product of reducing the threshold for expression of a conventional depressive disorder.

1877/W

Further evaluations of the NLGN3 and NLGN4 genes including a novel bioinformatic approach in autistic females with X inactivation skewness. *Z. Talebizadeh, M.F. Theodoro, M.G. Butler.* Section of Medical Genetics, Children's Mercy Hospital and Clinics and University of Missouri-Kansas City, MO.

We previously reported two novel splice isoforms in X-linked neuroligins, NLGN3 and NLGN4, with a potential role in the etiology of autism spectrum disorders (ASD). The objective of our current study is: 1) to perform mutation screening for NLGN3 and NLGN4 genes in additional autistic females with X inactivation skewness, and 2) to evaluate potential regulatory sequences influencing mRNA splicing. Autistic females were ascertained from the AGRE. X inactivation status was determined using the AR assay on genomic DNA from peripheral blood. Subsequently, cDNA from lymphoblastoid cell lines was used for mutation screening. Sequence alignments and a novel bioinformatic approach were applied to identify potential regulatory elements. X inactivation skewness was confirmed for a group of 10 of 30 autistic females. No new mutation was detected in the NLGN4 gene; however, our previous study identified a NLGN4 transcript missing exon 4 in one autistic female. For NLGN3, RT-PCR screening confirmed that exons 3 and 4 are subject to alternative splicing resulting in an in-frame inclusion or exclusion of 20 amino acids for each of these two exons. In addition, we isolated a novel NLGN3 transcript with intron 2 retention adding 639 nt and introducing an early stop codon. Intron retention (IR) is a very rare form of alternative splicing and recent bioinformatic analyses suggest that specific sequence motifs such as intronic Gs may play a role in IR. We are examining sequence of intron 2 to identify factors contributing to its inclusion during mRNA processing. Furthermore, quantitative RT-PCR is underway to evaluate expression levels of the NLGN3 transcript with IR in autism compared with controls. As susceptibility genes for autism, it is important to understand the role of alternative splicing and contributing factors for the neuroligin genes. Our study suggests that cis or trans-acting elements are likely to play a crucial role in regulating formation of the observed alternative splicing (exon skipping and IR) in NLGN3 and NLGN4 genes impacting on encoded proteins in autistic subjects.

1878/W

Detection of gene duplication(s) among Chinese X-linked mental retardation (XLMR) with multiplex ligation probe amplification (MLPA). *X-G. Tao[1], X-Z. Wang[1], J-M. Wang[1], Y-W. Jiang[1], N. Zhong[1,2].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Mental retardation (MR) is defined as a significant impairment of cognitive and adaptive function, with onset before age 18 years. It is estimated to occur in about 3-5% of the population. In a national wide survey conducted in 2000, there are about 30,000,000 MR patients among 0-6 years Chinese children in the mainland China, with an annual increase of 300,000. Because X-linked MR accounts for about 20-30% of the MR population, we anticipated that there must be a large number of XLMR patients. We have initialized a molecular testing among the XLMR patients to exclude fragile X syndrome (see Ju et al., Abstract control number 10089 in this conference). After fragile X syndrome is excluded, we employed multiplex ligation probe amplification (MLPA) to screen for gene deletion or duplication among clinically suspected XLMR patients. A mix of 43 probes (probe kit P106, MRC, Holland) detecting for 14 XLMR genes including FMR1, FMR2, GDI1, and SCL6A8 was hybridized to patients genomic DNA that was amplified. Among 51 cases we studied, two cases were identified to have gene duplication. One is carrying a duplication at exon 11 of FMR2 gene, and another, having a large duplication spinning genes GDI1 and SCL6A8. Here we report the first finding of a gene-duplication at FMR2 locus. Our results indicated that applying MLPA may detect 3.9% of clinically suspected XLMR cases. We believe that the detecting rate may be higher once the size of testing sample is increased.

1879/W

Identification of Late-Onset Alzheimer's Disease (LOAD) Susceptibility Alleles in the PAI1 Gene. *S. Wilcox[1], M. Carrasquillo[1], S. Younkin[1], M. Li[1], L. Younkin[1], D. Dickson[1], N. Graff-Radford[1], R. Petersen[2], S. Younkin[1].* 1) Dept Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Dept Neurology, Mayo Clinic, Rochester, MN.

Given that the plasmin system has been implicated in Aβ degradation and that accumulation of Aβ in the brain has an important role in AD, genes encoding proteins in the plasmin system are obvious AD functional candidates. In this study we focused on the role of the plasminogen activator inhibitor-1 (PAI1), a protease inhibitor that is believed to be produced in the brain. Previous reports of association of PAI1 with AD are conflicting. To further evaluate PAI1's contribution AD risk, we selected variants within the PAI1 genomic region which met minor allele frequency (MAF) and conservation criteria: MAF 1-45%, and human-mouse identity >70% over 100bp windows. Variants were tested for association with disease status by logistic regression analyses at the level of single variant, haplotypes and multilocus genotypes (MLGs) using gender, age-at-diagnosis and APOEε4 dosage as covariates in our LOAD case-control series (1,807 vs. 1,970). All 8 variants that met genotyping criteria fall within a haplotype block that encompasses the entire gene. Seven out of 8 variants tag a haplotype, and were tested individually under allelic dosage, recessive and dominant models. The dominant model yielded the most significant results. One of the 8 variants, rs12673157, showed nominal significance (p=0.04). Five others, including rs1799889 reported in 2006 by Shibata et al. to associate with LOAD, showed suggestive association (0.06<p<0.25). The haplotypic global p-value did not achieve significance (p=0.15), however, MLGs were highly significant (global p=0.002). Forty-three MLGs account for 94% of all individuals in our case-control series. Two of these MLGs seem to confer significant risk (OR=5.5, p=0.001; OR=3.3, p=0.03) and 12 MLGs showed suggestive evidence (0.05<p<0.25) of either risk (ORs 1.3-1.7) or protection (ORs 0.3-0.6). Overall, our results strongly suggest that PAI1 plays a role in AD pathogenesis. Additional genetic and functional studies ought to be carried out to further substantiate its involvement.

1880/W

Brain-Derived Neurotrophic Factor (BDNF) in autism. *C. Correia[1,2], A.M. Coutinho[1], M. Barreto[1,2], M. Martins[1,2], L. Lourenço[1,2], J. Almeida[3], C. Marques[3], T. S.Miguel[4], A. Ataide[4], G. Oliveira[3], A.M. Vicente[1,2].* 1) Instituto Gulbenkian Ciencia, Oeiras, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; 3) Hospital Pediátrico de Coimbra, Coimbra, Portugal; 4) Direcção Regional de Educação da Região Centro, Portugal.

Several lines of evidence implicate BDNF, a neurotrophin crucial for brain development and function, in autism. In this study, the role of BDNF in autism etiology was studied. We found that BDNF plasma levels in autistic children (N=146) were significantly increased compared with control children (H=51.69, P<0.00001) and positively correlated with serotonin levels (r^2=0.289; P=0.004), with an heritability of 30%. We therefore sought to identify genetic factors that might regulate BDNF distribution. Several candidate genes were assessed, including *BDNF* and its receptor *NTRK2* genes; the *HTR1A* gene region, since the HTR1A serotonin receptor regulates serotonin levels; and the *GAD1* gene, as it encodes a key regulator of glutamate which, neurotoxic when in excess, induces *BDNF* expression as a neuroprotective mechanism. We found an association of three markers in the *HTR1A* genomic region with BDNF levels (χ^2=5.06, df=1, P=0.0245; χ^2=4.93, df=1, P=0.0264; χ^2=4.25, df=1, P=0.0392). While only one marker in this region showed a strong association with autism in the overall population (χ^2=30.13, df=9, P=0.0004), the three markers associated with BDNF levels were also associated with autism in the subset of patients with high BDNF levels (Z=2.982, df=1, P=0.0029; Z=2.668, df=1, P=0.0076; Z=2.524, df=1, P=0.012). Unexpectedly these markers were located within *RNF180*, a recently identified gene mapping near *HTR1A*. *RNF180* encodes a protein belonging to the zinc ring finger family implicated in the ubiquitination signal pathway, which is important in maintaining protein homeostasis and in transcriptional regulation by histone ubiquitination. Our results corroborate previous reports, in smaller cohorts, of increased plasma BDNF in autistic patients. The results also suggest that *RNF180* may be involved in the regulation of BDNF levels through an as yet unexplained mechanism that may underlie autism etiology in a subset of patients.

1881/W

No association of selected SNPs in *GALP*, *PCK1*, *SERPINA13* or *TNK1* with Alzheimer's disease. *J.A. Figgins[1], S.T. DeKosky[2], R.L. Minster[1], M.I. Kamboh[1].* 1) Department of Human Genetics; 2) Department of Neurology, University of Pittsburgh, Pittsburgh, PA.

Alzheimer's disease (AD) is a complex and multifactorial disease with the possible involvement of several genes. With the exception of the *APOE* gene as a susceptibility marker no other genes have been identified for late-onset AD (LOAD). A recent genome-wide association study of 17,343 gene-based putative functional single nucleotide polymorphisms (SNPs) found 19 significant variants in several genes showing association with LOAD in several population samples (Hum Mol Genet 2007;16:865-73). We have set out to replicate these significant associations in a large case-control cohort of American Whites. Thus far we have examined SNPs in four of the genes: *GALP*/*rs3745833* (c.216C>G p.I72M), *PCK1*/*rs8192708* (c.799A>G p.I267V), *SERPINA13*/*rs11622883* (c.*42980T>A), *TNK1*/*rs1554948* (c.81T>A). The four SNPs were genotyped in up to 1003 Caucasian Americans with late-onset Alzheimer's disease and up to 868 age matched healthy Caucasian Americans. All four variants were genotyped using 5' nuclease assays. We did not observe a statistically significant association between the SNPs with the risk of AD, either individually or stratified by APOE. We are in the process of completing analysis on SNPs in 12 other genes noted in the paper. Our data suggest that the association of *GALP*/*rs3745833*, *PCK1*/*rs8192708*, *SERPINA13*/*rs11622883*, and *TNK1*/*rs1554948* with LOAD, if it exists, is not statistically significant in our population.

1882/W

OSTM1 and NR2E1: Positional candidate genes for psychosis in Alzheimer's and Schizophrenia. *V. Kodavali[1], R.A. Sweet[1], R.I. Kamboh[2], V.L. Nimgaonkar[1,2].* 1) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Psychotic symptoms, i.e., hallucinations and delusions, are common among patients with late onset Alzheimer's Disease (LOAD), with a reported three-year cumulative incidence of 51%. Psychotic symptoms define a more severe sub-group of LOAD that has more rapid cognitive decline, causes greater caregiver distress, and results in greater societal burden due to premature institutionalization. Current treatments for LOAD with psychosis (LOAD+P) are inadequate. We have found LOAD+P to be familial, with a heritability of 61-70%. We found suggestive linkage using data from the NIMH Alzheimer Disease Genetics Initiative, at chromosome 6q near marker D6S1021. This region may also harbor psychosis liability genes for schizophrenia and bipolar illness. We conducted follow-up genetic association studies of the Chr 6q region in a cohort of 200 subjects with LOAD+P and 350 unrelated individuals with LOAD without psychosis, using pooled DNA analysis. We detected significant association with a Single Nucleotide Polymorphism (SNP, rs218291) which is located in the upstream region of Osteopetrosis-associated Transmemberane Protein 1 (OSTM1) and Tailless (NR2E1) genes (p=0.009). This polymorphism could be potentially regulating the transcription of both OSTM1 and NR2E1 genes. Importantly, increased amyloid b protein deposition and neuronal loss was observed in osteopetroic mice. Similarly, Monaghan et al (2003) showed that disruption of the NR2E1 locus leads to impaired development of supragranular layers of neocortex, layers that may be selectively affected in psychosis. In order to further understand the genetic association and the role of the regulatory regions of OSTM1 and NR2E1 in psychosis, here, we are systematically characterizing OSTM1 and NR2E1 polymorphisms and their functional effects in US Alzheimer's and schizophrenia samples.

1883/W

Haplotype association of Monoamine Oxidase A Gene and Bipolar Affective Disorder in Han Chinese men. *Y.-M.J. Lin[1], F. Davamani[1], C.-H. Hsu[1], W.-C. Yang[2], T.-J. Lai[3], H.S. Sun[1].* 1) Inst Mol Medical Sci, National Cheng Kung Univ, Tainan, Taiwan; 2) Department of Biology, National Cheng Kung University, Tainan, Taiwan; 3) Department of Psychiatry, Chung Shan Medical and Dental College Hospital, Taichung, Taiwan.

Background/Aims: Monoamine oxidase A (MAOA) is a mitochondrial enzyme involved in degrading several different biological amines, including serotonin. Although several pieces of evidence suggested that MAOA is important in the etiology of bipolar affective disorder (BPD), associations for markers of the MAOA gene with BPD were not conclusive and the association has not been investigated in Taiwanese population. This study was designed to illustrate the role of MAOA in the etiology of BPD in Han Chinese. Methods: Two markers, a dinucleotide polymorphism in exon 2 and a functional uVNTR on the promoter of the MAOA gene, were used to study the genetic association in 108 unrelated patients with BPD and 103 healthy controls. Allelic distributions of two polymorphisms were analyzed and, caused the MAOA located at X chromosome, haplotype association was performed using haplotype unambiguously assigned in male participants. Results: While no difference in allelic distributions of two MAOA polymorphism was found, one common haplotype 114S was weakly associated with BPD in male patients (P = 0.05). The significance, however, was not found in female patients with 114S haplotype. Conclusions: Results from this study suggest that MAOA may have a gender-specific and small effect on the etiology of BPD in Taiwan. Due to the limited sample size, results from this study need to be confirmed in replicates.

1884/W

Contribution of *SHANK3* mutations to autism spectrum disorder. *C.R. Marshall[1], R. Moessner[1], J.S. Sutcliffe[2], J. Skaug[1], D. Pinto[1], J. Vincent[3], L. Zwaigenbaum[4], B. Fernandez[5], W. Roberts[6], P. Szatmari[7], S.W. Scherer[1].* 1) The Centre for Applied Genomics and Program in Genetics & Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Center for Molecular Neuroscience and Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN, USA; 3) Centre for Addiction and Mental Health, Clarke Institute and Department of Psychiatry, University of Toronto, Toronto, ON, Canada; 4) Department of Pediatrics, University of Alberta, Edmonton, AB, Canada; 5) Disciplines of Genetics and Medicine, Memorial University of Newfoundland, St. John's, NL, Canada; 6) Autism Research Unit, The Hospital for Sick Children, Toronto, ON, Canada; 7) Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada.

Mutations in *SHANK3* encoding the synaptic scaffolding protein have been described in cases with an autism spectrum disorder (ASD). The *SHANK3* gene (also termed ProSAP2) is preferentially expressed in the cerebral cortex where it functions as a scaffolding protein at the postsynaptic density of excitatory synapses. To assess the quantitative contribution of *SHANK3* to the pathogenesis of autism, we determined the frequency of DNA sequence and copy number variants in this gene in a cohort of 400 ASD cases ascertained in Canada. One *de novo* mutation and two gene deletions were discovered indicating a contribution of 0.75% in this cohort. In one of the deletion families, we also detected a *de novo* duplication in the proband spanning 1.4 Mb at 20q13.33. One additional *SHANK3* deletion was characterized in two ASD sibs from another cohort bringing the total number of published mutations in unrelated ASD families to seven. From these data there are no obvious correlation between phenotype and genotype suggesting all ASD forms are candidates for *SHANK3* testing. The combined data provides support for haploinsufficiency of *SHANK3* causing a monogenic form of autism in sufficient frequency to warrant consideration in clinical diagnostic testing.

1885/W

The Inheritance of Resistance Alleles in Multiple Sclerosis. *S.V. Ramagopalan[1,2], D.A. Dyment[1,2], B.M. Herrera[1,2], G.C. DeLuca[1,2], M.R. Lincoln[1,2], S.M. Orton[1,2], M.J. Chao[1,2], A.D. Sadovnick[3], G.C. Ebers[1,2].* 1) Department of Clinical Neurology, Oxford University, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom; 3) Departments of Medical Genetics and Neurology, University of British Columbia, Vancouver, Canada.

Multiple sclerosis (MS) is a complex trait in which alleles at or near the class II loci HLA-DRB1 and HLA-DQB1 contribute significantly to genetic risk. HLA-DRB1*15 and HLA-DRB1*17 bearing haplotypes and interactions at the HLA-DRB1 locus increase risk of MS but it has taken large samples to identify resistance HLA-DRB1 alleles. In this investigation of 7093 individuals from 1432 MS families, we have assessed the validity, mode of inheritance, associated genotypes and the interactions of HLA-DRB1 resistance alleles. HLA-DRB1*14, DRB1*11, DRB1*01 and DRB1*10 bearing haplotypes are protective overall but they appear to operate by different mechanisms. There are major practical implications for risk and for the exploration of mechanisms in animal models. Restriction of antigen presentation by HLA-DRB1*15 seems an improbably simple mechanism of MHC associated susceptibility.

1886/W

Analysis of a de novo balanced translocation [46,XY,t(2;9)(p13;p24)] in a patient with autism spectrum disorder reveals direct interruption of RAB11FIP5. *J. Roohi[1], D.H. Tegay[1], J.C. Pomeroy[1], G. Stone[2], R. Stanyon[2,3], S. Christian[4].* 1) Stony Brook University Medical Center, Stony Brook, NY; 2) Comparative Molecular Cytogenetics Core, National Cancer Institute, Fort Detrick, Frederick, MD; 3) Department of Animal Biology and Genetics, University of Florence, Florence, Italy; 4) Department of Human Genetics, The University of Chicago, Chicago, Illinois.

An apparently balanced de novo translocation between chromosomes 2 and 9 [46,XY,t(2;9)(p13;p24)] in a patient with pervasive developmental disorder not otherwise specified (PDD-NOS), significant fifth finger clinodactyly, slight hypotonia, and slight microcephaly was mapped using flow sorted chromosomes generated from Epstein-Barr Virus immortalized peripheral white blood cells. Chromosomes 2 and 9 isolated from a normal individual and the patient's derivative chromosome 9 were amplified with Phi29 DNA polymerase and the normal material hybridized against the patient's onto a tiling path BAC array. The translocation breakpoints were identified to within approximately 200kb and PCR used to map them in finer detail. RAB11 family interacting protein 5 (RAB11FIP5) on chromosome 2p13.2 was found to be directly interrupted by the translocation; no gene on chromosome 9 was involved. RAB11FIP5 is a Rab11 effector associated with the mitochondria and recycling endosomes. It regulates the recycling of plasma membrane receptors and is expressed at low levels in several organs, including the brain. Mutations that affect Rab11, a GTPase that regulates vesicle targeting and fusion, have been associated with autism and mental retardation. Given the potential importance of RAB11FIP5 in autism pathogenesis, we used Hi-Res melt analysis to investigate this gene in 607 autistic individuals and have identified several undescribed changes.

1887/W

Examination of Sortilin-related receptor SORL1 in Late-Onset Alzheimer Disease. *S.D. Turner[1], X. Liang[1], E.R. Martin[2], N. Schnetz-Boutaud[1], J. Bartlett[1], B.M. Anderson[1], S. Zuchner[2], H. Gwirtsman[1], D. Schmenche[3], R. Carney[3], J. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Miami Institute for Human Genomics, Miami, FL; 3) Center Human Genetics, Duke University, Durham, NC.

Late-onset Alzheimer disease (AD) is a neurodegenerative disorder with a genetically heterogeneous etiology. Accumulation of Aβ peptide in the brain is a key event in AD pathogenesis. Aβ is generated primarily by the endocytic pathway that processes amyloid precursor protein (APP) recycling from the cell surface. A component in this pathway, the sortilin-related receptor SORL1, was recently associated with AD in both family and case-control datasets. Here, we genotyped 6 previously associated SNPs in SORL1 in 518 cases and 527 age and gender matched controls in a Caucasian population from the Southeastern United States. All cases met NINDS-ADRDA criteria for probable or possible AD and all controls were cognitively normal. Intronic SNP rs3824968 in SORL1 showed both a significant genotypic association with an odds ratio of 1.41 (95% CI=[1.10, 1.79], p=0.006) and significant allelic association with an odds ratio of 1.24 (95% CI=[1.03, 1.50], p=0.025). Intronic SNP rs2070045 was significantly associated with AD with an odds ratio of 1.35 (95% CI=[1.05, 1.75], p=0.017). These data provide additional support for the role of SORL1 in AD pathogenesis and should be further investigated in additional replication datasets and functional studies.

1888/W

Uncovering Cln3 interacting partners with the Ubiquitin-based Split-System gives new clues on the pathogenesis of the juvenile neuronal ceroid lipofuscinosis (Batten's Disease). *A. Lloret, J.E Janice, V.C Wheeler, M.E MacDonald, S.L Cotman.* CHGR. 185 Cambridge Street. 5th Floor. Rm. 5300. Mass. General Hospital. Havard Medical School. Boston MA, 02114.

Juvenile neuronal ceroid lipofuscinosis is caused by mutation of Battenin, a novel endosomal/lysosomal membrane protein encoded by CLN3. Patients bearing a recessive mutation in this gene display severe widespread neuronal degeneration resulting in retinal atrophy and in massive loss of brain substance and massive autofluorescent lysosomal accumulations. The storage material consists of hydrophobic aggregates containing proteolipids, a predominant component of which in most NCLs is the mitochondrial ATP synthase subunit c. Despite all information provided by several models of the disease the function of battenin is still unknown. Until now, some attempts had been done to establish the protein interactors of Cln3. using the Y2H as the methods of choice, but the reported results are either negative or restricted to a very small fragment of the protein used as bait. Therefore, with the aim of uncover Cln3 protein interacting partners, we decided to apply a method that has proven extremely useful to monitor protein-protein interactions; the Ubiquitin-based Split-protein System (UBPS). This system allows detection of protein-protein interactions in vivo using fusions with two fragments of ubiquitin. We generated an CLN3-Ubiquitin (N-terminal part) fusion comprising the whole sequence of the CLN3 wild type human gene. This bait construct is also in frame with a sequence that encodes for a hybrid transcriptional factor, comprised by the LexA DNA binding domain and the transcriptional activator VP16. With it, we performed a screening using an adult brain cDNA library (fused to the C terminal part of ubiquitin) as our preys. As a result, we obtained 32 positive independent clones for the interaction, 10 of which correspond to the neuronal membrane glycoprotein M6-b (Gpm6B). Taken all together, results derived form this study will help to point out the pathways in which Cln3p is involved, and also will lead to a better understanding of the pathogenesis of the disease.*CLN3*.

1889/W

Family and twin studies of Restless Legs Syndrome. *L. Xiong[1], K. Jang[2], J. Montplaisir[3], A. Levchenko[1], P. Thibodeau[1], C. Gaspar[1], G. Turecki[4], G.A. Rouleau[1].* 1) Center for the Study of Brain Diseases, CHUM Research Center - Notre Dame Hospital, University of Montreal, Québec, Canada; 2) Department of psychiatry, University of British Columbia, Canada; 3) Centre d'étude du sommeil, Hôpital du Sacré-Cœur de Montréal and Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada; 4) Research Center, Douglas Hospital, McGill University, Québec, Canada.

Background and purpose: Restless legs syndrome (RLS) is a prevalent sensorimotor disorder characterized by an imperative urge to move the legs. It often aggregates in families, indicating a potential genetic component. The purpose of this study is to characterize the clinical features of familial RLS and estimate its heritability. **Subjects and methods:**We undertook a systematic full family study of 259 RLS probands and their family members during a period of 10 years by multidimensional assessments of the probands and structural questionnaire telephone interviews for evaluation of family members using standardized RLS diagnostic criteria. We also conducted a population survey of RLS in 272 adult twin pairs from Canada using the same structured standardized questionnaire. **Results:**Our data confirms that RLS aggregates in families with a familial rate of ~77% by family history. Data from twin study also confirms that RLS is a common disorder with a prevalence of 12.3% in the surveyed population. The concordance rate of RLS is 53.7% in monozygotic and 19.0% in dizygotic twins with same sex, predicting a heritability of 69.4%. There are significant positive correlations of age at onset and severity scores among the concordant twin pairs. However familial RLS is a chronic disorder with average disease duration about 24±16 years and a wide range of age of onset (AO: 28±15 yr) and variable phenotypic expressivity in terms of severity, clinical course and sleep disturbance. Younger AO seems to be the most decisive factor distinguishing the familial and sporadic forms. Familial RLS is further characterized by higher rate of restless arms, bilateral restless legs, progressive clinical course and higher measurement of periodic leg movements during sleep (PLMS).

1890/W

***PTEN* gene mutation causes megalencephaly with prominent Virchow-Robin spaces without cognitive delays or autism: New PTHS phenotype.** *L. Medne[1], A. Waldman[2], C. Bonnemann[2].* 1) Div of Hum Genetics & Neurology, Children's Hosp Philadelphia, Philadelphia, PA; 2) Div of Neurology, Children's Hosp of Philadelphia, Philadelphia, PA.

We report two patients with a pathogenic *PTEN* gene mutation as the cause of megalencephaly with prominent Virchow-Robin spaces who both demonstrated normal cognitive development for age. Patient 1 presented to neuromuscular clinic at 19 months of age for evaluation of hypotonia, motor skill delay and megalencephaly. Macrocephaly was noted shortly after birth. He had a febrile seizure at 18 mo of age. Repeat MRI showed prominent Virchow-Robin spaces, which lead to an extensive diagnostic work-up with normal results. At 19 months, his length was at -0.8 SD but his head circumference was +4 SD. He had hypotonia and ligamentous laxity but did not have PTHS stigmata. *PTEN* sequencing showed a de novo I101T missense mutation that has been previously reported in Cowden syndrome. At both his19 mo and at 4 yr evaluations, he had normal speech and cognitive development with delays limited to gross motor skills due to hypotonia. Patient 2 presented at 11 months for evaluation of post-natal macrocephaly. The head circumference was normal at birth but was above the 98% by 5 months of age. Brain MRI 14 months revealed prominence of Virchow-Robin spaces. Her length was at -1 SD with head circumference at +2.5 SD. She did not have lipomas or hypotonia and her development was age appropriate. *PTEN* sequencing revealed a novel nonsense mutation E285X that was not present in her mother. The occurrence of megalencephaly with prominent Virchow-Robin spaces is a known association that has been previously reported in patients with or without associated cognitive delays and mental retardation. The underlying genetic etiology is felt to be heterogeneous. We suggest that *PTEN* gene mutations should be considered in patients with apparently isolated megalencephaly and prominent Virchow-Robin spaces as well as those with additional previouly associated PTHS stigmata. Establishing the diagnosis would avoid extensive metabolic work-up, guide further management, and allow for appropriate genetic counseling.

1891/W

Neurobehavioral profile and brain imaging study of the 22q13.3 deletion syndrome. *A. PHILIPPE[1], N. BODDAERT[2], L. VAIVRE-DOURET[3], L. ROBEL[3], V. MALAN[1], O. RAOUL[1], M.C. de BLOIS[1], M. PRIEUR[1], V. CORMIER-DAIRE[1], S. LYONNET[1], B. BENZACKEN[4], D. HERON[5], B. GOLSE[3], M. VEKEMANS[1], M. ZILBOVICIUS[2], A. MUNNICH[1].* 1) INSERM U 781 & Département de Génétique, Hôpital Necker-Enfants Malades, Paris; 2) INSERM URM 0205, CEA, Orsay, France; 3) INSERM U483 et Service de Pédopsychiatrie, Hôpital Necker-Enfants Malades, Paris; 4) Service d'Histologie Embryologie Cytogénétique et Biologie de la Reproduction, Hôpital Jean Verdier, Bondy, France; 5) Département de Génétique, Hôpital de la Pitié Salpêtrière, Paris.

The 22q13.3 deletion syndrome (MIM 606232) is a neurodevelopmental disorder including hypotonia, severely impaired development of speech and language, autistic-like behavior and minor dysmorphic features. Although the number of cases reported is increasing, the 22q13.3 deletion remains under-diagnosed due to failure both to recognize the phenotype on clinical examination and to detect the 22qter deletion in routine chromosome analysis. Our objective is to improve the description of the neuro-behavioral and brain characteristics of this microdeletional syndrome. We report an overall neuro-behavioral assessment of 8 children with a 22q13.3 deletion. The assessment involved analysis of neuromotor, sensory, language, communication and social development, cerebral magnetic resonance imaging, and study of regional cerebral blood flow measured by positron emission tomography. Although the severity of symptomatology differs between children, the 22q13.3 deletion syndrome has a clinically distinctive developmental profile associated with hypoperfusion of left temporal polar lobe and amgydala. Although autism was suspected in 7/8 subjects in our cohort during the first year of their lives, it is not confirmed later subsequently. Our study shows that the paralinguistic clues of interaction and the nature of the repetitive behaviors suggested a particular pattern distinct from autism. More than clinical observation at a given time, the progression of symptoms and their assessment according to developmental and chronological age are particularly valuable for the diagnosis of deletion 22q13.3 syndrome.

1892/W

Psychiatric and substance abuse disorders in relatives of probands with schizophrenia and bipolar disorder utilizing a family history approach. *C.A. Bousman[1], L. Madlensky[2], M. Staton[1], S.J. Glatt[3], I.P. Everall[1], M.T. Tsuang[1].* 1) Center for Behavioral Genetics, Dept Psychiatry, Univ California, San Diego, San Diego, CA; 2) Cancer Prevention and Control Program, Moores UCSD Cancer Center, University of California, San Diego, San Diego, CA; 3) Dept Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, NY.

Previous family, adoption and twin studies have demonstrated that many adult psychiatric disorders, including schizophrenia and bipolar disorder, have a clear genetic component. To estimate this genetic component the family history approach is often utilized in psychiatry due to its simple application, low financial and time burden and exceptional reliability and validity. This study was designed to (1) examine differences in psychiatric and substance abuse family history in biological relatives of probands with schizophrenia and bipolar disorder and, (2) estimate the utility of the family history approach with schizophrenia and bipolar patients. Psychiatric and substance abuse disorders were collected utilizing a family history approach in a sample of schizophrenia (n =10) and bipolar (n =10) probands diagnosed using the Diagnostic Interview for Genetic Studies (DIGS). Results show that relatives of bipolar probands had significantly higher rates of bipolar and alcoholism than relatives of schizophrenia probands. In addition, results reveal that probands with schizophrenia were unable to provide history on 31% of their parents and grandparents, and bipolar probands were unable to provide history on 18% of these relatives. Implications of these findings for the utilization of family history and the potential shared heritability of bipolar and alcoholism are discussed.

1893/W

The LRRK2 G2019S mutation is both common and highly penetrant in a Tunisian non-familial Parkinson's Disease case-control study. *R.A. Gibson[1], J. Kachergus[2], L. Ishihara-Paul[1], M. Hulihan[2], R. Upmanyu[1], L. Warren[1], S. Oldham[1], R. Amouri[3], S. Ben Yahmed[3], M. Kefi[3], M. Zouari[3], S. Ben Sassi[3], P.A. Akkari[1], R. Elango[1], R. Prinjha[1], L. Ragone[1], L.T. Middleton[1], P.M. Matthews[1], F. Hentati[3], M. Farrer[2].* 1) Research and Development, GlaxoSmithKline, USA and UK; 2) Dept of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA; 3) Dept of Neurology, Institut National de Neurologie, Tunis, Tunisia.

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease, affecting approximately 1% of the population aged over 50. The majority of patients are considered sporadic with age, genetic and environmental risk factors as important determinants of disease penetrance. Studies of multiplex PD families have identified a number of genes in PD, which have provided insights into the mechanisms of the more frequent sporadic form of disease. One such example is the leucine-rich repeat kinase 2 gene (LRRK2) which is implicated in autosomal dominant forms of PD. We previously investigated the frequency of the most common substitution of LRRK2 (G2019S, 6055G>A), in Tunisian-based consanguineous PD families. The G2019S substitution was identified in 39 of the 88 families (44%) examined. To investigate the role of the Lrrk2 G2019S substitution in non-familial forms of PD, an additional 239 individuals with non-familial sporadic PD and 371 unrelated controls were recruited from the Institut National de Neurologie, Tunisia which provides a specialized neurological service to the entire country. All PD patients and controls received neurological examinations and completed standardized questionnaires collecting demographic and clinical information. All cases and controls recruited were screened for the G2019S substitution. In total 35% (73 of the 239) of the sporadic cases recruited and only 1.9% of the controls (7 out of the 371 recruited) were found to have the G2019S mutation. Of the 7 controls that were heterozygous for this change, 5 were younger than 60 and need further evaluation. This finding indicates the high level of penetrance of this substitution it's applicability to sporadic PD and the relevance for genetic screening.

1894/W

PTEN Sequencing Improves the Diagnostic Yield in a Clinical Sample of Patients Evaluated for Idiopathic Autism Spectrum Disorders. *E. Varga, K. Ratliff-Schaub, M. Pastore, K. McBride, G.E. Herman.* Children's Research Institute and Dept. of Pediatrics, The Ohio State University, Columbus, OH.

We performed a retrospective chart review of 108 unrelated, newly-referred patients with an isolated autism spectrum disorder (ASD) evaluated in Genetics Clinic over a period of 26 months. The referral population consisted of 81 patients who met DSM-IV criteria for autistic disorder, 16 with PDD-NOS and 7 with Asperger syndrome. There were 4 females with Rett syndrome (confirmed by MECP2 sequencing); these subjects were excluded since 3 were referred for developmental delay (no autism) and 1 for suspected Rett syndrome. Of included subjects (n=104), 89 were male and 16 female (ratio 5.6:1) with an age range of 18 m - 16.5 y. Genetic testing was performed at the clinicians' discretion based on tiered testing guidelines developed by geneticists and developmental pediatricians at our institution. First tier testing included a high-resolution karyotype, DNA for fragile X syndrome, plasma amino acid and urine organic acid analysis, homocysteine, lead level and hearing screening. Second-tier testing included chromosomal microarray, MECP2 gene sequencing, DNA methylation for PWS/AS, urine guanidinoacetate profile and purines/pyrimidines ratio, uric acid, and PTEN gene sequencing (if HC≥95%) as appropriate. The overall diagnostic yield of testing was 11% (12/105). Three subjects had a karyotypic abnormality (47, XYY; mos, 47, XX, +r(8)(p12q11.2)[28]/46,XX[4]; and 46,XY, del 17p11.2(SMS-). One abnormality was detected on chromosomal microarray, a submicroscopic deletion of 3 BAC clones in 1q21 that was also present in the patient's asymptomatic mother. One had a high lead level as an infant (documented level of 34 ug/dL; nl <3). None had fragile X syndrome or abnormal metabolic testing. Six patients had heterozygous mutations in the PTEN gene (3 de novo; 2 inherited; 1 parents pending). All patients with PTEN mutations had macrocephaly (≥98%). The disproportionate number of cases with PTEN mutations represents a referral bias. Nonetheless, these data suggest that PTEN sequencing in ASD patients improves diagnostic yield and is warranted in ASD patients with macrocephaly.

1895/W

Genome-wide association analysis of Attention Deficit Hyperactivity Disorder (ADHD). *J. Lasky-Su[1], K. Zhou[4], C. Lange[14], B. Neale[4], N. Laird[14], M. Daly[7], R. Ebstein[6], J. Buitelaar[3], M. Gill[8], A. Miranda[9], F. Mulas[9], R. Oades[10], H. Roeyers[11], A. Rothenberger[2], J. Sergeant[12], H-C. Steinhausen[13], E. Sonuga-Barke[5], B. Franke[3], P. Asherson[4], S.V. Faraone[1], IMAGE Consortium.* 1) SUNY Upstate Medical University, Syracuse, NY; 2) University of Göttingen, Göttingen, Germany; 3) Radboud University, Nijmegen Medical Center, Nijmegen, The Netherlands; 4) Institute of Psychiatry, London, UK; 5) University of Southampton, Highfield, Southampton, UK; 6) S Herzog Memorial Hospital, Jerusalem, Israel; 7) Broad Institute, Cambridge, MA; 8) St James's Hospital, Dublin, Ireland; 9) University of Valencia, Valencia, Spain; 10) University Clinic for Child and Adolescent Psychiatry, Essen, Germany; 11) Ghent University, Ghent, Belgium; 12) Vrije Universiteit, De Boelelaan, Amsterdam, Holland; 13) University of Zurich, Zurich, Switzerland; 14) Harvard School of Public Health, Boston, MA.

Although psychiatric geneticists have begun to produce replicated findings implicating specific genes or chromosomal loci in the etiology of ADHD, most of the genes underlying these disorders have remained elusive. One obstacle to gene discovery for ADHD has been the lack of a tool for screening the genome for genes of small effect. We used 958 parent-child trios with offspring meeting the DSM-IV combined-type criteria for ADHD from the IMAGE project. Families were collected in the Netherlands, Ireland, the UK, Germany, Belgium, Switzerland, Spain and Israel. Probands were European Caucasians aged 5 to 15 years. Genotyping of >550,000 SNPs was recently completed at Perlegen Sciences. In addition to an affection status analysis, we develop a quantitative phenotype at each SNP by weighting 9 inattentive and 9 hyperactive-impulsive symptoms. The weights are selected to maximize the heritability at each SNP and FBAT association tests are performed. For the significant SNPs, corrected for multiple testing using rankings based on conditional power, the correlation between each of the ADHD symptoms and the quantitative phenotype is used to determine what symptoms are driving the association.

1896/W

Association of the UBE3A Substrate ECT2 with Autism. *R.J. Delahanty[1], J.A. Smart[2], J.S. Sutcliffe[1,2], L.T. Reiter[3].* 1) Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Molecular Physiology and Biophysics, Vanderbilt Univ, Nashville, TN; 3) Department of Neurology, University of Tennessee Health Science Center, Memphis, TN.

Evidence indicates a predominantly genetic etiology for autism, but locus heterogeneity has confounded the identification of genes contributing to the idiopathic condition. Maternal duplications of 15q11-q13 are the most frequent chromosomal abnormality found in autism. Data indicate that dup(15) autism results in over-expression of contiguous loci including the maternally expressed E6-AP ubiquitin ligase (UBE3A) gene. Maternal deficiency of UBE3A causes the severe neurodevelopmental disorder Angelman syndrome (AS), which can share features with autism. Altered expression of UBE3A is hypothesized to result in dysregulation of UBE3A substrates, which are ultimately responsible for the phenotypic consequences of UBE3A over- or under-expression. However, few attempts have been made to identify the protein substrates regulated by UBE3A. We have used a proteomics approach in Drosophila to identify proteins affected by elevated UBE3A levels, with the aim of identifying genes that could be candidates for harboring susceptibility alleles for idiopathic autism. We previously demonstrated that a RhoGEF involved in cell migration and morphology was significantly down-regulated by UBE3A expression. The mammalian ortholog of pebble (ECT2; 3q26.1) was identified and examined for the presence of autism-associated alleles. Using tag SNPs to detect common alleles (>5%) at both loci, association analyses were performed on a sample of ~700 autism families. Association tests were conducted for six SNPs in ECT2 using FBAT and calculating exact P-values based on Monte Carlo simulations. Two SNPs in intron 22 and the 3'UTR of ECT2, showed significant association (P<0.02) to autism. The quantitative trait disequilibrium test (QTDT) by ascertainment site indicates that susceptibility alleles at this locus increase risk for social deficits and remain significant after Bonferroni correction. These studies support the hypothesis that the genes encoding proteins regulated by UBE3A may also contribute to idiopathic autism.

1897/W

Genome-wide scan of copy number variation in Attention deficit hyperactivity disorder (ADHD). *B. Franke[1,2], J. Hehir-Kwa[1], S. Vermeulen[1], J. Veltman[1], J. Lasky-Su[3], P. Asherson[4], M. Gill[5], J. Sergeant[6], R. Ebstein[7], A. Rothenberger[8], HC. Steinhausen[9], T. Banaschewski[10], R. Oades[11], E. Sonuga-Barke[12], A. Miranda[13], H. Royers[14], J. Buitelaar[2], S.V. Faraone[15] for the IMAGE consortium.* 1) Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2) Psychiatry, Nijmegen, NL; 3) Boston, MA, USA; 4) London, UK; 5) Dublin, Ireland; 6) Amsterdam, NL; 7) Jerusalem, Israel; 8) Göttingen, Germany; 9) Zurich, Switzerland; 10) Mannheim, Germany; 11) Essen, Germany; 12) Southampton, UK; 13) Valencia, Spain; 14) Ghent, Belgium; 15) SUNY Upstate Medical University, Syracuse, NY, USA.

Recent data suggest that copy number variants (CNVs) can contribute to complex disease susceptibility. The relative impact of CNVs compared to single nucleotide polymorphisms (SNPs) on one of the processes underlying disease vulnerability, variable gene expression, has been estimated to range around 18% (Stranger et al, Science 315:848-53). The involvement of CNVs in ADHD etiology has not been investigated. Within the International Multi-site ADHD Genetics Study (IMAGE), sponsored by the Genetic Association Information Network (GAIN), a whole genome association study investigating over 500.000 SNPs is currently carried out on 958 European Caucasian parent-child trios with offspring meeting the DSM-IV combined-type criteria for ADHD. Families were collected in the Netherlands, Ireland, the UK, Germany, Belgium, Switzerland, Spain and Israel. Using the intensity data from the SNP analysis carried out at Perlegen Sciences, copy number information will be extracted for each individual. In an overall analysis, we will identify known and new CNVs in the patients and their parents. By comparing parents with offspring we will investigate which CNVs are inherited, which are de novo. For inherited CNVs a TDT-based association study will be carried out. For those that occur de novo in the patients, we will investigate the gene content to find out if the CNV carries genes that can explain the presence of ADHD in the particular patient. In conclusion: CNV analysis in ADHD can potentially identify new candidate genes for this disorder.

1898/W

Candidate single nucleotide polymorphisms associated with case-control status from a genome-wide association study of Alzheimer's disease. *H. Li[1], S. Wetten[1], L. Li[1], P. StJean[1], R. Upmanyu[1], J. Williams[2], GenADA. Investigators[3], M. Plumpton[1], A.D. Roses[1], R.A. Gibson[1], M.C. Irizarry[1].* 1) GlaxoSmithKline, RTP and NFSP, USA and UK; 2) Cardiff University School of Medicine, Cardiff, UK; 3) Multi-Site Centres across Canada.

Identification of genes associated with more common forms of AD provides an opportunity to develop novel therapeutic targets, and to identify potential genetic factors influencing phenotypic expression of disease or responses to treatment. We performed a whole genome scan analysis in a case-control study of 753 AD patients and 736 non-demented control individuals from Canada as a hypothesis generating dataset. 100 SNPs with the strongest genetic association with AD were evaluated in a second dataset of 418 AD cases and 249 non-demented controls from Cardiff, UK, to assess generalizability and to reduce false negative associations. Association of each SNP genotype with AD was examined by multivariable logistic regression adjusting for age, sex, education, French Canadian ancestry (in Canada dataset), study site, and number of APOE ε4 alleles (The results from an age of onset analysis are presented in a separate abstract). In both datasets, the APOE ε4 allele was strongly associated with AD. In the multivariable adjusted logistic models, one ε4 allele had an OR for AD of 4.6 and two ε4 alleles an OR of 21.1-21.4 relative to 0 ε4 alleles ($p < 3.9 \times 10^{-24}$). Among the 100 most significant genes in the Canada dataset, there were four that replicated in the Cardiff dataset according to the following criteria: (1) allele frequencies sufficient to allow analysis; (2) nominal significance at $p < 0.05$; and (3) a risk genotype consistent with the Canada results. In conclusion, screening of the entire genome confirms that the APOE ε4 allele is the strongest risk factor for AD. There is evidence for additional SNPs associated with AD by replication in a second dataset, although these effects appear weak, and merit further evaluation.

1899/W

COPY-NUMBER VARIATIONS IN A CASE-CONTROL STUDY OF SCHIZOPHRENIA. *F. Martinelli Boneschi[1,2], F. Torri[1], S. Lupoli[1,2], A. Orro[3], C. Dal Fiume[1], G. Comi[2], D. Keator[4], J. Turner[4], J. Fallon[4], S. Potkin[4], C. Barlassina[1], F. Macciardi[1].* 1) University of Milan, Milan, Italy; 2) INSPE, Scientific Institute San Raffaele, Milan, Mi, Italy; 3) CILEA Consortium, Segrate Milan, Italy; 4) University of California, Irvine,Usa.

Recent studies have highlighted DNA copy-number variations (CNVs) as a largely under-explored source of human genetic variation, which could be responsible for the development of complex disorders. According to this hypothesis, evaluation of DNA copy number in schizophrenia may yield insights into the discovery of genetic risk factors for this disease, since CNVs can also be transmitted as mendelian traits(1). We have assayed 317.511 SNPs in 173 DNA samples from a case-control study of schizophrenia, including 91 controls and 82 schizophrenics, representing the first wave of a much larger sample, using the Illumina HumanHap300 Genotyping BeadChip®. In an effort to examine individual chromosomes for structural mutation, we used Homozygosity Detector and ChromoZone algorithms within BeadStudio v3.0.22 to detect respectively extended tracts of homozigosity and chromosomal aberrations in the single sample mode. Using the ChromoZone algorithm, we performed analyses at a genome-wide level, and found that some of the areas of CNV fall into regions previously known to be associated with schizophrenia. We also looked at the distribution of LOH regions larger than 2 Mb across the genome, and found that they seem to be equally distributed in cases and in controls. We selected CNV regions smaller than 4 Mbs and present in at least 1% of the screened sample, and tested if they were differentially distributed in cases and in controls. About 250 regions have been identified, and few of them are more frequent in cases than in controls. In our preliminary analyses, CNVs tend to be sparse in the genome, and they seem to be equally distributed between cases and controls. In the next step of our investigation, we will focus on the 250 already identified regions to get a prioritized list of those CNVs potentially associated to the disease. REFERENCES: 1. Sebat J. et al., Science 316, 445-49, 2007.

1900/W

Meta-analyses of Genetic Studies on Major Depressive Disorder. *C.M. van Duijn[1], S. Lopez-Leon[1], A.C.J.W. Janssens[2], A.M. Gonzalez-Zuloeta Ladd[1], J. Del Favero[3], S.J. Claes[4], B.A. Oostra[5].* 1) Epidemiology & Biostatistics, ErasmusMC, Rotterdam, Zuid Holland, Netherlands; 2) Department of Public Health, ErasmusMC, Rotterdam, Zuid Holland, Netherlands; 3) Applied Molecular Genomics Group, Department of Molecular Genetics, VIB, University of Antwerp, Antwerp, Belgium; 4) Department of Psychiatry, University of Leuven, Leuven, Belgium; 5) Clinical Genetics, ErasmusMC, Rotterdam, Zuid Holland, Netherlands.

The genetic basis of major depressive disorder (MDD) has been investigated extensively, but the identification of MDD genes has been hampered by conflicting results from underpowered studies. We reviewed all MDD case-control genetic association studies published before June 2007 and perform meta-analyses for polymorphisms that had been investigated in at least three studies. The study selection and data extraction were performed in duplicate by two independent investigators. The 183 papers that met our criteria studied 393 polymorphisms in 102 genes. Twenty two polymorphisms (6%) were investigated in at least three studies. Seven polymorphisms had been evaluated in previous meta-analyses, of which for five were new data available. Hence, we performed meta-analyses for 20 polymorphisms in 18 genes. Pooled odds ratios (ORs) with 95% confidence intervals (CI) were calculated. Statistically significant associations were found for the APOE ε2 (OR, 0.51), GNB3 825T (OR, 1.38), MTHFR 677T (OR, 1.20), SLC6A4 44bp ins/del S (OR, 1.11), alleles and the SLC6A3 40bpVNTR 9/10 genotype (OR, 2.06). Our meta-analyses found significant evidence for five MDD susceptibility genes (APOE, GNB3, MTHFR, SLC6A3 and SLC6A4). Together with our previously published meta-analysis on DRD4 there is evidence for six MDD susceptibility genes. The low coverage of genetic variants of candidate genes makes it impossible, however, to exclude that the other genes studied are not involved at all in MDD. Further, there is a need to standardize the methodology in research of MDD and other complex traits.

1901/W

Refinement of a candidate locus for dyslexia on chromosome 7q31-q34. *H. Matsson[1], K. Tammimies[1], H. Anthoni[1], M. Zucchelli[1], G. Schulte-Körne[2], J. Nopola-Hemmi[3], H. Lyytinen[4], M.M. Nöthen[5], A. Warnke[6], J.W. Gilger[7], G.W. Hynd[7], J. Kere[1, 8], M. Peyrard-Janvid[1].* 1) Dept of Biosciences & Nutrition, Karolinska Institute, Huddinge, Sweden; 2) Dept of Child & Adolescent Phychiatry and Psychotherapy, Univ of Marburg, Germany; 3) Dept of Pedriatrics, Jorvi Hospital, Finland; 4) Dept of Psychology & Child Reasearch Center, Univ of Jyväskylä, Finland; 5) Dept of Genomics, Life & Brain Center, Univ of Bonn, Germany; 6) Dept of Child & Adolescent Psychiatry and Psychotherapy, Univ of Würzburg, Germany; 7) Collage of Education, Dept of Educational Studies, Purdue Univ, West Lafayette, IN, USA; 8) Dept of Medical Genetics, Biomedicum, Univ of Helsinki, Finland.

Dyslexia is the most common childhood learning disorder and may have significant social consequences from early school years throughout life. The specific reading and spelling deficits are manifested in spite of normal intelligence, senses, education and social environment. At least nine loci (DYX1-9) contributing to dyslexia phenotypes have been mapped. We recently identified two genes from the DYX3 locus, C2ORF3 and MRPL19, associated with dyslexia and currently there are six candidate genes described. Our previous genome scan (Kaminen et al. 2003) suggested linkage to chromosome 7q31-q34 with a non-parametric linkage (NPL) score of 2.8 in 11 Finnish families with dyslexia as categorical diagnosis. Next, a more detailed analysis restricted the linkage peak to approximately 12 cM (max. NPL=2.5). In order to replicate these findings in an independent population we then genotyped 10 microsatellite markers throughout the region using 251 German families with a total of 429 dyslectics. The results did not support linkage of the markers to the German samples. To pinpoint the risk variants in the linked region suggested by the Finnish sample set, we have now saturated a 20 Mb region on chromosome 7q31-q34 with 158 SNPs, all with a minor allele frequency >0.25. Potential candidate genes were selected on the basis of known functions in brain development affected in dyslexia and were specifically targeted with tagging SNPs. We are currently analysing the genotyping results from 280 individuals of both Finnish as well as US origins.

1902/W

A Genome-wide association study using DNA pooling identifies evidence for novel susceptibility genes for Alzheimer's Disease. *R. Abraham[1], G. Kirov[1], V. Moskvina[1], A.R. Morgan[1], P. Hollingworth[1], L. Georgieva[1], S. Lovestone[2], M. O'Donovan[1], M. Owen[1], J. Williams[1].* 1) Dept Psychological Medicine, Cardiff University, UK; 2) Institute of Psychiatry, King's College London, UK.

Late-onset Alzheimer's Disease has a strong genetic component but the only replicable genetic risk factor identified to date is the (ε)4 allele of APOE. To identify additional susceptibility loci we have conducted a genome-wide association study using DNA pooling to reduce costs. DNA pools were constructed from 1000 LOAD cases and 1200 age, gender and ethnicity matched controls. Pools were hybridised to Illumina HH300 and HH240S arrays, assaying over 550,000 SNPs. For each SNP the ratios of intensities for allele A and allele B (A/(A+B)) were used to predict allele frequencies. To reduce technical error each hybridisation was replicated between 4 and 8 times and predicted allele frequencies averaged over high quality replicates.(

)In order to prove that our experiment was capable of detecting a true genetic association, we examined whether we were able to detect the known association at the APOE locus. Eight SNPs were identified in the APOE region with predicted allele frequency differences between cases and controls of 5-14%. Four of these 8 SNPs have been individually genotyped and show significant association with LOAD (p-values ~ 7 x 10-9 to 4 x 10-15), demonstrating the accuracy of our pooling method.(

)We are currently individually genotyping 100 SNPs with the greatest predicted differences in allele frequencies. Initially we focused on SNPs in clusters with other significant SNPs. To date we have identified 16 significant SNPs in our case / control sample (p (<)0.02). Our most significant SNP has p=0.00002 (Odds Ratio 1.298 CI: 1.151-1.463) and displays linkage disequilibrium with SNPs in a gene showing putative functional candidacy for AD. We are currently individually genotyping functional and tagging SNPs in this and other promising genes in addition to SNPs from the pooled experiment dataset.

1903/W

Two genome-wide association studies suggest DFNB31 as a risk locus for bipolar disorder. *A.E. Baum, M. Cabanero, N. Akula, F.J. McMahon.* National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

At this writing, two genome-wide association studies in three datasets have been published for bipolar disorder. Our group (NIMH) screened 555,235 SNPs in two independent samples (Illumina HumanHap550 platform, 1233 cases/1439 controls). We identified 88 SNPs in 80 genes (Baum et al 2007) and 78 SNPs in intergenic regions that met replication criteria in both samples. The Wellcome Trust Case-Control Consortium (WTCCC 2007) screened an independent sample (1868 cases/2398 controls) with the Affymetrix GeneChip 500K Mapping Array Set. They reported 14 SNPs associated with bipolar disorder at $p < 1x10^{-5}$. Since each study used a different platform, direct comparison of SNPs is largely impossible. Each study identified one SNP reaching genome-wide significance levels, but the two SNPs are on different chromosomes. However, the list of replicated SNPs from the NIMH study includes 2 SNPs (rs942518 and rs16929770) that tag a ~40 kb region on 9q32 which has been previously linked to bipolar disorder. This region also contains one SNP reported by WTCCC to be associated with bipolar disorder at $p = 8.8x10^{-6}$. The 2 NIMH SNPs are located 5.2kb and 7.4kb upstream of the promoter region of the gene DFNB31. The WTCCC SNP is located ~13 kb away in the first intron of the gene. DFNB31 encodes the neuronally-expressed protein whirlin, a component of the Usher protein complex, which affects neuronal morphogenesis and structural plasticity and is an effector of β-catenin. Additional high-density genotyping in this region is now underway to answer the question of whether the same allele is associated with bipolar disorder in both studies.

1904/W

Population-based WGA studies of cigarette smoking reveal novel genes for nicotine dependence. *W. Berrettini[1,2], X. Yuan[2], K. Song[2], D. Waterworth[2], H. Chilcoat[2], P. Vollenweider[3], G. Waeber[3], M. Preisig[3], P. Muglia[2], F. Tozzi[2], V. Mooser[2].* 1) Dept Psychiatry, Univ Pennsylvania, Philadelphia, PA; 2) GSK R&D, Upper Merion, PA, Research Triangle Park, NC and Verona, Italy; 3) CHUV University Hospital Lausanne Switzerland.

BACKGROUND: Cigarette smoking, a major risk factor for lung and cardiovascular diseases, is the single largest preventable source of morbidity and mortality in North America and Europe. Twin and adoption studies indicate that a majority of risk for nicotine dependence (ND) is genetic. The goal of the present study was to find novel genes associated with ND, in an effort to identify new drug targets for this addiction. METHODS: We conducted WGA analyses of cigarettes-per-day (CPD) as a quantitative trait in a cross-sectional population-based sample of 5641 European-origin subjects from the city of Lausanne, Switzerland, and in a second sample of ~974 European-origin persons, selected for the presence or absence of dyslipidemia. Genotyping was done with Affymetrix 500K chips. Finally, CPD analysis of ~ 8000 individuals of European origin were also completed for selected candidate genes. RESULTS: Combined analysis of the two datasets revealed ~ 20 genes (and 561 SNPs) containing one or more alleles which were nominally significant ($p < 0.05$) for CPD in both samples, with the same allele identified as conferring ND risk. These 20 genes included CHRNA3 ($p = 0.00007$), identified as a ND risk gene ($p = 0.0003$) by Saccone et al (HMG 16:36, 2007). Three additional genes were also nominally significant both in the present two studies ($p < 0.003$) and in the one ND WGA study published (Bierut et al, HMG 16:24, 2007, $p < 0.02$). Analyses of CPD in ~ 8000 additional individuals of European origin confirmed CHRNA3 ($p = 0.0000026$) as a risk allele for this quantitative trait of CPD. CONCLUSION: This comprehensive approach implicates several genes identified in one previous WGA study of ND. A more precise phenotypic definition of ND using specific questionnaires is currently ongoing which is expected to provide additional insight into genetic deteminants of ND.

1905/W

Genome-Wide Association Study of Bipolar Disorder in European Americans. *J. Li[1], L.J. Scott[3], D. Absher[1], R.C. Thompson[4], W. Guan[3], F. Meng[5], A. Southwick[1], M. Burmeister[4,5], H. Akil[5], S.J. Watson[5], R.M. Myers[2], M. Boehnke[3].* 1) Stanford Human Genome Center; 2) Department of Genetics, Stanford University, Palo Alto, CA; 3) Department of Biostatistics and Center for Statistical Genetics; 4) Department of Psychiatry; 5) Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI.

Bipolar disorder (BPD) is a common familial disease with poorly understood etiology. Despite clear evidence for a substantial genetic contribution, linkage and candidate gene studies have so far failed to generate well-replicated findings. We have performed a genome-wide association study of BPD in which we used the Illumina Infinium Beadchips to genotype >550,000 "tagging SNPs" in European American samples. We obtained from the NIMH Genetic Repository program ~1,200 Bipolar I cases, up to two BPD cases per sibship, and ~800 unrelated controls who reported no BPD, schizophrenia, or major depression. Cases and controls were frequency-matched by self-reported ancestry from different regions of Europe. The analysis of the first 466 cases and 467 controls showed that >99% of the samples had initial call rates of >98.5%, and >99.5% of SNPs passed all QC measures. Inferred ancestry showed reasonable agreement with self-reported ancestry, and confirmed adequate sample matching, with a genomic control inflation factor of 1.02. Allelic intensity data revealed extensive copy number variation (CNV), covering a broad range of CNV sizes and population frequencies. We have now completed genotyping for the ~2,000 samples, and data cleaning and association analysis is currently underway for the entire dataset. To increase statistical power, we will augment our analysis with publicly-available control datasets for Americans of European ancestry.

The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C.

1906/W

Cadherin 13, addiction, alternative splicing, and aging. *Q. Liu[1], D. Walther[1], A. Hishimoto[1], T. Drgon[1], C. Johnson[1], E. Roach[1], O. Pletnikova[2], J.C. Troncoso[2], G.R. Uhl[1].* 1) Molecular Neurobiology Branch, NIDA/NIH, Baltimore, MD; 2) Department of Pathology (Neuropathology), Johns Hopkins School of Medicine Baltimore, MD.

Genome-wide association studies comparing substance-dependent vs control and successful vs unsuccessful abstainers from smoking have each identified SNPs in the 3' region of the cadherin 13 (CDH13) gene. CDH13 is a "cell adhesion molecule" that anchors to plasma membranes through a glycosyl-phosphatidyl-inositol (GPI) anchor, is well positioned to play roles in regulation of synaptic connectivities that are of interest for brain disorders that include addictions and neurodegenerations. Analysis of the 1.2 Mb human CDH13 gene revealed novel human-specific CDH13 splicing isoforms that include GPI-anchored- and soluble isoforms with five and single cadherin motifs, respectively. The CDH13 splice sites that are involved in these differentially-spliced gene products near the addiction-associated SNPs. We thus sought relationships between CDH13 SNPs and the expression of mRNAs encoding CDH13 isoforms in mRNAs prepared from 135 frontal cortical specimens from individuals who died at ages ranging from 13 to 98. Substantial individual differences in expression of soluble isoforms were identified among these brains. Some individuals appeared to express two soluble isoforms, some expressed one soluble isoform, and some expressed none. We were not able to identify significant reproducible relationships between these expression patterns and addiction-associated SNPs. However, more of the individuals who died at younger ages displayed two soluble isoforms, while more of the individuals who died at older ages expressed no detectable mRNA encoding a soluble isoform. Membrane bound isoforms were expressed universally in samples from each age group tested. While we continue to seek other reproducible molecular correlates of the haplotypes associated with addiction, the differential age-related expression of CDH13 isoforms that we report here might contribute to the brain's age-related vulnerabilities to disorders of synaptic function. (Support: NIH IRP (NIDA)).

1907/W

DNA pooling in a Whole-Genome case-control study. *S. Lupoli[1], C. Barlassina[2], F. Martinelli Boneschi[1], A. Orro[3], F. Esposito[1], F. Torri[2], J. Turner[4], S. Potkin[4], G. Comi[1], F. Macciardi[2].* 1) ISPE, San Raffaele Scientific Institute, Milan, Italy; 2) University of Milan, Dept. of Science and Biomedical Technology; 3) CILEA Consortium, Segrate Milan Italy; 4) University of California, Irvine.

Pooling genomic DNA samples within clinical classes of disease, followed by genotyping on whole-genome SNP microarrays, allows for rapid and less expensive genome-wide association studies. Aims of the study: 1) to measure the correlation within pools replicates, and between pools and individual genotyping, 2) to explore whether the degree of correlation is influenced by other factors, such as the minimum allele frequency (MAF) of SNPs. We created a total of 8 pools of DNA samples within a case-control study of schizophrenic patients. To assess variance in allele frequencies attributable to the pooling procedure we created each pool twice: 2 case pools and 2 control pools, 28 and 35 subjects for pool. Moreover, to assess if the number of DNAs in each pool could influence the allele frequencies, we splitted our DNA samples into 4 pools: 2 case pools and 2 control pools, 14 subjects for the 2 case pools, and 17, 18 subjects for each control pool. Each pool has been replicated four times, leading to a total of 32 (four multiplied by eight) readings. Genotyping was performed using Illumina HumanHap300 duo. We restricted our initial analyses on chromosome 22, and we selected common SNPs (n=5355) between HumanHap300, on which individual genotyping was performed, and HumanHap300 duo. We infer allelic frequency of pools by using correction factors which take into account dye intensities in heterozygotes and homozygotes of individual genotyping (1). Pearson's correlation coefficient within pool replicates was 0.96. The assessment of the correlation coefficient between pools and individual genotyping is on going. 1 Baum et al. Molecular Psichiatry 2007, 1-11.

1908/W

Towards a Molecular Classification of Psychiatric Illness. *A.K. Malhotra[1], P. DeRosse[1], T. Lencz[1], K.E. Burdick[1], T.V. Morgan[2], J.M. Kane[1], R. Kucherlapati[2].* 1) Psychiatry Research, Zucker Hillside Hospital, Glen Oaks, NY; 2) Harvard Partners, Center for Genetics and Genomics, Boston, MA.

Schizophrenia (SCZ) is a complex neuropsychiatric disorder characterized by a range of clinical manifestations, including positive, negative, and disorganized symptoms, yet relatively limited work has been conducted to parse out the specific clinical phenomena associated with risk genotypes. Thus, a major goal of our research group is to not only identify candidate genes that predispose to SCZ but also to assess the relation between genotype and refined phenotypes that include symptom domains and neurocognitive function. Using this approach, our group identified a risk haplotype within the gene encoding for dysbindin (DTNBP1) that was not only associated with SCZ but was more specifically associated to a form of the disorder that is characterized by prominent negative symptoms and generalized cognitive impairment. Further, our group also found a relation between variation in DISC1 and risk for SCZ with additional analyses revealing associations to severity of positive symptoms as well as specific deficits in cognitive function (working memory). Candidate gene studies, however, are inherently limited in their scope and fail to adequately address the complexity of SCZ. Therefore, we have recently utilized more comprehensive approaches to identify disease-associated genes. Using the Affymetrix 500k array we conducted the first case-control whole genome association study of SCZ and were successful in identifying a novel risk locus. Using data obtained from the WGA analyses we have also begun to fine map regions of the genome which have previously been associated through linkage analyses with phenotypic variation within SCZ. These analyses have also identified novel loci that may contribute to the heterogeneity of SCZ. Combined with our previous work, these data represent the initial steps towards the molecular classification of psychiatric illness.

1909/W

A Whole Genome Association Study in Parkinson's Disease Using the Rosetta Syllego System and Publicly Available Genotype Data. *M.A. Peters, A.Q. Chen, C. Davis, E.S. Paegle.* Rosetta Biosoftware, Seattle, WA.

Our goal is to identify genetic variation associated with Parkinson's disease (PD) and to identify potential biomarker targets. Using publicly available genotype data, we performed a genome-wide association (GWA) study in a case control cohort to further analyze previous work (Fung et al. Lancet Neurology 2006). The study comprised 271 Parkinson's disease patients and 270 neurologically normal patients. The 408,803 markers genotyped include a combination of Illumina® Human-1 and HumanHap 300 arrays. The genotype data were obtained from the National Institute of Neurological Disorders and Stroke (NINDS) repository at the Coriell Institute (https://queue.coriell.org/Q/snp_index.asp). The analyses were carried out using the Syllego Genetic Data Management and Analysis system and PLINK, an open source whole genome association analysis toolset (http://pngu.mgh.harvard.edu/purcell/plink/). An initial analysis using PLINK identified 39 significant SNPs (P-value < 0.0001, maximum permutation) which confirmed 23 of 26 markers associated with PD identified by Fung et al. Examination of 800 kb regions surrounding the 39 significant SNPs identified 404 genes located on or near the SNPs. Five of these genes (GSTT1, MIF, CXCL12, UCHL1, and PHOX2B1) are reported in the Genetic Association Database (GAD) to be associated with neurological disorders and will be characterized further. A linear regression analysis on age of onset for the 271 PD patients and 39 significant markers found four significant markers (P-value < 0.05) in complete LD on GLT25D2 on chromosome 1. A logistic regression analysis on the same data set (271 PD and 39 markers) identified 2 significant markers (P-value < 0.05) associated with family history of PD. One of these is located on chromosome 13 in a gene poor region and the other is located on chromosome 4 near UCHL1 and NSUN7. UCHL1 is reported in the GAD to be associated with neurological disorders. We have confirmed previous potential associations with PD and have identified additional markers that merit further analysis. We have shown that genotype and phenotype data can be combined in the Syllego system to identify biomarker targets.

1910/W

Copy number variants and one novel susceptibility region identified in a genome-wide association study of autism in an isolated population. *K. Rehnström[1,2], H. Kilpinen[1], E. Gaál[1], T. Ylisaukko-oja[1], R. Vanhala[3], L. von Wendt[3], T. Varilo[1,2], L. Peltonen[1,2,4].* 1) Department of Molecular Medicine, National Public Heath Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland; 4) Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA, USA.

Autism spectrum disorders (ASDs) have a strong genetic component, but only a small number of rare genetic causes have been identified so far. Results of recent genome-wide association scans have highlighted the role of copy-number variations in the etiology of autism. To identify genes predisposing to autism, we genotyped 54 ASD probands from families originating from an internal isolate of Finland and 37 regionally matched controls using the Illumina HumanHap 300 BeadChip. All probands have at least two grandparents originating from Central Finland, and 19 of these families form an extended pedigree, that can be traced back to the same farm in a small village in Central Finland in the 18th century. This would provide an ideal setting for the use of shared haplotype strategy in the identification of high impact rare alleles, enriched in this isolate. Association analysis of single markers as well as shared allelic haplotypes performed using PLINK software identified a putative susceptibility locus at 6p22.2. Further, analysis of the 54 cases indicated five copy number variants (CNVs) exceeding 1Mb, resulting in a frequency of 9% for large CNVs. Three of the CNVs were gains of chromosome 15q11.2-q13.1, all exceeded 5Mb. Two other gains were also quite sizable, one at 1q42.13-42.2 spanning approximately 2 Mb and one at 9q21.33 approximately 1.5 Mb. None of the CNVs were identified in the controls. The results of CNV analysis support the role of CNVs in the etiology of autism. Additional SNPs at 6p22.2 are being genotyped in the whole Finnish autism sample to confirm the validity of the initial haplotype sharing.

1911/W

A genomewide association study of chronic fatigue syndrome. *A.K. Smith, S.D. Vernon, E.R. Unger, M.S. Rajeevan.* Centers for Disease Control, Atlanta, GA.

Chronic fatigue syndrome (CFS) is a complex disorder of unknown etiology. Current hypotheses suggest hypoactivity of the hypothalamic pituitary adrenal (HPA) axis leads to psychoneuroendocrine and immune alterations. While multiple studies support a genetic contribution to CFS, genomewide efforts to identify associated loci remain unexplored. This study addresses the role of genetic variation in CFS by evaluating 116,204 single nucleotide polymorphisms (SNPs) in 40 empirically-defined CFS cases and 40 non-fatigued (NF) controls identified in a population-based study. DNA extracted from peripheral blood mononuclear cells was amplified prior to genotyping. Chi-square tests were used to assess association between a marker and case status, and p-values were estimated using 10,000 Monte Carlo simulations. Case and control subjects did not significantly differ in age, sex, body mass index, or history of major depressive disorder.

Allelic association tests revealed 65 SNPs with p-values ranging from 0.00005-0.001 that were consistent with Hardy-Weinberg proportions in controls. Associated SNPs reside in or near genes related to brain function (*PPFIBP1*, *NPAS2*, *ARHGAP20*), glutamate neurotransmission (*GRIK2* and *GRIN2B*), immunity (*LILRB4*), inflammation (*NLRP13*, *NLRP11*, *PELI1*) and metabolism (*MTAP*) as well as expressed sequence tags identified from HPA axis tissues. To further interrogate the roles of these 65 SNPs with HPA axis function, association with morning serum cortisol levels was examined using analysis of variance adjusted for age, sex, and BMI. SNPs in *NLRP13*, *GRIK2*, and *MTAP* were also associated with cortisol levels (p= 0.0015-0.043). Further analysis of *NLRP13*, a gene that activates proinflammatory mediators that influence the neuroendocrine system, identifies a haplotype (33.9%) associated with CFS (p=0.001) and with decreased serum cortisol (p=0.005), consistent with hypoactivity of the HPA axis. This study, though limited by sample size, identifies candidate genes not considered in prior studies and supports psychoneuroendocrine and immune disruption in CFS. Further studies will be required for fine mapping, functional validation and to replicate these findings in additional populations.

1912/W

Genome-wide Scan for Association to Schizophrenia. *J.L. Stone[2,7], Intl. Schizophrenia Consortium[1,3,4,5,6,8,9,10].* 1) Inst. of Med. Sci., Univ. of Aberdeen, Aberdeen, UK; 2) Psych. Genet. Initiative, The Broad Inst. of Harvard and MIT, Cambridge, MA; 3) Dep. of Psychol. Med., Cardiff Univ., Cardiff, UK; 4) Windeyer Inst. of Med. Sci., UCL, London, UK; 5) Div. of Psych., Univ. of Edinburgh, Edinburgh, UK; 6) Dep. of Med. Epidemiology and Biostat., Karolinska Inst., Stockholm, Sweden; 7) Psych. and Neurodev. Genet. Unit, MGH, Boston, MA, USA; 8) Dep. of Psych., UNC, Chapel Hill, NC; 9) Dep. of Psych. and Behavioral Sci., USC, Los Angeles, CA; 10) Inst. of Molec. Med., TCD, Dublin, Ireland.

Despite evidence of a strong inherited genetic component in the etiology of schizophrenia, research suggests that each variant may have a small to modest contribution to overall genetic susceptibility. To obtain the number of samples necessary to have sufficient power to identify susceptibility loci of modest effect, researchers from institutions across Europe and the US have created a consortium to perform a case-control whole genome association study. Participating institutions are: Univ. of Aberdeen, The Broad Inst., Cardiff Univ., Univ. College London (UCL), Univ. of Edinburgh, Karolinska Inst., Massachusetts General Hospital (MGH), Univ. of North Carolina (UNC), Univ. of Southern California (USC), and Trinity College Dublin (TCD). Samples were collected at sites representing six countries. Schizophrenic cases, diagnosed using comparable semi-structured interviews, and an approximately equal number of population-based controls were contributed by seven of the above listed institutions (excluding the Broad Institute, MGH and UNC). Samples were genotyped on either the 500K (5.0 SNP Chip) or 1 million (6.0 SNP Chip) Affymetrix SNP genotyping platform. Data were cleaned and analyzed using the genetic analysis package, Plink. Approximately 3800 cases and 4200 controls have been genotyped in two waves at The Broad Institute of Harvard and MIT. The first wave of samples (n ~ 3300) was genotyped on 500,000 SNPs while the second wave of samples was genotyped on 1 million SNPs, reflecting the current rapid evolution of genotyping technologies. Here we present genotyping performance, quality control steps and preliminary association analysis.

1913/W

A genome-wide association study of autism finds association to a locus on chromosome 1. *L. Weiss[1,2], T. Green[1,2], S. Santangelo[1], P. Sklar[1,2], M. Daly[1,2].* 1) Center for Human Genetic Research, Harvard-MGH, Boston, MA; 2) Broad Institute, Harvard-MIT, Cambridge, MA.

Autism has been estimated to be the most heritable psychiatric disease, yet, to date, linkage and association studies have not explained a substantial proportion of the genetic susceptibility to autism. We have partnered with the Autism Genetics Research Exchange (AGRE) to conduct a large genome-wide association study on over 2500 members from more than 700 multiplex families from the AGRE family database using the Affymetrix 5.0 platform. Employing a new algorithm, Birdseed, to make genotype calls, and PLINK for QC and genome-wide analysis, we have obtained data for more than 400,000 polymorphic SNPs that pass strict QC thresholds. Association was evaluated with a family-based TDT. Preliminary analysis reveals one locus on chromosome 1 in a nongenic region that meets strict criteria for genome-wide significant association by the TDT. This will be followed up by integration of independently genotyped NIMH control samples to increase power and additional genotyping in the chromosome 1 region to finemap the association signal. Several other signals of suggestive significance will also be followed up. In addition, we are performing a high resolution evaluation of de novo copy number abnormalities and testing common copy number polymorphisms for association with autism, using SNP probes as well as an additional 420,000 distinct invariant oligonucleotide probes intended expressly for copy number estimation.

1914/W

Candidate single nucleotide polymorphisms associated with age of onset from a genome-wide association study of Alzheimer's disease. *S. Wetten[1], L. Li[1], P. St. Jean[1], R. Upmanyu[1], J. Williams[2], GenADA. Investigators[3], M. Plumpton[1], A.D. Roses[1], R.A. Gibson[1], M.C. Irizarry[1].* 1) GlaxoSmithKline, NFSP and RTP, USA and United Kingdom; 2) Cardiff University School of Medicine, Cardiff, UK; 3) Multi-site centre across Canada.

Twin and family studies support a strong genetic component to late-onset Alzheimer's disease (AD), for which the APOE ε4 allele is the major identified risk factor. Case-control association analyses have reported multiple genetic polymorphisms as potential risk factors for AD. Our objective was to identify candidate SNPs associated with age of onset in a primary population and replicate in a secondary population. We performed an association analysis with Cox proportional-hazards regression using the Affymetrix 500K Array stratified by education and adjusting for gender, French-Canadian ancestry, number of APOE ε4 alleles and study site. Our primary population consisted for 753 cases in Canada and 736 ethnically-matched controls. Our replication population consisted of 418 cases and 249 controls from the UK. 469,438 SNPs passed initial quality controls with >70% genotyping efficiency and HWE p>10-7. For SNPs with minor allele frequency (MAF) ≥ 0.10, dominant, additive, and recessive effects were examined in the multivariable adjusted Cox model; the Wald p-value for the optimal model adjusted for the 3 genetic tests is reported. For SNPs with 0.10 > MAF ≥ .005, only the dominant model was tested. One SNP was significant in the primary dataset after study-wide Bonferroni correction. The top 100 SNPs from the primary analysis were examined in the secondary dataset under the same genetic model. Two of these SNPs were nominally significant in the second dataset with the same risk genotypes. APOE ε4 was the main risk factor for AD, with HR 2.0-2.5 for 1 ε4 allele and 3.9-6.4 for 2 ε4 alleles relative to no ε4 alleles. These associations, from the first genome-wide assessment of AD age of onset, will be reported. Once published, results will be released into the public domain to aid the evaluation and discovery of further susceptibility loci which may have definitive but modest effects on AD.

1915/W

Polymorphisms in the AMPA receptor 1 gene region are associated with psychotic symptoms in bipolar disorder. *B. Kerner, A. Jasinska, J. DeYoung, M. Almonte, O.-W. Cho, N.B. Freimer.* Ctr Neurobehavioral Genetics, Univ California, Los Angeles, Los Angeles, CA.

Bipolar disorder with psychotic symptoms has been linked to a region on chromosome 5q31-33 in several studies by us and others. We report here the fine-mapping of this region in 56 nuclear families (299 individuals) from the National Institute of Mental Health Bipolar Genetics Initiative (NIMH-BPGI) data sets. 1134 single nucleotide polymorphism (SNP) markers were genotyped in those families that contributed to the original linkage finding on chromosome 5q across a 9.4 Mb region under the linkage peak, as well as in a replication sample. Family based association in the presence of linkage was then tested with the computer software package FBAT. Twenty-nine SNPs were significantly associated with the phenotype (empirical p-value <.05) in the initial sample. One of those SNPs was also marginally associated with psychotic bipolar disorder in the replication sample (p-value=0.06). These SNPs were located in the first and second intron of the α-amino-3-hydroxy-5-methyl-4-isoxasole proprionic acid (AMPA) subunit 1 receptor gene (GRIA1) (rs472792, rs524905), in the first intron of the IL2-inducible T-cell kinase (ITK) (rs452223), and in intron 6 of the Early B cell factor 1 gene (EBF1) (rs4244438). One SNP (rs2421050) was located in a gene poor region without known significance. The AMPA1 receptor has been shown to influence cognitive functions, such as working memory and reward learning. Variations in this gene have been implemented in the pathogenesis of schizophrenia (SZ). Our findings suggest that variations in this receptor may contribute to the pathophysiology of bipolar disorder with psychotic features.

1916/W

Analysis of the Fragile X Mental Retardation Genes in Autistic Individuals. *D. Okou, M. Zwick.* Dept Human Genetics, Emory Univ School of Medicine, Atlanta, GA.

Autism spectrum disorders (ASDs) are common, heritable neurodevelopmental disorders. The genetic architecture of ASDs appears to arise from the alleles at a large number of loci. One of the most striking aspects of ASD is the pronounced 4:1 male bias among affected individuals. This suggests that susceptibility alleles on the X chromosome may contribute to ASDs. Triplet repeat expansion mutations at the X-linked FMR1 and FMR2 loci have been shown to cause mental retardation in males. Interestingly, approximately 20-25% of patients with the FMR1 triplet repeat expansion mutation that leads to Fragile X also display symptoms characteristic of ASD. However, screening of the FMR1 and FMR2 genes and surrounding non-coding genomic regions is not routinely conducted. We are comprehensively resequencing the FMR1 and FMR2 loci in order to test the hypotheses that these loci harbor variation that contributes to ASDs. We have used high throughput chip-based resequencing to accurately identify all rare and common variants in male affected sibpairs (ASPs) from the Autism Genetic Resource Exchange (AGRE) collection. Our sample for resequencing consists of 314 cases and 314 controls who share the same region of the X chromosome that includes FMR1 and FMR2. One of the male ASPs is chosen as case, and the corresponding father is selected as control. To isolate our candidate region for resequencing, our novel Microarray-based Direct Genomic Selection (MGS) protocol is being used to isolate target DNA from each sample. Hybridization to a custom RA then determines the DNA sequence. Our initial analyses demonstrate call rates of > 90% with accuracy estimates of fewer than 1 error per 100,000 bases sequenced. SNPs identified are annotated and partitioned into functional classes (UTR, silent, replacement, intron, intergenic) and compared within and between these classes. We will present sequence data from the resequencing of 300 individuals, that describe both the normal levels of variation in addition to alleles that may contribute to ASD.

1917/W

Epilepsy and Mental Retardation Limited to Females (EFMR): A unique inheritance pattern and linkage to Xq22. *L.M. Dibbens[1,2], I.E. Scheffer[3,4], M.A. Bayly[1], S.J. Turner[3], K. Friend[1], B.L. Hodgson[1], K. Hynes[1,5], E.A. Haan[1], A. Mazarib[6], Z. Afawi[6], M.Y. Neufeld[6], P.I. Andrews[7], G. Wallace[8], S. Kivity[9], D. Lev[10], T. Lerman-Sagie[10], A.D. Korczyn[6], S.F. Berkovic[3], J. Gecz[1,2,5], J.C. Mulley[1,2,5].* 1) Department of Genetic Medicine, Women's and Children's Hospital, Adelaide, South Australia, Australia; 2) School of Paediatrics and Reproductive Health, the University of Adelaide, Adelaide, South Australia; 3) Department of Medicine (Neurology), The University of Melbourne and Austin Health, Heidelberg, Melbourne, Victoria; 4) Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Melbourne; 5) School of Molecular and Biomedical Sciences, University of Adelaide, South Australia, Australia; 6) Department of Neurology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 7) Sydney Children's Hospital, Randwick, New South Wales, Australia,; 8) Mater Medical Centre, South Brisbane, Queensland, Australia; 9) Department of Neurology, Schneider Children's Medical Centre, Petaq Tikvah, Israel; 10) Metabolic Neurogenetic Clinic, Wolfson Medical Centre, Holon, Israel.

Epilepsy and Mental Retardation limited to Females (EFMR) is a striking disorder following X-linked inheritance where females are affected and males transmit the condition. EFMR is characterized by early onset seizures in previously normal infants, followed by developmental regression of varying severity. The one previously described EFMR family was mapped to Xq22. We aimed to refine the clinical phenotype of the disorder and to ultimately determine the molecular genetic basis of EFMR. We ascertained four new unrelated EFMR families, two Australian and two Israeli, and haplotype analysis was consistent with linkage to Xq22 for each family. Detailed clinical assessment was performed on 58 individuals. EEGs showed generalized and focal epileptiform abnormalities. Five obligate male carriers had obsessional tendencies. We conclude that EFMR is more common than appreciated and the epilepsy syndrome occurs with intellectual disability and psychiatric features. The unique mode of inheritance suggests a novel genetic mechanism for human disease.

1918/W

Beyond maleness: The Y-chromosome as a risk factor for ADHD and autism. *J.R. ten Bosch[1,2], B. Merriman[1], R.M. Cantor[1], P.K. Gregersen[3], D.H. Geschwind[1], J.J. McGough[1], S.L. Smalley[1], S.F. Nelson[1].* 1) David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, NY.

ADHD and autism are complex neurobehavioral disorders with a strong male bias. Both disorders are approximately four-times more prevalent in males than females. For idiopathic autism, at least a portion of this bias appears to be attributed to risk factors present on chromosome 17 in affected males (Stone et al. 2004; Cantor et al. 2005). To further clarify the underlying etiology that gives rise to this sex bias in autism and ADHD, we examined Y-chromosome copy number and haplotype in affected and control individuals. We found XYY individuals were much more common among male probands, particularly those affected with ADHD (*P*-value < 0.00001), than among control subjects. In addition, we identified Y-chromosome haplogroups that were significantly associated with both these disorders, thus demonstrating that the Y-chromosome contribution to the acquisition of ADHD and autism is of greater significance than previously appreciated.

1919/W

Association study of neuroprotection genes erythropoietin, heme-oxigenase 2, and kallikrein 1 with stroke. *S. Violante[1], T. Krug[1], H. Manso[1,2], B.V. Fonseca[1], L. Gouveia[3], I. Albergaria[2], G. Gaspar[2], R. Taipa[4], M.R. Silva[5], M. Correia[4], M.V. Baptista[6], A. Pinto[7], R. Silva[7], G. Lopes[4], J.P. Gabriel[5], I. Matos[8], J.M. Ferro[3], A.M. Vicente[1,2], S.A. Oliveira[1].* 1) Instituto Gulbenkian de Ciência, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal; 3) H. Santa Maria, Portugal; 4) H. Geral de Santo António, Portugal; 5) H. São Pedro, Portugal; 6) H. Garcia de Orta, Portugal; 7) H. Fernando Fonseca, Portugal; 8) H. Distrital de Mirandela, Portugal.

Recent animal studies of cerebral ischemia, hypoxia and oxidative stress allowed the identification of several neuroprotective molecules, but most of the genes encoding for these proteins or hormones have not been tested as risk markers for stroke. In this study, we tested the association of erythropoietin (EPO), heme-oxigenase2 (HO2), and kallikrein1 (KLK1) genes with stroke in a Portuguese population. EPO, a critical cytokine in hematopoiesis, has been localized in the CNS where its upregulation by the presence of hypoxia has been observed after ischemia, protecting neurons by inhibiting their apoptosis. HO2 is believed to be an important endogenous neuroprotective agent against oxidative stress in the brain. It is constitutively expressed, but its activity can be modulated by phosphorylation. The KLK1 gene encodes a serine protease that catalyzes the release of vasoactive peptides and may be involved in hypertension and cardiovascular diseases. KLK1 gene seems to provide neuroprotection against cerebral ischemia injury by enhancing glial cell survival and migration and inhibiting apoptosis through suppression of oxidative stress and activation of signaling pathways. We genotyped 3, 3, and 5 tagging SNPs in the genes and flanking regions in EPO (2.9 kb), HO2 (33.9 kb) and KLK1 (4.6 kb), respectively, on 533 stroke patients (82% ischemic strokes) and 507 unrelated controls. We found weak evidence of association (OR=0.82, 95% CI=0.67-0.99, p=0.04) with stroke risk for SNP rs7702, located 0.5 kb downstream of HO2. Other allele or haplotype association tests did not reveal any significant findings, suggesting that these neuroprotection genes are not important risk factors for stroke.

1920/W

Family-Based Association of FKBP5 in Bipolar Disorder. *V.L. Willour[1], H. Chen[2], J. Toolan[1], P. Belmonte[1], D.J. Cutler[3], F.S. Goes[1], P.P. Zandi[4], D.F. MacKinnon[1], F.M. Mondimore[1], B. Schweizer[1], J.R. DePaulo, Jr.[1], E.S. Gershon[5], F.J. McMahon[6], J.B. Potash[1].* 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Dept Psychiatry, Univ of Michigan, Ann Arbor, MI; 3) Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 4) Dept Mental Health, Johns Hopkins Univ, Baltimore, MD; 5) Dept of Psychiatry, Univ of Chicago, Chicago, IL; 6) Mood and Anxiety Program, NIMH, Bethesda, MD.

The FKBP5 gene product forms part of a complex with the glucocorticoid receptor and can modulate cortisol-binding affinity. Variations in the gene have been associated with increased recurrence of depression and with rapid response to antidepressant treatment. We sought to determine whether common FKBP5 variants confer risk for bipolar disorder. We genotyped seven tag SNPs in FKBP5, plus two SNPs previously associated with illness, in 317 families with 554 bipolar offspring, derived primarily from two studies. Single marker and haplotypic analyses were carried out with FBAT and EATDT employing the standard bipolar phenotype. Association analyses were also conducted using eleven disease-related variables as covariates. Under an additive genetic model, rs4713902 showed significant over-transmission of the major allele (P = 0.0001) which was consistent across the two sample sets (P = 0.004 and P = 0.006). rs7757037 showed evidence of association that was strongest under the dominant model (P = 0.001). This result was consistent across the two datasets (P = 0.017 and P = 0.019). The dominant model yielded modest evidence for association (P < 0.05) for three additional markers. Covariate-based analyses suggested that genetic variation within FKBP5 may influence attempted suicide and number of depressive episodes in bipolar subjects. Our results are consistent with the well-established relationship between the hypothalamic-pituitary-adrenal (HPA) axis, which mediates the stress response through regulation of cortisol, and mood disorders. Ongoing whole genome association studies in bipolar disorder and major depression should further clarify the role of FKBP5 and other HPA genes in these illnesses.

1921/W

Influence of gender on mtDNA quantity in cerebrospinal fluid following traumatic brain injury. *Y. Conley, M. Henning, D. Ren, S. Alexander.* Univ Pittsburgh, Pittsburgh, PA.

Considerable variation exists in outcomes attained following a severe traumatic brain injury (TBI)and the nature of this variability is not well understood. Mitochondrial function is altered follwing TBI and amount or condition of mtDNA may play a role in this altered function, which may play a role in outcomes. This study was designed to obtain cerebrospinal fluid (CSF) drained as standard of care from TBI victims for the five days following injury and extract mtDNA for evaluation. Our preliminary evaluation of mtDNA quantity, measured using RT-PCR and normalized with simultaneous RT-PCR for betaglobin, indicates that considerable variation exists in mtDNA quantity over day 2-5 following a TBI and that females have an adjusted average of 40 times the amount of mtDNA compared to males. This difference almost reaches statistical significance (p=.063) with an n=19, 15 males and 4 females. Age did not impact quantity (age range 17-69) and initial extent of injury measured by Glasgow Coma Score did not impact quantity (GCS range 3-8). We are in the process of collecting data on additional subjects as well as investigating mtDNA quantity in reference to long term functional outcomes attained.

1922/W

Mitochondrial haplogroup H1 is protective for stroke. *A. Rosa[1], B.V. Fonseca[1], T. Krug[1], H. Manso[1,2], I. Albergaria[2], G. Gaspar[2], M. Correia[3], M.V. Baptista[4], R. Silva[5], J.R. Fontes[6], G. Lopes[3], J.P. Gabriel[7], I. Matos[8], R. Taipa[3], M.R. Silva[7], L. Gouveia[9], J.M. Ferro[9], A.M. Vicente[1,2], S.A. Oliveira[1].* 1) Instituto Gulbenkian de Ciência, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal; 3) H. Geral de Santo António, Portugal; 4) H. Garcia de Orta, Portugal; 5) H. Fernando Fonseca, Portugal; 6) H. São Marcos, Portugal; 7) H. de São Pedro, Portugal; 8) H. Distrital de Mirandela, Portugal; 9) H. de Santa Maria, Portugal.

Several neurological disorders have been associated with mutations/polymorphisms in mitochondrial DNA (mtDNA), which alter gene expression and ultimately compromise mitochondrial function. The best-known example is that of MELAS syndrome, characterized by stroke-like episodes, where the A3243G transition causes a respiratory chain deficiency through a generalized effect on protein synthesis. In addition, the mtDNA phylogenetic background was also shown to influence the expression of particular diseases (e.g. Parkinson's disease, LHON disease, and occipital stroke in migraine). In order to evaluate the role of the mitochondrial genome in stroke susceptibility, we tested the allelic and haplogroup association of 27 polymorphisms (tagging SNPs or SNPs defining European haplogroups) in 515 stroke patients and 476 controls, all Caucasians and of Portuguese nationality and ancestry. Haplogroup H1 was found to be significantly less frequent in stroke patients than in controls (OR= 0.56, 95% CI=0.41-0.77, p=0.001), when comparing each clade against all other haplogroups pooled together. Conversely, the pre-HV/HV and U mtDNA lineages emerge as potential genetic factors conferring risk for stroke (OR=2.74, 95% CI=1.21-6.16, p=0.013 and OR= 3.22, 95% CI=1.18-8.79, p=0.018, respectively). SNPs G3010A and C7028T strongly influence the risk, their allelic state in haplogroup H1 corroborating for its protective effect. Although the functional dynamics are not yet clear, these mtDNA substitutions most likely interact with the predisposing allele(s), either mitochondrial or nuclear, increasing the expression of the primary variant or predisposing the origin and fixation of the pathogenic alleles.

1923/W

Parkinson Disease in Russia: Analysis of Genetics Markers in Patients with Sporadic Parkinson's Disease. *S. Limborska[1], M. Shadrina[1], E. Semenova[1], G. Bagyeva[2], M. Partola[1], S. Illarioshkin[2], P. Slominsky[1].* 1) Human Molecular Genetics Dept, Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russian Federation; 2) Department of Neurogenetics, Institute of Neurology of Russian Academy of Medical Sciences, Moscow, Russian Federation.

Sporadic Parkinson's disease (PD) is a common neurodegenerative disorder, characterized by the loss of midbrain dopamine neurons and Lewy body inclusions. It is thought to result from a complex interaction between multiple predisposing genes and environmental influences, although these interactions are still poorly understood. A major breakthrough in recent years was identification of mutations in multiple genes causing inheritable form of the disease such as parkin, leucine-rich repeat kinase 2, DJ-1, PINK1 and other loci. Mutations in the parkin gene (PARK2) are a frequent cause of autosomal recessive, early onset Parkinsonism. Various mutations have been identified. s of PD development is mutations in this gene. The mains types of mutations in this gene are deletions and duplications of single exons or exon groups. We analyzed rearrangements in exons 1-12 of the PARK2 gene in 140 patients with early-onset Parkinson's disease (EOPD) and in 200 patients with classical sporadic Parkinson's disease. All the patients were from Russia. The frequency of these mutations in EOPD patients was 11,8%, in classical sporadic PD patients - 4,5%. Most frequent rearrangments were detected in exons 3, 4 and 5. Mutations in the gene Leucine-Rich Repeat Kinase 2 (LRRK2) have been identified in both dominant and sporadic cases affected by Parkinson's disease (PD). The LRRK2 Gly2019Ser mutation is the most frequent substitution in Caucasians, accounting for approximately 5-6% of familial and 0.5-2.0% of apparently sporadic PD cases. We investigated the frequency of the LRRK2 G2019S mutation in our sporadic PD patients and 13 autosomal dominant PD patients. We established that the frequency of this mutations in female patients was 7,7% and in sporadic patients was 0,8%.

1924/W

Gene Expression in Peripheral Lymphocyte Cells of Patients with Major Depressive Disorder Treated with Citalopram. *F. Mamdani[1], P.A. Sequeira[1], J. ffrench-Mullen[2], M.M Beaulieu[1], M. Berlim[1], G. Turecki[1].* 1) McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montréal, Quebec, Canada; 2) GeneLogic Inc., Gaithersburg, Maryland, USA.

Major depression (MD) is a psychiatric disorder that affects 5-10% of the population and is considered the second leading cause of disability by the World Health Organization. Moreover, 30 to 40% of patients treated with antidepressants do not present with an adequate response to treatment. In order to identify gene targets that may mediate response to Citalopram (CIT), a commonly used SSRI (selective serotonin reuptake inhibitor) antidepressant, we are conducting a large-scale gene expression study in lymphocytes of drug-naïve depressed patients treated with CIT for eight weeks, with Affymetrix U-133 Plus2 microarrays being performed pre and post treatment initiation. We are presenting preliminary results on 60 cases. Outlier detection is performed using several quality control variables as well as principal component analysis. Our analyses are based on individual response status determined at the end of eight weeks using severity of depression measures obtained throughout the trial with the Hamilton Depression Rating Scale (HAMD-24). We have found gender-specific gene subsets that are possibly implicated in the response to CIT; 394 differentially expressed genes (DEGs) in females and 359 DEGs in males. As well as, genes that may allow for the prediction of this response prior to treatment commencement. Gene ontology analysis of differentially expressed genes revealed several biological processes being affected by treatment; these include immune response, regulation of transcription and nucleic acid metabolism. These findings show promise for the eventual determination of possible biomarkers for classification of responders and non-responders to antidepressant treatment.

1925/W

Principal components analysis of signs and symptoms of Schizophrenia: Development of quantitative trait phenotypes for linkage and association analyses. *J.A. McGrath[1], D. Avramopoulos[1, 2], V.K. Lasseter[1], P.S. Wolyniec[1], J.R. Luke[1], M.H. Thornquist[1], M.D. Fallin[3], K.Y. Liang[3], D. Valle[2], G. Nestadt[1], A.E. Pulver[1].* 1) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; 2) McKusick Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.

Subjects with schizophrenia or schizoaffective disorder (SZ) experience a wide range of psychotic and behavioral signs and symptoms that are not pathognomic for these diagnoses. Data reduction techniques offer the possibility of defining a reduced number of dimensions to aid in the classification of more homogeneous groups of patients for linkage and association studies. Establishing the heritability of such dimensional phenotypes is an important first step. Principal components analysis was conducted in a sample of 1199 subjects with SZ (approximately half from European-Caucasian backgrounds and half from Ashkenazi Jewish background) using 73 dichotomous signs and symptoms from consensus diagnostic ratings and the Diagnostic Interview for Genetic Studies. Multiple imputation (Markov Chain Monte Carlo method) was used to impute missing data. Parallel Analyses and Velicer's Minimum Average Partial Correlation test provided statistical support for from 8 to 15 factors: a reasonable 9 factor solution (with varimax rotation) demonstrated basic similarities to a number of earlier studies. Sub-analyses revealed similar solutions for both ethnic groups. Heritability analyses of the 9 sets of factor scores using SAGE ASSOC software demonstrated significant estimates: affective 0.46; child/adolescent sociability 0.352; disorganization 0.582; hallucinations 0.427; impaired / disabled 0.51; negative 0.521; positive 0.365; prodromal 0.421; school / developmental 0.381. These quantitative traits will be used in both linkage and association studies.

1926/W

The Dlx1and Dlx2 genes and Susceptibility to Autism Spectrum Disorders. *X. Liu[1,5,6], N. Novosedlik[5], A. Wang[5], M. Hudson[1,5,6], I.L. Cohen[3,6], M.E.S. Lewis[4,6], J.J.A. Holden[1,2,5,6].* 1) Dept of Psychiatry , Queen's University, Kingston, ON, Canada; 2) Departments of Physiology, Queen's University, Kingston, K7L 3N6; 3) Department of Psychology, New York State Institute for Basic Research in Developmental Disabilities, Columbia, Island, NY 10314; 4) Department of Pathology and Medical Genetics, University of British Vancouver, BC, V6H 3N1; 5) Autism Research Program, Ongwanada, Kingston, ON, Canada, K7M 8A6; 6) www.autismresearch.ca.

An imbalance between excitation and inhibition in the cortex, which could be caused by altered regulation of the growth of specific populations of neurons, has been suggested as a possible aetiological factor for autism. The Dlx genes encode homeobox transcription factors that have been implicated in the development of the GABAergic system. The Dlx1 and Dlx2 genes lie head to head in 2q32, a region implicated in genome scans and cytogenetic studies as harbouring genes associated with autism susceptibility. We investigated 6 Tag SNPs in the region covering Dlx1 and Dlx2 genes in two cohorts of multiplex (MPX) families and a comparison group of 384 samples for association with autism. While no significant differences in allele, genotype, and haplotype frequencies between affected cases and the comparison group were observed, the family-based tests showed strong association for two of the SNPs. We found association of the common G allele at rs788172 (P=0.02) and the common G allele at rs813720 (P=0.015) in the first 178 MPX families. The allelic associations at Dlx1-Dlx2 variants were confirmed in a replication sample of 134 MPX families (P=0.008 and 0.002 respectively) and in the combined 312 MPX families (P=0.0007 and P=0.0002 respectively). Haplotype analysis also revealed significantly excessive transmission of haplotype rs788172_G-rs813720_G from parents to affected children in the two cohorts and the combined samples (P=0.01, 0.0007 and 0.00008 respectively). Further testing of the two SNPs in 290 Simplex families did not replicate these findings, suggesting that genetic variants in Dlx1/Dlx2 genes may affect susceptibility or cause autism in a large subset of familial cases.

1927/W

Paternal X-linked gene(s) associated with increased risk of autism spectrum disorder in females. *N. Gharani, R.A. Zimmerman, B.J. Smith, S. Buyske, D.M. Waterworth, L.M. Brzustowicz.* Dept Genetics, Rutgers Univ, Piscataway, NJ.

Autism is a serious neurodevelopmental disorder with a complex genetic basis. Males are at least four times more likely to be affected than females, suggesting a role for X-linked genetic and/ or epigenetic features in the etiology of the disease. One of the epigenetic processes that regulate X-chromosome gene expression in females is X-chromosome inactivation (XCI). We hypothesized that skewing of XCI may play a role in autism by modulate the threshold of risk in female members of autism families. To explore this possibility we investigated the XCI pattern of 337 ASD affected and unaffected female members from 145 autism families using a standard published technique that examines differential methylation of the Androgen Receptor gene on active and inactive X-chromosomes. Of the 337 samples assayed for XCI patterns, 40 were uninformative (homozygous for the CAG repeat). Our data in 297 informative individuals demonstrates skewed XCI in both affected and unaffected females with as many as 42% of samples showing a skewing ratio of >80:20, which is significantly higher (P <0.0001) than 9% observed in the normal female population. Since XCI skewing is observed in both affected and unaffected individuals we have investigated the parental origin of the active X-chromosome in 129 informative ASD affected probands and unaffected siblings (for whom parental origin of the X could be unequivocally assigned). This analysis has shown a higher proportion of affecteds (74%) in the group with a predominantly paternal active X-chromosome compared to ~50% of those with skewing towards a maternal active X-chromosome (Fisher's exact P=0.007; OR=2.80, 95% C.I. 1.34, 5.83). Individuals are ~3 times more likely to be affected if there was skewing towards a paternal rather than maternal active X-chromosome. These data implicate a paternal X-chromosome in the risk of autism in females, suggesting a role for imprinted gene(s). These results support the hypothesis that variable expression at critical X-linked imprinted gene(s) alter an individual's threshold of risk for ASD and that skewed XCI is the mechanism by which this threshold is altered in females.

1928/W

Analysis of X chromosome inactivation in autism spectrum disorders. *E. Maestrini[1], X. Gong[2], F. Blasi[1], E. Bacchelli[1], C. Toma[1], DM. De Luca[1], M. Rossi[3], I. Jarvela[3], T. Bourgeron[2], The International Molecular Genetics Study of Autism Consortium (IMGSAC).* 1) Dept Biology, Univ Bologna, Italy; 2) Institute Pasteur, Paris, France; 3) Dept of Medical Genetics, Univ Helsinki, Finland.

Autism spectrum disorders (ASD) are a group of complex neurodevelopmental disorders more frequent in males than females, with an approximate ratio of 4:1. Skewed X chromosome inactivation (XCI) is observed in females carrying mutations involved in several X-linked disorders. In this study, we aimed to estimate the role of X-linked genes in ASD susceptibility by ascertaining the XCI pattern in a large sample of mothers of children with ASD. The study sample included 547 informative mothers of ASD children (256 multiplex and 290 singleton families) and 181 affected females, from the Paris Autism Research International Sib-pair study, the Finnish study group for ASD, and the IMGSAC. The control group included 144 adult females with a similar age distribution to the mothers' group. To determine the pattern of XCI we examined the differential methylation status of the human androgen receptor gene. We did not identify a different distribution of skewed XCI rate between mothers of affected children and the control group. Interestingly, two mothers and one girl carrying known mutations in X-linked genes (NLGN3, ATRX, MECP2) showed highly skewed XCI, suggesting that ascertainment of XCI could reveal families with X-linked mutations. Linkage analysis was carried out in the subgroup of multiplex families with skewed XCI (≥80:20), using the available Affymetrix 10K SNP data generated by the Autism Genome Project (AGP). No significant linkage was detected in this subgroup, and only a modest increased allele sharing was detected in the Xq27-Xq28 region. Mutation screening of MECP2 failed to identify any causative mutations in the skewed XCI subgroup. In summary, our results suggest that there is no major X-linked gene subject to XCI conferring susceptibility to ASD. However , the possibility that rare mutations in X-linked genes could contribute to ASD cannot be excluded. We propose that the XCI profile could be a useful criteria to prioritize families for mutation screening of X-linked candidate genes.

1929/T

Association analyses of the 7q34-36 language region support *CNTNAP2* as an autism QTL. *M. Alarcon[1,2], B.S. Abrahams[1,2], J.L. Stone[3], R.M. Cantor[1,3,4], J.A. Duvall[1,2,3], J.V. Perederiy[2], J.M. Bomar[2], S.F. Nelson[2,3], D.H. Geschwind[1,2,3].* 1) UCLA Neurology, Reed Neurological Research Ctr. and UCLA CART, Semel Institute of Neuroscience and Program in Neurogenetics, Neurology, David Geffen School of Medicine, Los Angeles, CA; 2) UCLA Center for Neurobehavioral Genetics, Semel Institute of Neuroscience, Los Angeles, CA; 3) Human Genetics, David Geffen School of Medicine, Los Angeles, CA; 4) Pediatrics, David Geffen School of Medicine, Los Angeles, CA.

Autism Spectrum Disorders (ASD) is quickly becoming the most prevalent neurodevelopmental disorders in the United States. Characteristic features include social and communicative deficits, repetitive and stereotypic behaviors, and language impairments. About half of individuals with ASD have language difficulties. Considering the male to female ratio of ASD (approximately 3:1), and the increased frequency of language delays in typically-developing boys, sex may be a major contributor to heterogeneity of the disorder and, thus, hinder the identification of autism susceptibility genes, when unrecognized. Despite the complexity of the disorder, we have identified a region on chromosome 7q35 that is linked to a language trait (age at first word) in 291 families from the Autism Genetic Resource Exchange (AGRE). Here, we report results of a two-stage quantitative, block-based association analysis of 2758 single nucleotide polymorphisms that exhaustively cover 15Mb of this linked language region in over 400 AGRE trios. Evidence for quantitative association to Contactin Associated Protein 2 (*CNTNAP2*), specifically attributed to the families with affected males only, is observed. Moreover, we investigated the expression of genes in developing human brain and identified the same gene, *CNTNAP2,* as having a specific expression pattern in circuits involved in cognition and language. These results suggest that *CNTNAP2* may contribute to autism susceptibility as well as language development.

1930/T

The Alzheimer gene FE65 forms a transcriptional repressor complex with Teashirt-family proteins: Evidence for association of Teashirt genes with Alzheimer disease. *J.D. Buxbaum[1,2], G.W. Beecham, Jr.[3], J.L. Haines[4], M.A. Pericak-Vance[3], Y. Kajiwara[2].* 1) Psychiatry, Mount Sinai Sch Medicine, New York, NY; 2) Neuroscience, Mount Sinai Sch Medicine, New York, NY; 3) Miami Inst for Human Genomics, U Miami Miller Sch Medicine, Miami, FL; 4) Center for Human Genetics Research, Vanderbilt Univ Medical Ctr, Nashville, TN.

FE65, an Alzheimer amyloid precursor protein (APP)-binding protein, has been postulated to be involved in both nuclear signaling and in transcriptional regulation. Here we identified and characterized the interaction of the Teashirt family of transcriptional factors with the first phosphotyrosine binding domain (PTB1) of FE65. Teashirt proteins can function as transcriptional repressors and inhibit transcriptional activity mediated by FE65 in heterologous reporter assay. Moreover, we demonstrate that Teashirt proteins can directly recruit histone deacetylase (HDAC) 1 and 2, contributing to the repression activity of Teashirt. Moreover, components of inhibitor of acetyltransferase (INHAT) can be recruited to FE65, together with Teashirt/HDAC, leading to a powerful gene-silencing complex. Genetic association analyses indicate that two Teashirt genes (TSHZ1 and TSHZ3) are associated with Alzheimer disease in a first study (also see Beecham et al., this meeting). The data support a role for FE65 in regulating gene expression and provide further support for a genetic role for the APP-FE65 pathway in the etiology of Alzheimer disease.

1931/T

Genetic association of *PLAUR* with autism spectrum disorder and possible gene-gene interactions in the MET signaling pathway. *D.B. Campbell[1], J.S. Sutcliffe[2,3], C. Li[4], R. Sacco[5,6], A.M. Persico[5,6], P. Levitt[1,3].* 1) Pharmacology, Vanderbilt Univ, Nashville, TN; 2) Mol Physiology & Biophysics, Vanderbilt Univ, Nashville, TN; 3) Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt Univ, Nashville, TN; 4) Biostatistics, Vanderbilt Univ, Nashville, TN; 5) Lab of Mol Psychiatry & Neurogenetics, Univ Campus Bio-Medico, Rome, Italy; 6) Fondazione S Lucia, IRCCS, Rome, Italy.

We recently described association of a functional variant of *MET* with autism spectrum disorder (ASD). The ASD-associated variant resides in the promoter and alters the affinity of transcription factors encoded by *SP1* and *SUB1*. MET functions to influence development of the cerebral cortex and cerebellum. Analyses of transcript levels in ASD postmortem cortical tissue revealed decreased expression of *MET* and increased expression of three genes encoding proteins that activate MET signaling, *HGF*, *PLAUR* and *SERPINE1*. Because the *SP1*, *SUB1*, *HGF*, *PLAUR* and *SERPINE1* genes lie within chromosomal regions that have shown evidence for linkage to ASD, we hypothesized that these genes may contribute to ASD susceptibility. We screened all exons and regulatory regions for variants in each of the five genes in 48 individuals with ASD. Identified variants were genotyped in 629 ASD pedigrees and 312 unrelated controls. The *MET* promoter variant rs1858830 allele C was associated with ASD in this sample by family-based association test (FBAT; P=0.008) and case-control analyses (P=0.015). The *PLAUR* promoter variant rs344781 allele G was also associated with ASD by both family-based (FBAT; P=0.006) and case-control analyses (P=0.007). The *PLAUR* promoter rs344781 relative risk was 1.932 (95% CI: 1.128, 3.308) for genotype GG and 2.422 (95% CI: 1.380, 4.251) for genotype AG compared to genotype AA. Exploratory gene-gene interaction analyses suggested interactions of *MET* with *PLAUR* and *HGF* and interactions of *PLAUR* with *HGF*, *SERPINE1* and *SP1*. Although functional variants have not yet been identified, the evidence of association of *PLAUR* provides further support that disruption of the MET signaling pathway contributes to ASD susceptibility.

1932/T

Genetic Analysis of Serum BDNF Levels. *M. Carless[1], D. Glahn[2], J. Curran[1], H. Goring[1], L. Almasy[1], M. Johnson[1], T. Dyer[1], E. Moses[1], J. Blangero[1].* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) University of Texas Health Science Center at San Antonio, San Antonio, TX.

Serum levels of brain-derived neurotrophic factor (BDNF) represent an important biomarker for a growing number of brain-related disorders, particularly major depression. However, little is known about the genetic factors influencing normal BDNF serum level variation. To examine the genetic determinants of this quantitative endophenotype, we measured serum BDNF using a commercial ELISA assay in existing samples from 867 Mexican American individuals who are members of approximately 40 large extended pedigrees. All of these individuals have been previously genotyped for a 10cM genome-wide scan. Using variance-component-based analysis, we estimated the heritability of BDNF serum levels to be 0.286 ± 0. 067 ($p = 2.1 \times 10^{-8}$). A genome-wide linkage analysis identified a single QTL in chromosomal region 22q13 (LOD score=3.90, nominal p-value=1.1×10^{-5}; genome-wide p-value=0.004). Three other genomic regions (chromosomal regions 1p32, 3p26, and 19p13) exhibited suggestive evidence for linkage with LOD scores greater than 2. To empirically nominate positional candidate genes in this region, we examined lymphocyte-derived transcriptional profiles in relation to serum BDNF levels. In the 6Mb 1-LOD support interval for the QTL on chromosome 22, we identified a *cis*-regulated (p-value=0.002) transcript for the gene *UPK3A*, whose expression level was significantly correlated with serum BDNF (p-value=0.019). We are currently examining sequence variation within the *BDNF* structural locus and the *UPK3A* candidate gene to identify specific genetic variants that may influence BDNF levels. Our demonstration of a significant genetic component to BDNF levels provides support for its utility as a quantitative endophenotype that may be useful as a measurable risk factor for various brain-related disorders.

1933/T

A de novo 3.3 Mb deletion on 1p34.2 in a patient with autism and microcephaly. *W.B. Dobyns[1], J. Sudi[1], R.A. Kumar[1], J. Conroy[2], D. McQuaid[2], N.J. Nowak[2], S.L. Christian[1].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Roswell Park Cancer Institute, Department of Cancer Genetics.

Autism spectrum disorder (ASD) is characterized by impaired social interactions, communication deficits, and restricted and repetitive behaviors and interests. Cytogenetic and array-based studies show that ASD may be associated with chromosome abnormalities are smaller copy number variants. We report a new ASD locus detected by array comparative genome hybridization (aCGH) in a boy with ASD and postnatal microcephaly. He was born at term and appeared normal except for right hydronephrosis and vesicoureteral reflux, which both resolved. His birth OFC was 34 cm (10-25%), but his head growth was slow so his OFC dropped to -3 SD by 15 months and thereafter followed a curve at the same percentile. His motor development was normal at first, but he walked late at 16 months. He used his first words at 15 months and slowly increased to 50-75 words by 2.5 years, but then stopped using almost all speech by age 3 years. Has has poor social communication including poor eye contact and limited interactive play, striking anxiety, difficulty with transitions, constant chewing, short attention span, poor sleep pattern, and repetitive activities such as bouncing on an exercise ball. Neurpsychological testing supported a diagnosis of ASD. Chromosome analysis was normal, but no other genetic tests were obtained. We performed aCGH on the patient using a 19K whole genome tiling path BAC microarray, and detected a 3.3 Mb deletion extending from RP11-769L8 to RP11-483I17 (chr1:39587288-42908332). FISH studies confirmed the deletion and microsatellite analysis determined that the deletion was de novo. This region contains 44 RefSeq genes, including several potential candidate genes. Given that microcephaly is uncommon among patients with ASD, we hypothesize that different genes in this region are responsible for the ASD and microcephaly.

1934/T

Genetic dissection of idiopathic generalized epilepsy: SNP discovery and genotyping in genes encoding ion channels in case and control populations. *R.A. Gibbs[1,2], D.A. Wheeler[1], A. Goldman[3], D.M. Muzny[1], S.E. Scherer[1], C. Davis[3], J.L. Noebels[3].* 1) Hum Genome Seq Ctr, Baylor College Medicine, Houston, TX 77030; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; 3) Department of Neurology, Baylor College of Medicine, Houston TX.

Epilepsy is the second most common neurological disorder, with a life-time prevalence of approximately 1-3% world-wide. Since mutations in ion channel genes account for the vast majority of rare inherited forms of idiopathic epilepsy they could be an equally important cause of the much more common, sporadic forms of the disorder. To investigate this problem, we are resequencing the exons of 240 ion channel genes in patients and matched controls. To date, we have generated sequence data across 2950 exons sampled from 256 patients and 54 controls. The study has produced over 4800 novel markers, 20% of which cause non-synonymous protein coding changes (nsSNP). From an initial set of SNPs we chose 1500 markers for a genotyping panel. The panel included 290 novel nsSNPs and putative splice variants from our study and an additional 503 nsSNPs and splice junction variants from dbSNP. We have tested 100 cases and 200 Caucasian controls in a first round of genotyping. A broad allele frequency spectrum was observed with over 50% of the polymorphic sites found at frequencies less than 5% but as yet no statistically significant associations have emerged. The project has also discovered termination mutations in 14 genes, nearly half of which have already been validated by an independent sequencing technology (pyrosequencing). These mutations are confined to cases in all but 2 of the genes, although the sample size of controls is still small. One of these genes, CLCN2, is also implicated in Mendelian forms of epilepsy. These findings are of great interest given the large number of spontaneous termination mutations in SCN1A, which are found in cases of severe myoclonic epilepsy of infancy. We are now focusing attention on the other nsSNPs found in these genes.

1935/T

Recognition of the first causative gene for epilepsy with continuous spike-and-waves during slow-wave sleep, a late-childhood epileptic encephalopathy. *C. Godfraind[1], M. Coutelier[2], S. Andries[3], G. van Rijckevorsel[3, 4], C. Raftopoulos[4], S. Gargani[3], F. Scaravilli[5], M. Vikkula[2].* 1) Neuropathology Laboratory, Université catholique de Louvain, Brussels, Belgium; 2) Laboratory of Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 3) Centre William Lennox, Université catholique de Louvain, Ottignies, Belgium; 4) Cliniques universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium; 5) Department of Neuropathology, Institute of Neurology, London, UK.

Point mutations in the gene neuroserpin or PI12 (protease inhibitor 12), located at 3q26, have been associated to 5 familial cases of autosomal dominant presenile dementia named Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB: OMIM #604218). Severity of clinical course is linked to the location of point mutations. Exon 2 PI12 mutations have been associated to later clinical onset than exon 9 mutations. Clinical spectrum includes progressive cognitive decline, myoclonus, seizure, tremor, dysarthria and chorea. Neuropathology is characterized by intra-cytoplasmic neuronal inclusions of polymerized mutant protein called Collin's body. These inclusions are strongly periodic acid-Schiff (PAS) positive and diastase resistant, similar to the ones observed in the liver of alpha-1-antitrypsin deficiency. We report a female patient, who at 8-years of age developed aggressive behaviour, intellectual decline and intractable epilepsy with continuous spike-and-waves during slow-wave sleep, for which she underwent neurosurgical sub-pial transections. A brain biopsy was performed and showed classical histological aspects of FENIB. PI12 sequencing revealed a novel exon 9 mutation. Paternity test was in concordance with a de novo mutation. This is the first causative gene implicated in continuous spike-and-waves during slow-wave sleep. It is the first proof of a de novo mutation in this gene and the youngest reported patient. Thus neuroserpin should be considered as candidate gene in this and other refractory epilepsies associated with cognitive impairment. catherine.godraind@anpg.ucl.ac.be.

1936/T

Association Analysis of Adenosine A1 receptor (ADORA1) and Dopamine D1 receptor (DRD1) genes with schizophrenia in the Japanese population. *L. Gotoh, H. Kawasaki, H. Mitsuyasu, Y. Kobayashi, N. Oribe, A. Takata, S. Kanba.* Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan.

Antipsychotic agents used for the treatment of schizophrenia affect dopamine D2 receptor (DRD2) mediated neurotransmissions, suggesting that dopaminergic dysfunction plays an important role in the pathophysiology of schizophrenia. On the other hands, it is shown that SCH23390, a selective DRD1 antagonist, inhibited PCP-induced schizophrenia-like behaviors. It is also reported that adenosine neurotransmitter system has functional interaction with dopaminergic neurotransmission. It is also known that N6-cyclopentyladenosine, ADORA1 agonist functionally involved in inhibition of PCP-induced behavior of schizophrenia model rats, as well as DRD1 antagonist. Therefore, it is possible to hypothesize that the functions of ADORA1 and DRD1 could be some part of the pathophysiological mechanisms of schizophrenia. To clarify the relationship between ADORA1, DRD1 and schizophrenia, the single nucleotide polymorphisms (SNPs) of these two receptor genes were analyzed in both schizophrenia patients and normal controls. For genotyping experiments, total 16 fragments were amplified by PCR from each subject consisting of the schizophrenia patients (n=200) and normal controls (n=210). Primers were designed according to the positions of SNPs and the database of JSNP (Japanese Single Nucleotide Polymorphisms). Using the amplified DNA fragments, all the subjects were genotyped with 27 SNPs and 2 deletions in ADORA1 gene, 1 SNP in DRD1 gene. Based on the results, genotyping and allele frequencies were calculated. Association analysis of each polymorphism was performed between schizophrenia patients and normal individuals. Two SNPs indicated statistically significant difference between the two populations (P>0.05). However, after Bonferroni correction, those were disappeared. Further analysis such as haplotype prediction, linkage disequilibrium calculation, sliding window analysis and multi-variate statistical analysis will be carried out. All subjects were given informed consent before blood collection strictly based on the ethical regulations of Kyushu University.

1937/T

Array comparative genomic hybridization and quantitative PCR identify a novel recurrent 16p11.2 microdeletion in autism spectrum disorder. *R.A. Kumar[1], J. Sudi[1], J. Conroy[2], D. McQuaid[2], S. KaraMohamed[1], J.A. Badner[3], C. Gilliam[1], N.J. Nowak[2], E.H Cook Jr.[4], W.B. Dobyns[1], S.L. Christian[1].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 3) Psychiatry, University of Chicago, Chicago, IL; 4) Psychiatry, University of Illinois at Chicago, Chicago, IL.

Autism spectrum disorder (ASD) is characterized by impaired social interaction, communication deficits, and restricted and repetitive behaviors and interests. Cytogenetic and array-based studies indicate that ASD may be associated with chromosomal abnormalities, including copy number variants (CNVs), which may be flanked by low copy repeats (LCRs). To identify additional rearrangements associated with ASD, we investigated 180 ASD probands and 260 controls by array comparative genomic hybridization (aCGH) using a novel 19K whole genome tiling path BAC microarray. We discovered a recurrent de novo ASD-specific 16p11.2 microdeletion in 2 probands. We screened an additional 479 probands and 465 controls by quantitative PCR using two probes specific for the deletion and identified 2 probands, but no controls, deleted for both probes. In one family, we detected the 16p11.2 microdeletion in 2 affected siblings but not in an unaffected sibling nor in the unaffected parents, suggesting germline mosaicism. We confirmed all 16p11.2 deletions using FISH, microsatellite analyses, and/or aCGH. In total, we found 4 ASD probands and an affected sibling with a recurrent 16p11.2 deletion that was not identified in 725 controls ($p < 0.047$). The deletion spans ~500 kb, includes 29 genes, and is flanked by ~140-kb LCRs that are >99% identical. High resolution characterization of the deletion breakpoints using a custom designed high-density oligonucleotide array is underway to elucidate the mechanism of the rearrangement. To rank the 29 genes within the deleted region, we used the molecular triangulation method and mapped 8 genes to putative disease-related interaction networks. Our work represents one of the largest case-control CNV analyses in autism and defines 16p11.2 deletions as a novel, recurrent genomic disorder that we hypothesize is causal for ASD.

1938/T

Frequent 22q11 aberrations in patients with non-syndromic autism spectrum disorders shown by SNP array based segmental aneuploidy screening. *M. Poot[1], N. Verbeek[1], R. van 't Slot[1], M.R. Nelen[1], B. van der Zwaag[3], E. van Daalen[2], W. Staal[2], J.A.S. Vorstman[2], M.V. de Jonge[2], P.F. Ippel[1], M.J. van den Boogaard[1], F.A. Beemer[1], J. van der Smagt[1], E.H. Brilstra[1], G.R. Jalali[4], B.S. Emmanuel[4], H. van Engeland[2], J.P.H. Burbach[3], H.K. Ploos van Amstel[1], R. Hochstenbach[1].* 1) Dept. Medical Genetics, UMC Utrecht, Utrecht, Netherlands; 2) Child and Adolescent Psychiatry, UMC Utrecht; 3) Pharmacology and Anatomy, Rudolf Magnus Institute of Neuroscience, UMC Utrecht; 4) The Division of Human Genetics, The Children's Hospital of Philadelphia and the Joseph Stokes Jr Research Institute, Philadelphia, USA.

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by impaired reciprocal social interaction, communicative deficits, and restricted behavioral patterns. ASD occurs in syndromic forms (e.g. FRAX, del(22q13), Rett), and as non-syndromic cases frequently involving cytogenetic abnormalities. Recently, array-based genome-wide screens have demonstrated frequent copy number variation in non-syndromic ASD. Screening 50 patients with autism and additional major or minor anomalies with the Infinium HumanHap300 SNP platform (Illumina, Inc., San Diego, CA) we found in 17 patients 10 regions with deleted and 19 with duplicated signals. Aberrant signals were distributed among 26 distinct chromosomal loci. Eleven of those have previously been reported as regions of significant linkage to or association with ASD. Apart from 14 patients with unique aberrations, 2 patients carried duplications and a 3rd patient a deletion within the 22q11 region, of 0.726, 2.966, and 0.388 Mb, respectively. The duplications were confirmed by multiplex ligation-mediated probe amplification and are likely to involve LCRs A, B and D. We conclude that SNP array-based screening of ASD patients uncovers an appreciable number of CNVs, which in part overlap with loci already discovered by other approaches. Our finding that 3 out of 50 ASD patients carried aberrations within the 22q11 region is highly unexpected. The relatively small size of CNVs found in this study may allow us to pinpoint candidate genes for ASD.

1939/T

Association Study of Diacylglycerol Kinase Eta (DGKH) Gene with Bipolar Disorder Patients in Japanese Population and Biological Function Analysis of DGKH Isoform 2 Val1201Ala Polymorphism. *A. Takata, H. Kawasaki, H. Mitsuyasu, Y. Kobayashi, L. Gotoh, N. Oribe, S. Kanba.* Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan.

Bipolar disorder (BD) is a common, severe, chronic, and life-threatening illness where patients alternate between episodes of depression and mania. Detailed pathophysiology of BD is still unclear, but family, twin and adoption studies consistently indicate a strong genetic component. Therefore, a number of genetic studies of BD have been conducted and recent genome-wide association study of BD revealed several candidate genes those influence disease risk. Among such genes, diacylglycerol kinase eta (DGKH), a member of diacylglycerol kinase (DGK) gene family, showed strong association statistically. In addition, DGK plays an important role in the lithium-sensitive phosphatidyl inositol pathway and DGKH is located within the bipolar disorder linkage region on chromosome 13q14.Thus, in order to elucidate the pathophysiological mechanisms of BD, it should be a good approach to examine detailed function of DGKH. In this study, we carried out association study with several polymorphisms selected from exons and exon/intron boundaries of DGKH between BD patients matched to DSM-IV criteria and healthy controls in Japanese population. We focused on a single nucleotide polymorphism (SNP) in exon 30 of DGKH. DGKH has two splicing variants (DGKH1 and DGKH2) those manifest gene expressions and biochemical features in different manners respectively and above described SNP causes nonsynonymous amino acid substitution (valine to alanine) only in DGKH2. Therefore, cell biological experiments of this amino acid change of DGKH2 are now in progress. In this study, we show detailed results of genetic association study and cell-biological experiments. All subjects were given informed consent before blood collection strictly based on the ethical regulations of Kyushu University.

1940/T

Serotonin Related Genes in Autism. *B.M. Anderson[1], N. Schnetz-Boutaud[1], M.L. Summar[1], J. Bartlett[1], M. Cuccaro[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Miami Institute for Human Genomics, Miami, FL.

Introduction: Autism is a severe neurodevelopmental disorder with a strong genetic component. Despite numerous genome screens and individual candidate gene studies, the underlying genetic etiology remains largely unknown. Increasing evidence suggests that autism is more genetically complex than previously thought, and that single gene approaches toward dissecting autism genetics may not be informative. We are taking the alternative approach of testing for interactive effects of multiple genes within the serotonin pathway. Methods: We tested 75 SNPs within 13 different genes related to serotonin including TPH1, TPH2, HTR1A, HTR2A, HTR3A, SLC6A4, SLC7A5, YWHAZ, and DDC. SNPs were chosen to represent the linkage disequilibrium patterns across each gene, and included when possible common coding variants. The dataset consists of over 345 multiplex families and 292 parent-child trios collected at two centers in the southeast United States. Initial analyses included single locus family-based association tests, considering both parental and proband gender. Subsequent analyses examined explicitly for gene-gene interactions using multifactor dimensionality reduction (MDR). Results: Single locus analyses generated marginally significant results for YWHAZ and HTR3A, however, these did not survive correction for multiple comparisons. Preliminary two-way interaction analysis with MDR did not identify any significant interactive effects; higher-order interaction analyses are ongoing. Conclusions: As expected, none of the tested genes generated significant results when considered individually. The lack of a strong two-locus interactive effect suggests that either interactions among these genes do not exert a strong effect on autism, or the effect requires a higher order interaction.

1941/T

Analysis of the MET and Neurexin Genes in Autism. *J. Bartlett[1], N. Schnetz-Boutaud[1], B. Anderson[1], K. Gainer-Luci[1], M. Cuccaro[2], J. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Miami Institute for Human Genomics, University of Miami, Miami, FL.

Autism, Asperger's Syndrome, Childhood Disintegrative Disorder, Rett Disorder and Pervasive Developmental Disorder (PDD-NOS) are all classified as Autism Spectrum Disorders (ASD). ASD's are complex neurodevelopmental disorders with an onset early in childhood and 4:1 male:female ratio. It is characterized by impairments in language, reciprocal social interactions, combined with repetitive and stereotypic behaviors. Concordance rates for MZ twins have been estimated between 60%-90% and 0%-10% for DZ twins, suggesting a strong genetic etiology. Despite substantial efforts, very little of the genetic etiology has yet been explained, and no common genetic variation has been universally associated with ASD. Two different studies have recently suggested that variations in the MET gene on chromosome 7 and the Neurexin gene on chromosome 2 are associated with ASD. We used a dataset consisting of 730 Caucasian families (578 trios, 152 multiplex) to test these associations. We performed 2pt linkage analysis using FASTLINK and family association tests using PDT and FBAT. We genotyped 8 SNPs in MET and 12 SNPs in Neurexin. The analysis of the MET SNPs revealed only a marginally significant p-value in RS39748 (FBAT p-value=0.051), but this does not survive correction for multiple comparisons. Our preliminary analysis of the Neurexin gene found a recessive LOD score of 1.55 at RS1045874 and a marginally significant association between ASD and RS7606758 (p-value-0.020), which again does not survive correction. Thus these data suggest that any effect of these two genes is likely to be very modest.

1942/T

Expression and association study of histidine triad nucleotide-binding protein 1 with schizophrenia. *Q. Chen[1], X. Wang[1], F.A. O'Neil[2], D. Walsh[3], K.S. Kendler[1], X. Chen[1].* 1) Psychiatry, Virginia Commonwealth University, Richmond, VA; 2) The Queens University, Belfast, Northern Ireland; 3) The Health Research Board, Dublin, Ireland.

Background: The histidine triad nucleotide-binding protein 1, HINT1, hydrolyzes adenosine 5'-monophosphoramidate substrates such as AMP-morpholidate. Recently it was found to be associated with dysregulation of postsynaptic dopamine transmission, thus suggesting a potential role in several neuropsychiatric diseases. The human HINT1 gene is located on chromosome 5q31.2, a region implicated in linkage studies of schizophrenia. HINT1 has been shown to have different expression in postmortem brains of schizophrenia patients versus controls.

Methods: In this work, we studied 8 SNPs (rs7735116, rs2526303, rs4696, rs3864283, rs2551038, rs2189663, rs7728773, rs3891636) covering 66.7kb of the HINT1 gene region using the Irish study of high density schizophrenia families (ISHDSF, 1350 subjects and 273 pedigrees) and the Irish case-control study of schizophrenia (ICCSS, 655 affected subjects and 626 controls). Expression studies of HINT1 were carried out in postmortem brain cDNAs from the Stanley Medical Research Institute from 35 schizophrenic patients, 34 with bipolar disorder and 35 unaffected controls by real time PCR.

Results: We found significant differences in allele frequencies in several SNPs for the ISHDSF and ICCSS samples in sex-stratified analyses; however the sex effect differed between the two samples. In expression studies, we found that affected male subjects had lower expression than female (p=0.0063). For subjects with a 1/1 genotype at rs386428, a marker showing male-specific association in the ICCSS, affected male subjects had lower expression than normal male controls (p = 0.036).

Conclusion: Data from both association and expression studies suggested that variants at HINT1 may impact on risk for schizophrenia.

1943/T

Serotonin Transporter Polymorphism and Depression in Costa Rican Schizophrenic Patients. *J. Contreras[1,2], P. Quezada[2], A. Dassori[2], R. Medina[2], M. Escamilla[2], R. Salazar[1], H. Raventos[1].* 1) Centro de Investigacion y Biol, University of Costa Rica, San Jose, San Jose, Costa Rica; 2) University of Texas, Health Science Center at San Antonio, TX.

Context. Variation in serotonin transporter gene (5HTT) has been shown to influence depression in psychosis.

Objective. We tested the association between variation in the 5-HTTLPR promoter region (s/l variation) lifetime depression in Costa Rican schizophrenics.

Design. We tested if having an "ss" or "sl" genotype was associated with lifetime depression.

Setting. Subjects were originally recruited for a family based linkage disequilibrium study of schizophrenia and schizoaffective disorder. A best estimate consensus process was used to assign diagnoses and to determine major depressive syndrome in the patients'lifetime.

Patients. For the 155 schizophrenic subjects we found Undifferentiated (68), Paranoid (48), Disorganized (22) and Residual Type (17). Of the total sample, 87 individuals (56.1%) had had at least one full episode or syndrome of major depression after the onset of schizophrenia.

Intervention. We genotyped each subject at the s/l promoter region polymorphic site.

Main Outcome Measure. Lifetime history of depressive syndrome (DSMIIIR).

Results. We found a significant association between the "ss" or "sl" genotype history of depression: X2=5.4 (p=0.02). Schizophrenic subjects with "ss" or "sl" genotype are 2.7 times more likely to develop depression: OR=2.7 (1.15-6.3) than those with the "ll" genotype.

Conclusions. This supports our previous finding that having an "ss" or "sl" genotype increases the risk of schizophrenic patients to develop depression.

1944/T

Developmental regression, autism and GABA receptor genes. *M.L. Cuccaro[1], D. Ma[1], E.R. Martin[1], R.K. Abramson[2], H.H. Wright[2], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Miami Inst for Human Genomics, Univ of Miami Miller School of Medicine, Miami, FL; 2) Univ of South Carolina SOM, Columbia, SC.

Autism (AUT) is characterized by significant genetic heterogeneity. Clinical subsetting on diagnostic features (e.g., language) has yielded several candidate loci. We have extended subsetting to an associated feature-developmental regression (DR). DR occurs in 20-49% of individuals with AUT and may co-exist with seizures. The relationship between DR and seizures implicates the GABAergic system, as it believed that GABA transmission influences seizure susceptibility. We reported association in AUT for SNPs in GABA receptor genes on chromosome 4 (GABRA4) in Caucasians (CA) and confirmed these results in an independent African American (AA) sample, although the associated SNPs differed in the two racial groups. We hypothesized that DR may have an effect on association with GABA receptor genes and could clarify the differences in associated SNPs in AA and CA families. Using the ADI-R, a standard interview for features of AUT, we identified a DR subset (N = 263; 35%) from our overall sample of 606 AUT families (54 AA, 552 CA). DR families were those with positive ADI-R regression scores in at least 1 affected individual. Using the pedigree disequilibrium test (PDT) we tested for association in the DR and non-DR subsets in 36 SNPs in GABRA4 and GABRB1. Data were analyzed by race and contrasted with overall findings. In the AA-DR subset (N=17), allelic association (p<.05) was detected for the two SNPs in GABRA4 (rs2280073, rs16859786) previously identified in the overall AA group as well as for two additional SNPs in GABRA4 (rs13151769, rs2351299). In contrast, no SNPs identified in the overall CA group showed association in the CA-DR subset (N=246) although three SNPs (rs17599165, rs1912960, rs17599416) in the overall set remained significant in the CA non-DR complement. It appears that subsetting on DR had an effect on association and that DR subsetting may explain the different patterns of associated SNPs in the two racial groups. This is the first study of DR, GABA genes, and AUT. Our results suggest that DR should be explored in other candidate gene studies of AUT.

1945/T

Association between Neuregulin 1 and Schizophrenia in the PAARNTERS Study. *M.R. Dickson, H. Wiener, R. Perry, Z. Chen, R.C.P. Go, on behalf of The PAARTNERS Study Group.* Epidemiology, UAB, Birmingham, AL.

Neuregulins are essential for neuronal development. Neuregulin 1 (*NRG1*), located at 8p13, is a candidate gene for schizophrenia (SCZ) first identified by linkage studies with replication in different populations. Association studies have shown specific polymorphisms in *NRG1* to be associated with development of SCZ. The PAARTNERS study is a familial study of SCZ liability among African Americans. For this initial study, DNA samples were available for 486 families with ≥1 proband with SCZ. Eight SNPs within the *NRG1* gene, that were examined in previous studies, were genotyped and tested for association using FBAT. We performed two analyses, first using as affected only those determined to have SCZ (DSM-IV code 295.xx excluding 295.7), and second including those with schizoaffective disorder (including DSM-IV code 295.7). When an underlying additive effect of SNP genotype on risk of development of SCZ was assumed, allele G of SNP rs6988339, located in the 3' region, was observed to be transmitted to cases more frequently than expected by chance in both analyses (p=.028 with 77 informational families in the first, p=.033 with 79 informational families in the second). The assumption of a dominant model yielded an association with the same SNP in the first analysis (p=.043 with 75 informational families) with allele G, again, being the one transmitted more frequently. In the second analysis, two SNPs were seen to be associated under a dominant model: rs6988339 (p=.049 with 77 informational families), and rs6994992, located within the 5' region, (p=.047 with 116 informational families) with the T allele at this locus being transmitted more frequently than its C allele. Using the full genotype model available in FBAT, which does not impose a simpler a priori genetic model between genotype and phenotype, confirmed what was seen in the simpler models above. *NRG1* haplotype association with neurocognition in the PAARNTERS set will also be presented. Variation within the *NRG1* gene provides preliminary evidence of modest association with SCZ liability, and awaits confirmation in a larger PAARTNERS set and replication in other African American populations.

1946/T

Putative Neurexin 1 and Neuroligin 4 mutations in U.S. Caucasian patients with autism or Asperger syndrome. *J. Feng[1], K. Noltner[1], J. Yan[1], J. Sebastian Saldivar[2], S. Martinez[2], M. Hua[2], J. Picker[3], S. Sommer[1,2].* 1) Dept Molecular Genetics, City Hope Natl Medical Ctr, Duarte, CA; 2) Department of Molecular Diagnosis, City of Hope National Medical Center, Duarte, CA; 3) Department of Medical Genetics, Children's Hospital, Boston, MA.

The neuroligins are post-synaptic membrane cell-adhesion molecules that bind to pre-synaptic beta-neurexins, a family of proteins that act as neuronal cell surface receptors. A triad of studies reported associations between mutations in the Neuroligin 4 (NLGN4) gene and autism, Asperger syndrome, and mental retardation. Yan et al estimated an attributable risk of 3% in U. S. Midwest and Portuguese Caucasian families. Recently, two studies reported an association of neurexin 1 beta with autism. Feng et al. estimated an attributable risk of 3%. Herein, 78 patient samples with autism or Asperger syndrome submitted for clinical testing from throughout the U.S. were tested comprehensively by direct sequencing for mutations in the NLGN4 gene. In addition, 94 patient samples with autism were sequenced comprehensively for the neurexin 1 alpha gene. Three cases were positive for mutations in the NLGN4 gene that are very likely to be deleterious (~4%). An analysis of the neurexin 1 alpha gene reveals putative mutations. NLGN4 mutations occur at a significant level in a clinical sample of U.S. Caucasian patients with autism and Asperger syndrome. We conclude that NLGN4 and neurexin 1 may have an aggregate attributable risk for autism/Asperger syndrome of 6% or more.

1947/T

An E3 Ubiquitin Ligase, Rnf41, is associated with anxiety-like behavior, major depression, and b-carboline-induced seizure. *H.K. Gershenfeld[1], S. Kim[1], S. Zhang[2], K. Choi[3], R.L. Reister[1], A.F. Baykiz[4].* 1) Dept. of Psychiatry and Integrative Biology, UT Southwestern, Dallas, Tx; 2) Dept. of Psychiatry, Univ. of Conn., Farmington CT; 3) Stanley Laboratory of Brain Research, Rockville, MD; 4) Elazyg Asker Hastanesi Psikiyatri Klinigi, 23300 Elazig, Turkey.

Using an unbiased genetic approach, Quantitative Trait Loci (QTL) influencing anxiety-like behaviors and b-carboline-induced seizure have been mapped to the distal portion of mouse chromosome 10. An interval specific congenic mouse line containing the telomeric region of the C57BL6/J chromosome 10 on the A/J background narrowed down the chromosomal region of interest (66 cM to telomere), defined the behavioral influences of this region, and permitted gene expression profiling to identify a candidate gene. Ring Finger 41, (Rnf41 / Neuregulin Degrading Protein; Nrdp), an E3 Ubiquitin Ligase, was the only gene differentially expressed comparing the hippocampi of A/J vs C10 congenic mice as well as A/J vs B6 mice by microarray studies. RNF41 expression levels were significantly reduced in the hippocampi of A/J mice compared to C57BL6/J and the congenic mice. In addition, expression levels of Rnf41 mRNA and proteins were reduced in A/J mice compared to B6 mice across postnatal development. Rnf41 hippocampal mRNA expression levels were significantly correlated with open field behavior in the LxS recombinant inbred panel of mice, providing an independent replication. As anxiety and depressive disorders share a genetic predisposition, RNF41 was a potential candidate gene for psychiatric illness. Using human, post-mortem prefrontal cortex (Brodmann's Area 46/10) tissue of patients and controls, RNF41 mRNA expression levels were reduced significantly in patients with major depression and bipolar disorder compared to unaffected controls. Overall, RNF41 is a pleiotropic candidate gene for anxiety-like behaviors and beta-carboline induced seizure response. This RNF41 E3 Ubiquitin ligase and its physiologic binding partners are discussed as potentially novel mechanisms for influencing behavior variation relevant to psychiatric illness.

1948/T

Association between polymorphisms in catechol-O-methyltransferase (*COMT*) and cocaine-induced paranoia in European-American and African-American populations. *R. Hirunsatit[1,4,5], H.R. Kranzler[6], C. Ittiwut[1,4,5], R. Weiss[7], K. Brady[8], V. Hesselbrock[6], B. Rounsaville[1,4], L.A. Farrer[9], J. Gelernter[1,2,3,4].* 1) Yale Univ. Sch. Medicine, Dept Psychiatry; 2) Genetics; 3) Neurobiology, New Haven, CT; 4) VA CT Healthcare System, West Haven, CT; 5) Chulalongkon Univ. Thailand; 6) Univ. CT Sch. Medicine, Farmington, CT; 7) Harvard Medical Sch., Boston, MA; 8) Medical Univ. of SC, Charleston, SC; 9) Boston Univ Sch. Medicine and Public Health, Boston, MA.

COMT (genetic locus,*COMT*) is a major enzyme involved in catecholamine metabolism that has been reported to be associated to numerous psychiatric phenotypes and endophenotypes. We studied the association of 17 *COMT* SNPs with cocaine-induced paranoia (CIP) in 319 African-American (AA) and 302 European-American (EA) nuclear families ascertained for cocaine or opioid dependence, using family-based association methods (FBAT/HBAT). SNP rs737865 was nominally associated with CIP in AA families (p=0.05 in additive and p=0.03 in dominant and recessive models). In EA families, rs737866 was significantly associated with CIP in dominant and recessive models (p=0.02). SNP rs174696 also showed significance in all models (p=0.02 additive, p=0.004 dominant and recessive). The best-known marker, rs4680 (Val158Met), was nominally significant in all models (p=0.03 additive, p=0.005 dominant and recessive). Haplotype analysis including rs737866, rs4680, and rs174696 revealed an association of haplotype A-A-T with increased CIP risk in EA families (p=0.0044) and an association of haplotype G-G-T with decreased risk of CIP in AA families (p=0.0014) after Bonferroni correction [corrected p<0.008 for significance] for testing of six major haplotypes in both populations. We conclude that COMT is associated with cocaine-induced paranoia in both AAs and EAs. Different haplotypes composed of the same SNPs were associated in the two populations, which suggests that the actual risk variant (or variants) were introduced on different chromosomal backgrounds in the different populations. COMT is the second enzyme involved in dopamine metabolism (after DBH) that has been shown to be associated with CIP.

1949/T

High-throughput genotyping of the duplicated gene encoding dopamine receptor 5. *D.J.E. Housley[1], M. Nikolas[2], K.A. Jernigan[1], P.J. Venta[1], J.T. Nigg[2], K.H. Friderici[1].* 1) Dept of Microbiology and Molecular Genetics, Michigan State University; 2) Dept of Psychology, Michigan State University, East Lansing, MI.

Several independent association-based studies have implicated the *DRD5* locus in contributing to attention deficit hyperactivity disorder (ADHD). However, as promising as this locus is, its coding region has not been fully evaluated in most study populations, most likely due to the presence of two highly similar pseudogenes. Our goal was to develop a high-throughput approach to evaluate the entire *DRD5* coding region for new variants and to make accurate genotyping calls for common SNPs. A restriction enzyme site present in both pseudogenes, but absent in *DRD5*, presented an opportunity to use an enzyme treatment prior to amplification to eliminate co-amplification of the duplicated loci. Sequencing of PCR products from 31 trios of an ADHD affected child and both parents confirmed the purity of the amplicons, allowed for discovery of new variants, and enabled confident genotyping calls. Two common variants were genotyped in the trios, which enabled haplotype construction and determination of frequencies in the population. In addition, two previously described rare variants were found: one non-synonymous substitution and one nonsense mutation. We also compared the recorded SNPs in the UCSC browser and HapMap to mismatches between gene and pseudogenes and found that many SNPs from the databases in this region most likely represent gene/pseudogene mismatches. A bioinformatics approach, used to evaluate the extent of the duplicated region, revealed that two separate chromosomal segments, near and including *DRD5*, were duplicated onto HSA1 and 2. The promoter region of *DRD5* was part of a separate duplication event involving 16.6 kb of sequence upstream from the transcription start site, which includes a microsatellite commonly used for association-based studies. This analysis illustrates the importance of using caution when choosing SNPs from databases in regions of suspected duplications. Additionally, we provide a simple and relatively inexpensive method to analyze sequence variation using a high-throughput approach, which can be easily adapted to other duplicated genomic regions.

1950/T

Association analysis of human cAMP-GEFII gene polymorphisms with Japanese schizophrenia patients. *H. Kawasaki, H. Mitsuyasu, L. Gotoh, Y. Kobayashi, N. Oribe, A. Takata, S. Kanba.* Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan.

We have previously reported novel genes of second-messenger regulated Rap1-GEF (guanine nucleotide exchange factor) gene family whose GEF activities are positively regulated by the binding of second-messenger molecules such as cAMP, calcium and diacylglycerol (DAG), indicating that three major second messengers transduce their signals to target molecules different from protein kinases. A part of this gene family includes cAMP-GEFI and cAMP-GEFII, CaIDAG-GEFI and CaIDAG-GEFII. Both cAMP-GEFI and cAMP-GEFII have binding domains for cAMP as well as GEF domains. Since antipsychotics have antagonistic actions for dopamine receptors regulating intracellular cAMP concentrations in central nervous system, cAMP-GEFs can be good candidates for molecular studies of schizophrenia. In order to investigate the contribution of cAMP-GEFII to the pathophysiological mechanisms of schizophrenia, we carried out a genetic analysis of single nucleotide polymorphisms (SNPs) of the cAMP-GEFII gene. Information of a total 21 SNPs was collected based on the databases of both dbSNP and JSNP and our genotyping experiments, which included 3 coding SNPs and 17 intron SNPs and one regulatory SNP. We found two novel non-synonymous ones. We genotyped 96 schizophrenic patients and 140 healthy controls with the 21 SNPs by direct sequencing method. Two SNPs were shown to be significant difference between schizophrenia and healthy controls in Japanese population. However, after Bonferroni correction, those were disappeared. We are now trying to genotype more samples. The results of haplotype prediction, linkage disequilibrium calculation, and multi-variate statistical analysis are now being analyzed. Although there was slightly different distribution of the regulatory SNP between schizophrenia and controls, no statistical significant association were found. All subjects were given informed consent based on the ethical regulations of Kyushu University.

1951/T

Mutation screening and asscoiation study of the *FMR1* gene in Thai boys with autism. *P. Limprasert[1], C. Maharat[1], N. Ruangdaraganon[2], T. Hansakunachai[3], R. Sothanayongkul[2], T. Somboontham[2], T. Sripo[1], W. Maisrikhaw[1], V. Praphanpol[4].* 1) Prince of Songkla University, Hat Yai, Songkhla, Thailand; 2) Ramathibodi Hospital, Bangkok, Thailand; 3) Thammasat University, Pathumthani, Thailand; 4) Rajanukul Institute, Bangkok, Thailand.

Autism is a common neurodevelopmental disorder characterized by impairments in communication and social interactions, and repetitive and stereotypic behaviors. The presence of a genetic contribution to autism has been indicated by sibling and twin studies. Fragile X syndrome (FXS) is one of the most common single gene defects associated with autism. Some patients diagnosed as having autism or PDD-NOS have had FXS with expanded CGG repeats in the *FMR1* gene. Normally, FXS patients show some autistic behaviors and may be difficult to distinguish at a young age from autistic children. In an attempt to elucidate these connections, we screened 108 unrelated boys with autism or PDD-NOS, age < 15 years, using FXS PCR screening and EcoRI/EagI southern blot with StB12.3 probe. One patient was confirmed to have FXS, giving a frequency of FXS in autism of ~1% in our study. We also analyzed SNP haplotypes (WEX5-ATL1-rs25702) of the *FMR1* gene using biallelic ARMS-PCR in 77 autistic and 30 PDD-NOS patients, comparing them to 126 normal control males. The three major haplotypes in the combined cases and controls respectively were G-G-A (55% vs 59%), C-A-G (22% vs 22%) and C-G-A (13% vs 12%). No statistically significant differences between cases and controls were found for these haplotypes using the chi-square test ($P > 0.05$). When we analyzed the autistic or PDD-NOS subgroups, the frequencies of major haplotypes were still similar in both groups and not significantly different compared to the controls. To summarize, we did not find any association between SNP-haplotypes of the *FMR1* gene and Thai males with autism and/or PDD-NOS.

1952/T

Extreme Phenotype Candidate Gene Association Study within Endogenous Opioid System in Major Depressive Disorder. *S. Mee[1], C. Reist[1,2], L. Mee[1,2], R. Moyzis[3], W.E. Bunney[2].* 1) Dept Psychiatry, VA Long Beach , Long Beach, CA; 2) Dept Psychiatry, University of CA Irvine, School of Medicine; 3) Dept Biochemistry, University of CA Irvine, School of Medicine.

Background: Major depression plagues approximately 10% of the worldwide population. Depression is widely understood to be a complex genetic disorder with heritability estimated as high as .50. No genes conclusively predisposing to this complex genetic disorder have been identified. There is substantial evidence of a link between chronic pain and depression, including the observation that affective states directly influence pain intensity, higher rates of depression in chronic pain patients and analgesic properties of some antidepressants. The endogenous opioid system, central to the experience of pain, has also been implicated in the pathophysiology of depression both in animal models and clinically. Recently, investigators have utilized extreme discordant phenotype-based study designs for diseases presumed to be influenced by multiple genetic factors of individually small effect. We are investigating whether genetic variants within POMC, proenkephalin, mu, and delta opioid receptors are associated with major depressive disorder in a case-control design of rigorously defined phenotype. Methods: Subjects were recruited from the outpatient psychiatric population of the VALBHCS medical center and screened with the full SCID-DSM IV, chart review and clinical interview with an experienced psychiatrist. Approximately 5 markers per gene, selected on the basis of population frequency from HapMap published data, were genotyped in subjects and healthy, ethnically matched controls. Polymorphism frequencies and mutation screening were compared and tested for allelic association. Results: Variants within the delta opioid receptor indicate tentative evidence of association with the case group. Ongoing analysis will be completed by 10/2007. Conclusion: Converging Evidence of links between the endogenous opioid system and depression suggests the utility of selecting candidate genes in pain neuropathways for genetic association studies of depression.

1953/T

Schizophrenia candidate gene association study in a large European ancestry sample. *A.R. Sanders[1], J. Duan[1], M. Martinez[2], D. He[1], G.J. Burrell[1], N.G. Buccola[3], B.J. Mowry[4], R. Freedman[5], F. Amin[6], D.W. Black[7], J.M. Silverman[8], W.F. Byerley[9], R.R. Crowe[7], C.R. Cloninger[10], D.F. Levinson[11], P.V. Gejman[1].* 1) ENH & Northwestern Univ, Evanston, IL; 2) INSERM, Toulouse, France; 3) LSU Health Sci Ctr, New Orleans, LA; 4) QCSR & Univ Queensland, Brisbane, Australia; 5) Univ Colorado Health Sci Ctr, Denver, CO; 6) Atlanta VA Med Ctr & Emory Univ, Atlanta, GA; 7) Univ Iowa, Iowa City, IA; 8) Mt. Sinai School of Medicine, New York, NY; 9) UCSF, San Francisco, CA; 10) Washington Univ, St. Louis, MO; 11) Stanford Univ, Palo Alto, CA.

Introduction: We now present all data from a study of 14 schizophrenia candidate genes: *RGS4*, *DISC1*, *DTNBP1*, *STX7*, *TAAR6*, *PPP3CC*, *NRG1*, *DRD2*, *HTR2A*, *DAOA*, *AKT1*, *CHRNA7*, *COMT*, and *ARVCF* (data for 336 SNPs in a smaller sample were previously presented). The experimental design included a large sample size, dense gene coverage, and use of AIMs to ensure ancestral similarity of cases and controls. Methods: The European ancestry (EA) sample included 1,870 cases (90% schizophrenia and 10% schizoaffective; 20% familial) and 2,002 controls screened for psychosis. Outliers were excluded based on analysis of 194 AIMs. SNPs (N=789, chosen for tagging, from previous reports, or functionality) were genotyped using SNPlex and Taqman, with 648 passing extensive data cleaning procedures. Results: There were 31 SNPs (27 not previously reported as associated) with nominal p<0.05 for single-SNP tests of association (Armitage), of which 3 had nominal p<0.01 (two in *STX7*, one in *NRG1*). None were significant after correction for the number of tests. Haplotype analyses did not demonstrate increased significance. For tag SNPs, the q-q plot deviated slightly from the null line, but was under that line, consistent with a lack of evidence for association. Conclusions: We have not found significant evidence for association to these genes in our sample. The fact that these genes are not robustly associated in a large sample bolsters the case for systematic large-scale association studies, including linkage disequilibrium (LD) mapping of candidate regions, genome-wide association (GWA) approaches, and large-scale sequencing.

1954/T

Testing for association between Alzheimer's disease with psychosis and variations in candidate genes for psychosis. *R. Sims[1], P. Hollingworth[1], A. Morgan[1], V. Moskvina[2], S. Lovestone[3], C. Brayne[4], D. Rubinsztein[4], M. O'Donovan[1], M. Owen[1], J. Williams[1,2], R. Abraham[1].* 1) Department of Psychological Medicine, Cardiff University, Wales College of Medicine, Cardiff, United Kingdom; 2) Biostatistics and Bioinformatics Unit, Cardiff University, Wales College of Medicine, Cardiff, United Kingdom; 3) Institute of Psychiatry, King's College, London, United Kingdom; 4) University of Cambridge, United Kingdom.

As Alzheimer's disease (AD) progresses many sufferers experience additional behavioural and psychological symptoms such as psychosis. Psychotic symptoms are reported to affect 30-60% of individuals with AD and are associated with more rapid cognitive and functional decline, more severe cognitive impairment, premature institutionalization, and increased risks for agitated and aggressive behaviour. Evidence suggests that AD with psychosis shows greater familiality and evidence of linkage to specific chromosomal regions. As at least one of these overlaps with regions of linkage to psychotic disorders schizophrenia and bipolar affective disorder (BPAD), we set out to identify genes increasing risk of psychotic symptoms across diseases. Variants from nine genes (DAOA, GRM3, OLIG2, CNP, BDNF, DISC1, GRIK2, COMT, and DTNBP1) which show some evidence of influencing risk in psychotic disorders were individually genotyped in a sample of 1205 Caucasian cases of late onset AD (NINCDS-ADRDA criteria) and 1361 aged matched controls from the UK. Preliminary results show evidence for a psychosis susceptibility gene which modifies psychotic symptoms in Alzheimer's disease (allelic p = 0.0061; OR = 1.37, 95% CI; 1.0895 < OR < 1.7276).

1955/T

Haplotypic Variants in DRD2, ANKK1, TTC12 and NCAM1 co-Regulate the Comorbidity of Alcohol and Drug Dependences. *B.Z. Yang[1,4], H.R. Kranzler[5], H. Zhao[2], J.R. Gruen[3], X. Luo[1,4], J. Gelernter[1,4].* 1) Psychiatry; 2) EPH; 3) Pediatrics, Yale Univ Sch Med, New Haven, CT; 4) VA CT Healthcare Center, West Haven, CT; 5) Univ CT Health Center, Farmington, CT.

DRD2 is a functional candidate gene for substance use disorders (SUDs), including alcohol dependence (AD) and drug dependence (DD). Many studies of the association of *DRD2* with SUDs have been conducted, but the results have been inconsistent. The odds ratio of comorbid DD with AD, adjusted for demographic characteristics, is as high as 18.7 (Compton et al 2007). This comorbidity apparently generates heterogeneity of SUD phenotypes that could confound the mapping of genes. We hypothesized that prior inconsistent results were influenced by the heterogeneity of the SUDs and the presence of multiple risk variants for SUDs mapped in a small region close to *DRD2* (Gelernter et al, 2006). We conducted two separate association studies of comorbid AD and DD in 1220 European-American subjects using family-based and case-control designs and 43 single nucleotide polymorphisms (SNPs) mapped to the gene cluster of *NCAM1*, *TTC12*, *ANKK1* and *DRD2*. We used a generalized linear model and haplotype score tests for the case-control sample, and the family-based association test for the family sample. The result of haplotype associations centered on *TTC12* exon 3 both in the case-control and family samples (optimal individual haplotype simulated $p(p_{oihs})$ = 0.000015). Another associated haplotype extended from *ANKK1* exon 8 to *DRD2^C957T* in both designs (p_{oihs} = 0.0028). *NCAM1* exon 12 markers showed global significance in both case-control and family samples, but were significant for a specific haplotype association (p_{oihs} = 0.0029) only for the family sample. Population stratification was excluded as a possible cause of false positive association. LD contrast tests between cases and controls support selection at *TTC12* exon 3 and *ANKK1* exon 2. We conclude that variants in exon 3 of *TTC12*, exon 12/intron 13 of *NCAM1*, and the two 3' ends of *ANKK1* and *DRD2* co-regulate risk for the comorbidity of alcohol and drug dependence. Compton WM et al Arch Gen Psychiatry V64, 2007. Gelernter et al Hum Mol Genet. 2006 V15, 2006.

1956/T

Analysis of a neuregulin 1 missense mutation in families of Mexican and Central American origin. *A. Davelos Baines[1, 2], A. Figueroa[1, 2], C. Walss-Bass[1, 3], R. Salazar[4], A. Dassori[3], J. Peters[3], A. Ontiveros[5], H. Nicolini[6], R. Mendoza[7], M. Escamilla[1, 3], H. Raventos[4].* 1) South Texas Medical Genetics Group, UTHSCSA, Edinburg, TX; 2) UTPA, Edinburg, TX; 3) UTHSCSA, San Antonio, TX; 4) Universidad de Costa Rica, San Pedro, Costa Rica; 5) INFOSAME, Monterrey, Mexico; 6) Medical and Family Research Group, Carracci, Mexico D.F; 7) David Geffen School of Medicine at UCLA, Torrence, CA.

A missense mutation (Val to Leu) in exon 11 of the neuregulin 1 gene has been associated with schizophrenia in a population from the Central Valley of Costa Rica (CVCR). DNA genotyping and association studies were performed for 793 individuals with psychosis (536 of whom had a diagnosis of schizophrenia) from families of Mexican and Central American origin. Association analysis by the Family Based Association Test (FBAT) revealed that the missense mutation was transmitted more often than expected to affected persons, but the association was not significant in this sample (p = 0.28 for psychosis and p=0.47 for schizophrenia). A previous finding of an association of a missense mutation in the neuregulin 1 gene was not replicated in this independent sample of Hispanic individuals. Further analyses of samples from different populations should be conducted to determine the prevalence of this mutation and its relation to schizophrenia spectrum disorders.

1957/T

A High-Density SNP Genome-wide Linkage Scan in a Large Autism Extended Pedigree. *K. Allen-Brady[1], J. Miller[2], N. Matsunami[3], J. Stevens[3], H. Block[2], M. Farley[2], L. Krasny[2], C. Pingree[2], J. Lainhart[2], M. Leppert[3], W.M. McMahon[2], H. Coon[2].* 1) Dept of Biomedical Informatics, Univ of Utah, Salt Lake City, UT; 2) Dept of Psychiatry, Univ of Utah, Salt Lake City, UT; 3) Dept of Human Genetics, Univ of Utah, Salt Lake City, UT.

We performed a high-density, single nucleotide polymorphism (SNP), genome-wide scan on a six-generation pedigree from Utah with seven affected males, diagnosed with autism spectrum disorder. Using a two-stage linkage design, we first performed a non-parametric analysis on the entire genome using a 10K SNP chip to identify potential regions of interest. To confirm potentially interesting regions, we eliminated SNPs in high linkage disequilibrium (LD) using a principal components analysis method and repeated the linkage results. Three regions met genome-wide significance criteria after controlling for LD: 3q13.2-q13.31 (NPL= 5.58), 3q26.31-q27.3 (NPL=4.85) and 20q11.21-q13.12 (NPL=5.56). Two regions met suggestive criteria for significance 7p14.1-p11.22 (NPL=3.18) and 9p24.3 (NPL=3.44). All five chromosomal regions are consistent with other published findings. Haplotype sharing results showed that five of the affected subjects shared more than a single chromosomal region of interest with other affected subjects. Although no common autism susceptibility genes were found for all seven autism cases, these results suggest that multiple genetic loci within these regions may contribute to the autism phenotype in this family, and further follow-up of these chromosomal regions is warranted.

1958/T

MTSS1 and TRPS1 As Potential Susceptibility Genes for Alzheimer's Disease in the Chromosome 8q. *J. Deng[1], Y.J. Li[2], G.M. Mayhew[3, 4], J. Grimsley[2], X. Hu[1], M.A. Pericak-Vance[3, 4], J.M. Vance[3, 4].* 1) Neurobiology, Duke Univ, Durham, NC; 2) Center for Human Genetics, Duke Univ, Durham, NC; 3) Center for Molecular Genetics and Genomic Medicine, Univ of Miami, Miami, FL; 4) Miami Institute for Human Genomics, Univ of Miami, Miami, FL.

We previously conducted a whole genome screen for age-at-onset (AAO) of Alzheimer's Disease (AD) using 449 families and identified several linkage regions including chromosome 8q and 4q. The initial linkage region of chromosome 8q covers 46cM interval (between 119 to 165cM; LOD>1.0) with a peak multipoint LOD score of 2.09. At the Stage Two study, we re-genotyped the same microsatellite markers under the chromosome 8q and 4q linkage regions on the additional new 158 families to refine the intervals. We applied the variance component linkage analysis method implemented in SOLAR (Almasy and Blangero 1998) using this expanded dataset (607 families). The chromosome 4q peak disappeared, while 8q peak was strengthened with an updated two-point LOD score of 2.34 at the marker D8S1179. In addition, the new analysis narrowed the peak region to about 20cM (between 128 to149cM; LOD>1.0). We then designed our Stage Three study to follow up the chromosome 8 interval using an Illumina Goldengate custom SNP arrays. We selected 1536 SNPs to cover ~24mb area at one LOD score below the peak marker (1-LOD down region between q23.3-q24.23), resulting in a density of one SNP per 10000 bps in most part of the region. We included 1192 samples selected from 279 families with the highest linkage score from Stage Two study. The APL and PDT were used for detecting risk gene(s) for AD and QTDT was used for detecting genes regulating AAO of AD. After genotyping quality controls and correction for multiple testing using qvalue, we identified two SNPs that are the most significantly associated with AD risk. The best SNP is located in metastasis suppressor 1 (MTSS1), with p-values of 2.8×10^{-5} by APL and 0.0063 by PDT. The second significant SNP is in the front of a zink finger transcription factor, TRPS1, with a p-value of 0.0019 by APL and 0.0001 by PDT. Additional SNPs are being tested in these gene regions to identify the key areas of association.

1959/T

Analysis of variation in the pituitary adenylate cyclase-activating polypeptide (PACAP/ADCYAP1) gene and susceptibility to bipolar disorder. *F.W. Lohoff, A.E. Weller, P.J. Bloch, T.N. Ferraro, W.H. Berrettini.* Department of Psychiatry, Univ Pennsylvania, Philadelphia, PA.

Background: Linkage studies in bipolar disorder (BPD) suggest that a susceptibility locus exists on chromosome 18p11. The pituitary adenylate cyclase-activating polypeptide (PACAP/ADCYAP1) gene maps to this region. PACAP is a neuropeptide involved in PNS and CNS neurotransmission and is required for catecholamine secretion. Animal models of PACAP mutations show remarkable behavioral defects, including hyperactivity and increased exploratory behavior. We hypothesize that genetic variations in the human PACAP gene contribute to BPD. **Methods:** Genotypes for 4 SNPs (rs2846811; rs8192595; rs2856966; rs1610037) across the PACAP gene in BPD patients (n=570) and healthy controls (n=710) were obtained. Genotypes and allele frequencies were compared between groups using Chi square contingency analysis. LD between markers was calculated and estimated haplotype frequencies were compared between groups. **Results:** We did not observe any significant differences between groups on the allele, genotype or haplotype level for any of the tested SNPs. **Conclusion:** Our results provide no evidence for an association of the PACAP gene with BPD in this group of patients and controls. Additional studies are necessary to elucidate the BPD susceptibility locus on chromosome 18p.

1960/T

Extended meta-analysis of genome-wide linkage studies in Schizophrenia. *M.Y.M. Ng[1], C.M. Lewis[1], D.F. Levison[2], Schizophrenia Meta - Analysis Consortium.* 1) Medical and Molecular Genetics, King's College London, London, UK; 2) Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, CA, USA.

Introduction: We previously reported that a Genome Search Meta-Analysis (GSMA) of schizophrenia linkage scans provided evidence for linkage in 10 chromosomal regions. We performed an updated analysis to incorporate multiple new studies.

Method: Results (LOD, NPL scores or p-values) were obtained from investigators for 31 genome-wide linkage scans: 16 from the previous analysis (including 6 with new genotyping, some with expanded samples) and 15 new scans, totaling 3,215 pedigrees with 7,212 genotyped schizophrenia or schizoaffective cases, compared with 1,208 and 2,945 previously. GSMA is a non-parametric method which ranks the strongest evidence for linkage from chromosomal regions (bins) of equal width (Rutgers map), and sums ranks across studies to assess evidence for linkage. A primary analysis (all families and 30 cM bins) was compared with results for the subset of 22 scans of European-ancestry samples and for bin widths of 20 and 40 cM.

Results: Aggregate significance criteria identified the regions most likely to contain linked loci, including a region of chromosome 2q identified previously (but now extending across a broader region), and regions of chromosomes 8p (30-90 cM), 5q (150-210 cM), 1p (120-150 cM), 16p (30-60 cM) and 4q (120-150 cM). Much stronger evidence for linkage was identified in this analysis on chromosome 8p, particularly in European-ancestry samples. Further studies of empirical significance thresholds are in progress.

Discussions: Linkage regions supported by meta-analysis may contain schizophrenia susceptibility loci, which could include common SNPs, copy number variants and/or multiple rare variants. Intensive studies are warranted to identify these loci.

1961/T

Construction of a SNP Chip Containing 94 Candidate Genes for Schizophrenia and Schizophrenia-Related Phenotypes. *T.A. Greenwood[1], G.A. Light[1], M.F. Green[2], R.E. Gur[3], K.H. Nuechterlein[2], A. Olincy[4], L.J. Seidman[5], D.W. Tsuang[6], N.J. Schork[1,7], D.R. Weinberger[8], D.L. Braff[1].* 1) Dept of Psychiatry, Univ of California, San Diego, La Jolla, CA; 2) Dept of Psychiatry and Biobehavioral Sciences, Univ of California, Los Angeles, Los Angeles, CA; 3) Dept of Psychiatry, Univ of Pennsylvania, Philadelphia, PA; 4) Dept of Psychiatry, Univ of Colorado Health Sciences Center, Denver, CO; 5) Dept of Psychiatry, Harvard Medical School, Boston, MA; 6) Department of Psychiatry and Behavioral Sciences, Univ of Washington Seattle, WA; 7) Scripps Genomic Medicine, San Diego, CA; 8) Clinical Brain Disorder Branch, Genes, Cognition, and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

We have constructed a gene chip containing 1536 SNPs in 94 genes of relevance to schizophrenia that were chosen based on knowledge of biological systems, as well as an extensive review of published association and linkage studies. Many of these genes have also been reputed to be involved in P50 suppression, prepulse inhibition, neurocognitive functioning, brain development, and bipolar disorder. These genes cluster into several pathways, including cell signal transduction, amino acid metabolism, and glutamate, serotonin, dopamine, and GABA receptor signaling. In order to efficiently interrogate these genes, we have chosen to make primary use of Caucasian haplotype-tagging SNPs. Of the 1427 tagging SNPs that were selected for 89 of the genes, many also had reported associations in the literature, and 18 were nonsynonymous cSNPs. For the 5 genes for which tagging SNPs were not available, 29 SNPs were chosen for even coverage. We have also included an additional 80 SNPs that were reported to be associated with schizophrenia in the literature, many of which had been replicated by separate groups, and 10 of which were nonsynonymous cSNPs. Our preliminary analyses of three neurophysiological and nine neurocognitive endophenotypes for schizophrenia using the SNPs from this chip in a sample of 140 families have provided many interesting associations. This gene chip may be of interest to many groups studying schizophrenia and related phenotypes.

1962/T

Molecular mapping of a balanced translocation involving 11q24.2 associated with severe bipolar affected disorder. *S.T. Holden[1], A.S. Davies[2], S. Bint[2], A. Dunlop[3], R. Blennerhassett[4], R.C. Trembath[5], W. Reardon[3].* 1) Clinical Genetics, Guy's and St Thomas' NHS Trust, London, United Kingdom; 2) Cytogenetics, Guy's and St Thomas' NHS Trust, London, United Kingdom; 3) National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland; 4) St Ita's Psychiatric Hospital, Portrane, Co. Dublin, Ireland; 5) Medical and Molecular Genetics, King's College, London, United Kingdom.

The molecular characterization of the breakpoints of chromosome rearrangements associated with disease phenotypes, in conjunction with data from linkage and association studies, is a powerful approach to identifying candidate genes for complex genetic disorders. This approach has led to the identification of candidate genes for schizophrenia and bipolar disease, conditions associated with episodes of psychosis, and has highlighted pathways which begin to explain how these phenotypes, which show overlapping features and can be manifest within the same kindred, might be related at the molecular level. Linkage and association studies have shown that the region 11q22-24 is a disease locus for schizophrenia, and have highlighted FXYD6 and GRIK4 as candidate susceptibility genes. Interestingly, two patients have been reported, one with recurrent episodes of psychosis, and a second with severe bipolar disease, who have unbalanced chromosome deletions of 11q (Jacobsen syndrome) encompassing the interval of this disease locus. We report our progress in mapping the chromosome breakpoints in an intellectually normal patient with an apparently balanced chromosome rearrangement, 46,XX,t(11;15)(q24.2;q26.3), who has had recurrent episodes of severe bipolar disease associated with psychosis. The chromosome 11 breakpoint maps close to, but is distinct from, GRIK4, raising the possibility of further genetic heterogeneity for susceptibility at this disease locus.

1963/T

An association analysis of 13 anxiety disorder candidate genes. *I. Hovatta[1,2,3], J. Donner[1,2], S. Pirkola[4], K. Silander[1], L. Kananen[1,2], J. Lönnqvist[4], L. Peltonen[1,3,5].* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Research Program of Molecular Neurology, Biomedicum Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Finland; 4) Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland; 5) Broad Institute of MIT and Harvard, Boston, MA.

We have taken a cross-species approach to identify susceptibility genes for anxiety disorders. As a model, we used inbred mouse strains that differ in their innate anxiety levels. We chose an unbiased gene expression-based approach to identify candidate genes. We first assessed anxiety behavior of six inbred strains by behavioral tests, and conducted gene expression profiling using microarrays of seven brain regions. We identified 17 genes that had an expression pattern that correlated significantly with the behavioral phenotype across all strains. We carried out functional studies and showed by lentivirus-mediated gene transfer that two candidate genes, glyoxalase 1 and glutathione reductase 1, regulate anxiety-related behavior in mouse in vivo (Hovatta et al. Nature 2005). We have now investigated if any of the identified genes predispose humans to anxiety disorders as part of the Finnish population-based Health 2000 study. A representative sample (n=6005) of Finland's adult population was interviewed with CIDI for the presence of anxiety disorders. In addition, blood samples were collected for DNA extraction. The annual prevalence for the studied anxiety disorders was 5.7% (n=339) (Pirkola et al. 2005). Controls were matched according to age, sex, and home province. Thirteen of the 17 genes we identified in mouse have a homolog in the human genome. We have genotyped 207 SNPs including all non-synonymous SNPs, SNPs that alter potential microRNA binding sites, and additional gap-filling SNPs selected from the HapMap data in our anxiety disorder cohort. Statistical analyses using both allele and haplotype based methods are currently being carried out in order to investigate whether the studied SNPs associate to anxiety disorders in the Finnish population.

1964/T

Screening multiplex Tunisian kindreds for known PD mutations. *M. Hulihan[1], J. Kachergus[1], A. Soto[1], J. Stone[1], S. Lincoln[1], L. Ishihara-Paul[2], S. Oldham[2], R. Amouri[3], R. Gibson[2], F. Hentati[3], M. Farrer[1].* 1) Dept of Neuroscience, Mayo Clinic Jacksonville, Jacksonville, FL, USA; 2) Research and Development, GlaxoSmithKline, USA and UK; 3) Dept of Neurology, Institut National de Neurologie, Tunis, Tunisia.

Mutations in several genes have been implicated in familial Parkinson's disease (PD). We looked for mutations within five of these genes in 88 multiplex Tunisian families with PD. Population frequencies for all coding changes were assessed in a case/control series with similar ethnic origins. Exonic deletions/multiplications in four of the genes were also screened for within these families.

All samples were screened for the LRRK2 G2019S mutation. The proband from each of the 88 families was also sequenced for PRKN, PINK1, and DJ-1. Taqman probes were then designed for all coding changes seen in the sequenced genes so that remaining family members could be checked; subsequently, the probes were run in the case/control series. Additionally, variations in SNCA, PRKN, PINK1, and DJ-1 exonic copy number were assessed in the probands. Dosage abnormalities were checked in family members for consistency with disease segregation. Results were analyzed to determine which families appear to have PD-causing mutations consistent with disease segregation and what the estimated frequency of these mutations are within the Tunisian population.

Of the 88 families screened, 39 have G2019S mutations; 12 are homozygous for PINK1 mutations; and 5 are homozygous for PRKN mutations. One of the PRKN families had two different homozygous mutations for that gene. Each of these changes was seen in less than 2% of Tunisian controls. While 64% of the pedigrees now have an identified genetic cause of Parkinson's within the family, the remaining families have an as-yet unknown cause of disease. Further studies will be conducted to determine the novel gene(s) responsible for PD within these remaining kindreds.

.

1965/T

Linkage disequilibrium mapping for schizophrenia susceptibility genes on 8p23.3-p12 in a large European ancestry sample. *J.B. Duan[1], A.R. Sanders[1], M. Martinez[2], D. He[1], J. Li[3], G. Burrell[1], N.G. Buccola[4], B.J. Mowry[5], R. Freedman[6], F. Amin[7], D.W. Black[8], J.M. Silverman[9], W.F. Byerley[10], R.R. Crowe[8], C.R. Cloninger[11], D.F. Levinson[3], P.V. Gejman[1].* 1) ENH/Northwestern Univ, Evanston, IL; 2) INSERM, Toulouse, France; 3) Stanford Univ, Palo Alto, CA; 4) LSU Health Sciences Center (HSC), New Orleans, LA; 5) QCSR and Univ Queensland, Brisbane, Australia; 6) Univ Colorado HSC, Denver,CO; 7) Atlanta VA Med Ctr & Emory Univ, Atlanta, GA; 8) Univ Iowa, Iowa City, IA; 9) Mt. Sinai School of Med, New York, NY; 10) UCSF, San Francisco, CA; 11) Washington Univ, St. Louis, MO.

In our previous linkage genome scan of 409 schizophrenia (SZ) ASPs, the largest signals were observed across a ~60 cM region of 8p23.3-p12. We report here a dense LD mapping association study of this region in a large EA sample that includes 1765 cases (SZ or schizoaffective disorder) and 1956 controls screened for psychosis. Ancestral similarity of cases and controls was established with 194 ancestry informative SNPs. The 2,757 SNPs achieved an average 4 kb/tag SNP density for 236 RefSeq genes, capturing >80% of HapMap CEU common SNPs (r^2>0.8), and included tags within all conserved intergenic sequences plus all non-synonymous SNPs. A q-q plot of observed vs. expected p-values showed a small departure from (elevation above) the null line, consistent with association with variant(s) with small effects. Five single SNPs in or near CSMD1, MFHAS1, PSD3 or EBF2 produced nominal p<0.001, with the lowest value (p=0.0002, OR=1.21) for rs2059527, 48kb telomeric to EBF2. Genotyping 334 additional SNPs in these genes plus MCPH1 improved HapMap coverage (~90%) but not significance. Global haplotypic p-values were not more statistically significant. Empirical significance analyses are in progress. We also analyzed copy number variants, and found a rare 72 kb deletion (rs9650391 to rs418920) in MSR1 overrepresented in cases (p= 0.029). We did not detect region- or genome-wide evidence for association. One or more of the nominally associated genes could be involved in SZ susceptibility. Replication in other independent samples is essential.

1966/T

***GABRG1* and *GABRA2* Variation Associated with Alcohol Dependence in African American Population.** *C. Ittiwut[1,3,4], H.R. Kranzler[5], R. Anton[6], R. Hirunsatit[1,3,4], J. Covault[5], J. Gelernter[1,2,3].* 1) Dept Psychiatry, Yale Univ Sch. Medicine, New Haven, CT 06519, USA; 2) Genetics and Neurobiology, Yale Univ Sch. Medicine, New Haven, CT 06519, USA; 3) VA CT Healthcare System, West Haven, CT, USA; 4) Chulalongkorn Univ., Inter-Dept Program of Biomed Science, Bangkok, Thailand; 5) Dept Psychiatry, Univ CT Sch. Medicine, Farmington, CT, USA; 6) Medical Univ. of SC, Charleston, SC, USA.

GABRG1 and *GABRA2*, which encode the γ1 and α2 subunits, respectively, of the GABA-A receptor, are located in a cluster on chromosome 4p. Although markers located at the 3' region of the *GABRA2* locus have been associated with alcohol dependence (AD), one recent study suggests the possibility that the signal may be attributable to the adjacent gene, *GABRG1*, located 90kb distant in the 3' direction. To elucidate the association with AD, we genotyped 13 single nucleotide polymorphisms (SNPs) that span *GABRG1* and *GABRA2* in 276 African-Americans (AAs) with AD and in 242 AA controls (some of whom were included in an earlier report). Six tag SNPs were identified using the htSNP approach in HAPLOVIEW. Individual SNP associations were tested by chi-square. Nominally significant allele frequency differences were identified for rs10938426, at *GABRG1* intron 1, with p=0.044. Significant differences in both genotype and allele frequency (p=0.008 and 0.007, respectively) were observed at rs279869, located at *GABRA2* intron 6. We performed haplotype association analysis by means of PHASE. Haplotypes combining six SNPs from both gene loci showed frequency differences between controls and AD subjects, p=0.0027, significant after Bonferroni correction. A two-SNP haplotype composed of rs10938426 and rs279869 showed greater significance (p=0.00013). Association analysis of haplotypes defined within each gene showed no other association between any other *GABRG1* or *GABRA2* haplotype and AD risk. This finding suggests that there is an interrelationship between these two genes and that each may contain risk loci. A two-SNP haplotype composed of one SNP from each gene is consistent with an interaction of these genes and supports the involvement of both in susceptibility to AD in AAs.

1967/T

The first genome-wide inter-population linkage study of migraine families points to a locus on chromosome 10q22-q23. *V. Anttila[1,2,3], D.R. Nyholt[4], M. Kallela[3,5], V. Artto[3,5], S. Vepsäläinen[3,5], A. Sarahonka[1,2,3], P. Tikka-Kleemola[1,2,3], E. Hämäläinen[2,3], J. Terwilliger[2,3], L. Peltonen[2,3,6,7], M. Färkkilä[3,5], N.G. Martin[4], M. Wessman[2,3], A. Palotie[1,2,3,7].* 1) Finnish Genome Center, University of Helsinki, Helsinki, Finland; 2) Biomedicum Helsinki, Research Program in Molecular Medicine, University of Helsinki, Helsinki, Finland; 3) Center of Excellence in Complex Disease Genetics, University of Helsinki, Helsinki, Finland; 4) Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia; 5) Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland; 6) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 7) The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

To date, several genetic loci have been linked to common forms of migraine, though consistency between studies remains a problem. We performed a joint analysis of two new, large, well-characterized study samples from Finland and Australia. Some 400 microsatellite markers were genotyped in 1675 subjects, from 77 large independent Finnish multigenerational and 125 independent Australian nuclear migraine with aura families. Parametric and non-parametric qualitative two-point and multipoint linkage analyses, as well as QTL analyses, were performed using the migraine end-point diagnosis as well as phenotypes from trait component analysis and latent class analysis. We found significant evidence of linkage to a locus on chromosome 10q22-q23 in both samples separately, as well as in a joint analysis. In the Finnish study sample, the highest two-point HLOD of 4.37 was reached at marker D10S2470, and the highest multipoint LOD 5.52 was reached at 104.5 cM. In the Australian study sample, QTL analysis positions the peak at 102 cM, with a multipoint LOD of 3.68. This locus has now been detected in five separate genome-wide scans (including those reported here), providing by far the best consistency for any locus reported in common forms of migraine. In addition, our results also confirm six previously detected loci on 4q31, 5q21, 13q21, 15q11-q13, 17p13, and 18q12.

1968/T

Identification of *NRG3* (neuregulin 3) as a quantitative trait locus for the positive symptoms of schizophrenia. *P. Chen[1], D. Avramopoulos[2], J. McGrath[2], V.K. Lasseter[2], G. Nestadt[2], M.D. Fallin[3], A. Pulver[2], D. Valle[1].* 1) Inst Genetic Medicine, Johns Hopkins Sch Medicine, Baltimore, MD; 2) Dept Psychiatry; 3) Dept Epidemiology, School of Public Health.

Schizophrenia (SZ) is a complex disorder with a strong genetic component. Our previous genomewide linkage scan on families of Ashkenazi Jewish (AJ) descent (Fallin *et al.* Am J Hum Genet, 2003) showed the strongest linkage signal at chromosome 10q22 (NPL score: 4.27, P=0.00002). To further narrow down the susceptibility gene(s), we obtained a SNP-based fine mapping study with 1536 SNPs across the 12.5 Mb region. All subjects are of AJ background and were analyzed as 305 trios in family-based or 458 cases and 487 controls in population-based study. We used the UNPHASED statistic package (Dudbridge, Genet Epidemiol, 2003). The phenotypes for analysis included the disease status (either affected or non-affected) and 9 quantitative traits ("factors") derived from the 73 items of our consensus diagnostic ratings and direct assessment interviews (unpublished data) using the principal component factor analysis method. This 9 factor model is statistically supported and yields a number of factors consistent with other dimensional studies in the literature. Using the "positive symptoms" factor (e.g. thought insertion, delusions of influence, somatic hallucinations) as the quantitative trait, we found strong evidence of association at 3 nearby SNPs (rs10883866, rs10748842 and rs6584400) which are in strong linkage disequilibrium (LD) with each other. Our best P value from TDT analysis was 0.0000025 and the best P value from population-based association study was 0.000022. These 3 SNPs are located in a 13 kb interval in the first intron of *NRG3*, with an underlying LD block covering the proximal promoter, exon 1 and a part of intron 1 (total ~160 kb). *NRG3* is primarily expressed in the CNS and is one of 3 paralogs of *NRG1*, a gene strongly implicated in SZ in Celtic populations (Stefansson *et al.* Am J Hum Genet, 2002). These biological properties and our linkage and association results strongly implicate NRG3 as a candidate gene for SZ and justify functional and sequencing studies that are currently underway.

1969/T

GENETIC ASSOCIATION OF THE CHRNA3-CHRNB4 GENE CLUSTER WITH BEHAVIORAL DISINHIBITION IN YOUNG ADULTS. *I.R. Schlaepfer[1,2], A.C. Collins[1], R.P. Corley[1], T.J. Crowley[4], J.K. Hewitt[1,3], N. Hoft[1], C.J. Hopfer[4], J. Lessem[1], S.H. Rhee[1,3], M.C. Stallings[1,3], S.E. Young[1], M.A. Ehringer[1,2].* 1) Institute for Behavioral Genetics, University of Colorado, Boulder, CO; 2) Department of Integrative Physiology, University of Colorado, Boulder, CO; 3) Department of Psychology, University of Colorado, Boulder, CO; 4) Division of Substance Dependence, Department of Psychiatry, University of Colorado, Denver, CO.

Behavioral disinhibition is a manifestation of impulsive behavior that is important in the psychopathology of disorders like drug addiction, ADHD and conduct disorder (CD). Previous research from our lab has shown that genomic variations between the CHRNA3 and CHRNB4 genes are associated with the early age of initiation of tobacco and alcohol use, suggesting inherited vulnerability markers for behavioral disinhibition leading to early age of drug experimentation. Here we report our findings with markers in the CHRNA3-B4 region and CD and behavioral disinhibition composite phenotypes in young adults from Colorado. Our behavioral disinhibition variables include CD, ADHD, substance experimentation and novelty-seeking scores from young adults aged 17 to 21 years. Since SNP frequency calculations revealed ethnic-specific allele distributions in Caucasians, African-Americans and Hispanics, we conducted the genetic analysis including ethnicities as covariates in the statistical genetics program WHAP. Two SNPs previously shown to be associated with age of initiation for tobacco use were associated also with lifetime symptoms of CD scores ($p \leq 0.03$). Additionally, an adjacent synonymous SNP in the Exon 2 of the CHRNA3 gene was found to be associated with CD ($p = 0.01$), behavioral disinhibition ($p = 0.008$) and typical patterns of alcohol ($p = 0.004$) and tobacco use ($p = 0.04$). The patterns of use followed an additive model, where the more risk alleles of the Exon 2 variation significantly predicted the number of times using alcohol and tobacco in both males and females of our sample. In conclusion, our results emphasize the potential relationship between genetic variations in the CHRNA3-B4 region and behaviors that promote early age of experimentation with drugs.

1970/T

Effects of maternal-fetal genotype combinations on schizophrenia depend on offspring sex. *C.G.S. Palmer[1], E. Mallery[1], J.A. Turunen[2], H.J. Hsieh[3], L. Peltonen[2,4,5], J. Lonnqvist[3], J.A. Woodward[6], J.A. Sinsheimer[1].* 1) Univ Cal Los Angeles; 2) Natl Publ Hlth Inst; 3) Genentech Inc; 4) Univ Helsinki; 5) Broad Inst MIT; 6) Univ Cal Merced.

Previous studies suggest that maternal-fetal genotype incompatibility (MFG) at RHD and HLA-B loci increases risk for schizophrenia (SZ) in offspring (Palmer et al 2002; Palmer et al 2006; Hollister et al 1996; Insel et al 2005) by creating an adverse prenatal environment, and that the effects may depend on offspring sex. Although not tested by Palmer et al 2002, the effect of RHD MFG may be limited to male offspring (Hollister et al 1996; Insel et al 2005), while that of HLA-B MFG may be limited to female offspring (Palmer et al 2006). If true, this would suggest that sex differences during fetal neurodevelopment should be investigated to fully elucidate the etiology of SZ. The purpose of this study is to use a genetic approach to determine if the effect of RHD MFG is limited to male SZ offspring in the Finnish Schizophrenia Study Sample. The sample contained 277 nuclear families with ≥ 1 child affected with SZ or related disorder (affected offspring: 303 males, 202 females). Three models were evaluated using a general joint log-linear conditional model to test for association of RHD MFG and SZ risk in males (Kraft et al 2004). The null model (Model 0) constrains the relative risks for incompatible males (μ_M) and incompatible females (μ_F) to the null value (μ_M=μ_F =1). Model 1 assumes the relative risk of incompatibility is independent of offspring sex (μ_M=μ_F). Model 2 limits the relative risk to incompatible males (μ_F=1). A significant MFG effect was found in the sample (Model 0 vs Model 1; χ^2 =2.86, 1df, 1-sided p=.04), consistent with earlier analysis of a subset of these families (Palmer et al 2002). Furthermore, there was significant evidence that the effect of RHD MFG incompatibility on SZ is limited to male offspring (Model 0 vs. Model 2; χ^2=3.27, 1df, 1-sided p=.03), with μ_M=1.4. These results provide further support that RHD MFG increases SZ risk and that the risk is limited to males. Gender differences in fetal neurodevelopment should be considered in future studies of SZ.

1971/T

Dopamine Transporter 3'-UTR VNTR Genotype and Migraine. *F.B. Atac[1], U. Can[2], H. Verdi[1], A.C. Yazici[3], G. Celiker[2], R. Ocal[2], U.S. Benli[2].* 1) Dept Medical Biol & Genetics, Baskent Univ Fac Medicine, Ankara,Turkey; 2) Dept Neurology,Baskent Univ Fac Medicine,Ankara,Turkey; 3) Dept Biostatistics,Baskent Univ Fac Medicine,Ankara,Turkey.

Migraine is a debilitating neurovascular disease characterized by nausea vomiting, photophobia, phonophobia, neurological disturbances and severe recurrent headache. Although environmental factors could influence migraine susceptibility, the evident data indicates the importance of the genetic component. However, the pathophysiological mechanisms causing migraine is still remained to be elucidated. Therefore the continuing molecular identification of key proteins involved in migraine will refine our understanding of this common disease that strikes during the most productive years of a person's life. Recent pharmacological studies points out the effect of dopamine receptor antagonist in treatment. This treatment improves the involvement of the dopaminergic system in migraine. Dopamine uptake is mediated by dopamine transporter (DAT). Therefore the polymorphisms in DAT gene may be the candidates as migraine susceptibility modifier. In this study we aimed to elucidate the role of 40-bp variable number of tandem repeats (VNTR) in the 3' untranslated region (3'UTR) of the DAT gene (DAT3'UTR) in 100 migraine cases and 81 healthy controls. The G test and two proportion z test results indicate that DAT3'UTR polymorphism is a susceptibility factor for migraine in our population ($p<0.001$) since, the 10/11 ($p<0.01$), 11/12 and 12/12 ($p<0.001$) genotypes are strongly associated with migraine. When we performed allele comparison a significant difference was found between migraine cases and healthy controls (Fisher exact's $p<0.001$) with respect to allele 11 and 12 (OR= 2.872, 95% CI 1.828-4.514; OR= 56,568, 95% CI 7.723-414.347) respectively. In contrast to previous reports from other ethnic groups, our result may suggest that the genetic variability at the DAT gene is involved in the predisposition to migraine.

1972/T

NOS3 gene Polymorphysims in Turkish Stroke Patients. *U. Can[1], H. Verdi[2], A.C. Yazıcı[3], K. Beksac[2], G. Celiker[1], E. Derle[1], U.S. Benli[1], N. Ozbek[4], F.B. Atac[2].* 1) Neurology, Baskent University School of Medicine, Ankara, Turkey; 2) Dept Medical Biology and Genetics,Baskent University School of Medicine, Ankara, Turkey; 3) Dept Biostatistics,Baskent University School of Medicine, Ankara, Turkey; 4) Dept Pediatric Heamotology,Baskent University School of Medicine, Ankara, Turkey.

Impaired endothelial-mediated vasodilation is a common feature of many vascular risk factors, and experimental evidence strongly supports a role for impaired NO-dependent vasomotor reactivity in the pathophysiology of stroke. Nitric oxide (NO), synthesized by endothelial constitutive NO synthase (ecNOS-NOS3) plays a key role in vascular regulation and atherosclerosis. There are contradictory results existing on concerning the role of the ecNOS gene as a risk factor for brain infarction. In view of the location and proposed biological effect of NO we felt it was prudent to evaluate further the relation NOS3 gene intron 4 VNTR and exon 7 894 G/ T polymorphysims in Turkish Stroke Patients. 118 stroke cases and 100 controls were included in this study. There was an insignificant relationship between the intron 4 VNTR genotype distribution and allele frequencies in the stroke and control group. In contrast to this finding the distribution of exon 7 894 G/T genotype was significantly different between cases and controls, the TT genotype was more frequent in stroke cases (35.65%) than in controls (16.84 %); where as GG genotype was more frequent in controls (32.63 %) than in stroke cases (15.65%) ($p<0.001$). This data may suggest the importance of NOS3 exon 7 894 TT genotype as a pre-disposition factor for stroke in our population. Stroke is likely to be a multifactorial disease, several genes with weak or moderate effects are likely to be involved, and other candidate genes should also be investigated in order to understand the cause of the arteriolopathy which may have have future implications in treatment and prevention.

1973/T

FINE-MAPPING AN 18Mb ALCOHOL DEPENDENCE SUSCEPTIBILITY LOCUS ON 4q22-q32 IN THE IRISH AFFECTED SIB-PAIR STUDY OF ALCOHOL DEPENDENCE (IASPSAD). *G. Kalsi[1], P-H. Kuo[1], J. Alexander[1], P.F. Sullivan[2], E.J.C.G. van den Oord[1], D.G. Patterson[3], D. Walsh[4], C.A. Prescott[5], K.S. Kendler[1], B.P. Riley[1].* 1) Dept Molecular Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept of Genetics, University of North Carolina, Chapel Hill, NC; 3) Shaftesbury Square Hospital, Belfast, Northern Ireland, UK; 4) Health Research Board, Dublin, Eire; 5) Dept of Psychology, University of Southern California, Los Angeles, CA.

A genome wide linkage scan conducted in the IASPSAD produced significant evidence for linkage to number of DSM-IV alcohol dependence (AD) symptoms over a broad region of chromosome 4, from 4q22 to 4q32 (peak multipoint LOD=4.59, P=2.1 x 10-6, at D4S1611). A one-LOD interval under this peak delineates a 17.6Mb region defined by the markers D4S1572 and D4S427. We fine-mapped a region extending 18.5Mb region which includes the one-LOD interval and flanking regions on either side to include full genes. The region contains 65 genes and ESTs in the Jan '06 build of HapMap (hg18, NCBI Build 36.1). We used Tagger and Phase 2 HapMap data to select a minimum set of gene- and EST-based tagSNPs based on pairwise r2 to efficiently extract maximum information with the smallest genotyping burden. This approach identified 523 tagSNPs, of which 460 (88%) were suitable for high-throughput multiplex genotyping in a sample of 562 cases and 569 controls. Single marker and haplotype analyses were done in Haploview v3.3. Genes which pass the nominal significance level of $p<0.05$ include the PAPSS1 (rs9569, p=0.0155), ANK2 (rs313956, p= 0.0141; rs1351998, p=0.0162), ARSJ (rs12645879, p=0.0266; rs4441820, p=0.0124) and KIAA1627 (rs298998, p=0.0149). Haplotype analyses produced nominal statistical significance. Other genes, which showed single marker, but not haplotype, significance include DKK2, ALPK1, CAMK2D and SEC24D. The data is being analysed for possible multilocus interactions. Fine-mapping of the linkage region has produced evidence of association with AD. Functional roles of genes identified include cell architecture, binding with neurotransmitters and sulfonation, all of which are processes with possible physiological link to alcohol dependence.

1974/T

Contribution of the neurotrophic tyrosine kinase receptor type 3 (NTRK3) gene to genetic susceptibility to obsessive-compulsive hoarding. *M. Alonso[1], M. Gratacos[1], J.R. Gonzalez[1], J.M. Menchon[2], R. de Cid[1], C. Segalas[2], M. Bayes[1], A. Pertusa[2], E. Real[2], J. Labad[2], J. Vallejo[2], X. Estivill[1,3].* 1) OCD Clinic, Department of Psychiatry, Hospital Universitary de Bellvitge, Barcelona, Catalonia, Spain; 2) Genes and Disease Program, CeGen and CIBERESP, Center for Genomic Regulation (CRG-UPF), Barcelona; 3) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Hoarding, defined as the collecting of, and inability to discard, excessive quantities of useless or valueless items, is present in approximately 18-42% of those suffering from obsessive-compulsive disorder (OCD). Recent work suggests that hoarding syndrome may constitute a neurobiologically distinct variant of OCD with specific clinical and neuroanatomical correlates as well as a different pattern of genetic inheritance involving basically the dopaminergic pathways. To test the involvement of neurotrophic tyrosine kinase receptor type 3 (NTRK3), the high affinity receptor of neurotrophin 3 (NT-3), in obsessive-compulsive hoarding, we have performed an association study of 52 TagSNPs covering the whole gene in a sample consisting on 120 patients (OCD Clinic of Bellvitge University Hospital) and 342 controls. TagSNPs were selected from the HapMap project (Phase I data freeze, dbSNP b124) considering the genotypes corresponding to the 60 individuals from the 30 HapMap trios of European ancestry. We performed both single case-control association and haplotype analyses. Thirty-six of our patients (30%) exhibited hoarding obsessions and compulsions. A significant association of two SNPs in the 3' downstream region of NTRK3 gene and obsessive-compulsive hoarding was identified ($p = 0.0002$), with a protective effect associated with both of them. No other significant results emerged from the haplotype analysis. Our findings suggest that NTRK3, which plays a role in survival and differentiation of dopaminergic neurons, may contribute to genetic susceptibility to obsessive-compulsive hoarding. Supported by the Catalan and the Spanish Governments.

1975/T

Transmission of class I / II multi-locus MHC haplotypes and multiple sclerosis susceptibility: accounting for linkage disequilibrium. *M.J. Chao[1], M.C.N.M. Barnardo[2], G.Z. Liu[1], M.R. Lincoln[1], S.V. Ramagopalan[1], B.M. Herrera[1], D.A. Dyment[1], A.D. Sadovnick[3], G.C. Ebers[1].* 1) Department of Clinical Neurology, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK; 2) Nuffield Department of Surgery, Churchill Hospital, Oxford, OX3 7LJ, UK; 3) Department of Medical Genetics, University of British Columbia, Vancouver, V6T 1Z4, Canada.

The human MHC class II region is associated with genetic susceptibility to MS. Roles for HLA class I loci have been supported in several case-control studies, but this methodology does not consider the known LD between class I and II loci. In 1258 individuals from 294 MS families we analysed class I and II interactions. Using haplotype TDT, we found positive associations between MS and several *HLA-DRB1*15-HLA-A* haplotypes including *HLA-DRB1*15-HLA-A*02* ($P=2.41\times10^{-05}$) and *-HLA-A*03* ($P=8.42\times10^{-06}$), and several *HLA-DRB1*15-HLA-B* haplotypes including *HLA-DRB1*15-HLA-B*07* ($P=2.23\times10^{-10}$).

*HLA-DRB1*15* haplotypes divergent for reported *HLA-A* allelic associations were equally over-transmitted, illustrating no detectable effect of *HLA-A* or *-B* alleles in *cis* on susceptibility. *HLA-A* and *-B* alleles on haplotypes not bearing *HLA-DRB1*15* were not over-transmitted. Similarly, general over-transmission of *HLA-DRB1*15* haplotypes was independent of the *HLA-B* allele present. Furthermore, *HLA-B*07* haplotypes from *HLA-DRB1*X-HLA-B*X/HLA-DRB1*X-HLA-B*07* heterozygous parents were transmitted per random expectation giving no indication of *HLA-B* independence or *trans* complementation of *HLA-DRB1*15* by *HLA-DRB1*X-HLA-B*07* haplotypes. These results imply that many reports of class I allelic associations in MS are class II dependent, secondary to LD with class II loci. The lack of independent class I associations suggests that virus-related class I-antigen complexes are not T-cell targets in MS. The inability to replicate confirmed case-control associations highlights the importance of family-based analyses. The high frequency that allelic associations cannot be replicated emphasises the requirement for constructing multi-locus haplotypes in dissecting associations in regions of tight LD as illustrated by these results.

1976/T

Association of *MET* gene variants with autism susceptibility. *I. Sousa[1], N. Sykes[1], T.G. Clark[1], C. Allan[1], J. Lamb[2], K. Kobayashi[1], A. Pagnamenta[1], A.J. Bailey[3], A.P. Monaco[1], IMGSAC[4].* 1) Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 2) CIGMR, The University of Manchester; 3) University Department of Psychiatry, Warneford Hospital, Oxford, UK; 4) http://www.well.ox.ac.uk/~maestrin/iat.html.

Autism is a common severe neurodevelopmental disorder, with evidence from twin and family studies for a complex genetic predisposition. The IMGSAC genome screen for linkage in affected sib-pair families identified a principal susceptibility locus on chromosome 7q (AUTS1) covering approximately 40Mb (with ~ 200 genes), that has subsequently shown evidence of increased sharing in several independent multiplex samples and in two meta-analyses. Taking into account its location under the linkage peak (7q31) and the fact that it has been recently reported to be associated with autism, we carried out a family based study on the *MET* gene, which encodes for a pleiotropic receptor tyrosine kinase. Therefore, using HapMap data (phase II - release 21) to assess the patterns of linkage disequilibrium across the gene, 28 haplotype-tagging SNPs were selected using Tagger from Haploview 3.2 (with $r^2 > 0.8$, MAF > 0.05). We have genotyped 27 SNPs using the Sequenom and Illumina platforms, on a sample of 1702 individuals from 355 multiplex IMGSAC families, with sample and SNP genotyping success rates of ~99% and 100% respectively. Association analysis performed so far, using both single locus and haplotype approaches, showed significant results with the intron 1 SNP rs38845 (*P<0.003*) and with the intronic haplotype rs38845/rs38846 (*P<0.001*). In addition, a promoter SNP (rs1858830) previously reported to be associated with autism (its C allele resulting in a decrease in *MET* promoter activity) is being genotyped using an RFLP assay in our families, and association analysis will be performed and presented. Overall, these results provide further evidence that the *MET* gene plays a role in autism susceptibility.

1977/T

Association of SNPs in the 5' Upstream Regulatory Region of the α7 Nicotinic Acetylcholine Receptor Subunit Gene with Schizophrenia, an Endophenotype of Schizophrenia and Alcohol Use. *S. Stephens, A. Franks, S. Leonard.* Department of Psychiatry, University of Colorado Health Sciences Center, Aurora, CO.

The α7 neuronal nicotinic acetylcholine receptor subunit gene (*CHRNA7*) is localized in a region linked to schizophrenia in multiple independent studies and was selected as the best candidate gene for an endophenotype of schizophrenia, the P50 sensory processing deficit, by both genetic linkage to the region and human and animal studies. Mutation screening of the *CHRNA7* coding region and intron/exon splice junctions revealed multiple synonymous variants and rare non-synonmous variants that were not associated with schizophrenia or the P50 deficit; however, this screening also revealed a large number of functional mutations in the upstream regulatory region of *CHRNA7*, particularly, the core promoter. Studies show the prevalence of functional polymorphisms in the *CHRNA7* core promoter to be statistically greater in schizophrenics versus controls. Further, the presence of a promoter polymorphism was associated with the P50 deficit in control subjects. The current study sought to further investigate 12 SNPs in the core promoter and upstream regulatory region of *CHRNA7* via association studies with schizophrenia, the P50 deficit, smoking, smoking in schizophrenia, and alcohol use. Family-based and case-control association studies were performed on samples from 123 families as well as 348 schizophrenic patients and 144 controls. SNP markers upstream of the *CHRNA7* gene were genotyped using Denaturing High-Performance Liquid Chromatography. Family-based association analyses were performed using UNPHASED. Case-control analysis was evaluated by $\chi 2$, and endophenotypic analyses for P50 ratios by t-tests. The presence of *CHRNA7* core promoter polymorphism(s) was associated with the P50 deficit in control subjects. Additionally, the -1831 bp SNP (rs3087454), located in the upstream regulatory region of *CHRNA7,* is associated with schizophrenia in the case-control sample and supported by results in family members. The -1831 bp SNP was also found to be associated with alcohol use. These data support the α7 nicotinic receptor as a candidate gene for schizophrenia, and the P50 deficit.

1978/T

A genomic approach to studying repeat instability in schizophrenia. *D.E. Dickel[1], M-C. King[1,2], J.M. McClellan[3].* 1) Dept of Genome Sciences; 2) Dept of Medicine (Medical Genetics); 3) Dept of Psychiatry, University of Washington, Seattle, WA 98195.

Schizophrenia is a severe, debilitating psychiatric disorder of unknown cause. Some cases of schizophrenia may be caused by rare, often de novo, highly penetrant mutations. Sporadic cases of schizophrenia in previously unaffected families may most likely harbor such mutations. Potentially unstable oligonucleotide repeats are among the most vulnerable of genomic features. Repeat expansions are potentially intriguing in schizophrenia given the disorder's neurological phenotype, paternal age bias, and possible anticipation.

The purpose of this project is to identify repeat expansions with large effect on schizophrenia. Using a bioinformatics search, we identified 327 tri-, tetra-, or pentanucleotide repeats that overlap transcribed features and are sufficiently long to likely be polymorphic. By screening genomic DNA from individuals with schizophrenia, we are identifying repeats with more apparent homozygotes than expected based on Hardy-Weinberg equilibrium (HWE) criteria. To exclude loss of heterozygosity due to technical artifacts, we genotype repeats that fail HWE using multiple primer pairs, in cases and ancestry-matched controls.

In preliminary experiments, we identified a repeat with a significant excess of homozygosity among cases but perfect fit to HWE among controls. This excess is observed with multiple non-overlapping primer pairs and after identifying short repeats by capillary electrophoresis and medium length repeats by long-range PCR. We screened cases and controls at this locus by Southern blot to identify any expansions >300 repeats and any deletions of the entire repeat.

We will evaluate repeats elsewhere in the genome in the same way. If either large expansions or deletions are associated with schizophrenia at any repeat, we will sequence genes harboring schizophrenia-associated repeats in multiple unrelated cases to identify other classes of mutations such as frameshifts or point mutations. We will also undertake preliminary experimental characterization of the mutant sequences.

1979/F

Regional differences in SNPs associated with susceptibility to pulmonary tuberculosis in Mexican mestizo populations. *I. Aguilar-Delfin, C. Rangel-Escareño, J. Estrada-Gil, R. Goya, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Most individuals within the Mexican population are considered mestizo, having originated from the admixture of Amerindian groups with Spaniards and, to a lesser extent, Africans. This complex admixture process has resulted in genetic differences between geographical regions. The purpose of this study was to assess the existence of regional differences in Mexican mestizo populations in polymorphisms associated with susceptibility to pulmonary tuberculosis (PTB), analyzing individual genotypes as well as higher order interactions i.e. combinatorial genotypic categories. We called our method **C**ombinatorial **G**enotype **B**ins (CGBs) which also ranks PTB risk scores according to a probability matrix using the reported ORs for individual SNPs. Among the functional polymorphisms known to influence susceptibility to PTB we selected ***MCP1, IL10, IL12RB1*** and ***SLC11a1*** and used Taqman to genotype *MCP1-2518A>G* (rs1024611), *IL10-1082A>G* (rs1800896), *IL12RB1-111A>T* and *SLC11A1+1627G>A* (rs17235409) in 1,150 healthy mestizos from six different states. Results show that *MCP1-2518* GG genotype has a higher frequency in Mexican mestizos (0.276 ± 0.073 CI 0.195-0.368) compared to Europeans and Africans (0.017) suggesting that the G allele may have been contributed by Amerindian populations. The state of Sonora exhibits a significantly lower frequency of GG genotype (0.150) compared to the rest of the states (0.302 ± 0.043, CI 0.208 - 0.378; F_{ST} p<0.001) and also exhibits the lowest Amerindian contribution in the 100 kb genomic region that contains *MCP1*. The higher-order analysis showed that 3 of the CGBs corresponding to bins with high and moderate PTB risk scores have frequency differences between Mexican mestizos and a subset of 50 individuals with significantly higher Amerindian ancestral component. Overall, the results support the existence of regional differences in genomic determinants associated with susceptibility to PTB in Mexican mestizos which could have resulted from heterogeneity in the proportion of Amerindian ancestry.

1980/F

Genetic prediction of asthma exacerbation in children. *B.E. Himes[1,2], A.L. Berninger[2,3], S.T. Weiss[2,4,5], M.F. Ramoni[1,2,3,5].* 1) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 2) Harvard Partners Center for Genetics and Genomics, Boston, MA; 3) Children's Hospital Informatics Program, Boston, MA; 4) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 5) Harvard Medical School, Boston, MA.

Exacerbations are the major cause of morbidity and mortality in asthma, a complex lung disease that affects 6.2 million American children. In this work, we used Bayesian networks to create a multivariate predictive model of asthma exacerbations using genetic data. The Childhood Asthma Management Program (CAMP) was a clinical trial that followed asthmatic children for approximately four years. A subset of Caucasian CAMP participants not randomized to steroid treatment, who have been followed for an additional 6 years, were selected (n=290). An asthma exacerbation case (n=83) was defined as a subject with at least one reported hospitalization; a control (n=207) was defined as a subject with no hospitalizations or emergency room visits. To handle the large amount of genetic data available for these subjects, 2443 SNPs in 349 candidate genes, a specialized Bayesian network algorithm that focuses the search process around the phenotype of interest was developed. It found that 132 SNPs in 55 genes predict asthma exacerbation status. The models' predictive accuracy was assessed using fitted values and a 20-fold cross-validation. Fitted values were obtained by predicting exacerbation status in each subject used in the model creation. A 20-fold cross-validation was performed by splitting all subjects into 20 groups, and using each group as an independent dataset to predict exacerbations while the remaining 19 groups were used to quantify model parameters. Predicted and observed exacerbation statuses were compared using areas under receiver operating characteristic curves (AUROCs). The AUROC for fitted values is 0.97 and for 20-fold cross-validation is 0.84. The model's good predictive accuracy is shown to be superior to single gene analysis. The results of this work demonstrate the promise of using Bayesian networks to study the genetic architecture of complex traits.

1981/F

Gene Selection for Classification of Microarray Data Based on the Bayes Error. *J.G. Zhang[3], H.W. Deng[1,2,3].* 1) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China; 2) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P. R. China; 3) Dept Basic Medical Sci, Univ Missouri, Kansas, Kansas City, MO.

Background: With DNA microarray data, selecting a small subset of discriminative genes from thousands of genes is a critical step for accurate classification of phenotypes for, e.g., disease diagnosis. Several widely used gene selection methods often select top-ranked genes according to their individual discriminative power of each gene in classifying samples into distinct categories, without considering correlations among genes. A limitation of these gene selection methods is that they may result in gene sets with some redundancy and yield an unnecessary large number of candidate genes for classification analyses. Some latest studies show that incorporating gene to gene correlations into gene selection can remove redundant genes and improve classification accuracy. Results: In this study, we propose a new method, Based Bayes error Filter (BBF), to remove irrelevant and redundant genes in classification analyses of microarray data. The effectiveness and accuracy of this method is demonstrated through analyses of five publicly available microarray datasets. The results show that our gene selection method is capable of achieving better accuracies than previous studies, while being able to effectively remove irrelevant and redundant genes and obtain efficient and small gene sets for sample classification purposes. Conclusion: The proposed method can effectively identify a compact set of genes with high classification accuracy. This study also indicates that application of the Bayes error is a feasible and effective way for removing redundant genes in gene selection.

1982/F

The Use of Preferential Imbalance to Identify Skin Cancer Susceptibility Loci. *A.M. Dworkin[1], D. Bautista[6], K. Ridd[2], D. Pinkel[2], B. Bastian[2, 3], A.E. Toland[4, 5].* 1) OSU IBGP; 2) UCSF Cancer Research Institute; 3) UCSF Dept. of Dermatology; 4) OSU MVIMG; 5) OSU CCC; 6) OSU Coll. Math & Physical Sci.

Cutaneous squamous cell carcinoma (SCC) is the second most common type of nonmelanoma skin cancer. Organ transplant recipients (OTR) have an increased incidence and aggressiveness of SCC. The mechanisms underlying this phenomenon are unknown, but effective treatments or prevention methods for these skin tumors would have a significant impact upon morbidity in this group of patients. This project is assessing the genetic components of SCC susceptibility by comparing multiple tumors from organ transplant recipients (OTR) to look at repeated aberrations within an individual in which allelic imbalance occurs. Preferential imbalance (gain or loss) of an allele may indicate the presence of a polymorphism that is driving the imbalance which could then be considered a candidate for SCC susceptibility. Studies using array comparative genomic hybridization in 133 tumors from OTRs led to the identification of regions of frequent copy number changes in multiple tumors from OTR. We used microsatellite markers from these regions to determine if there were alleles showing preferential imbalance in individuals with multiple SCCs. Forty-five patients with at least 4 tumors each were genotyped for 28 microsatellite markers mapping to regions of frequent copy number aberrations. To test whether the observed imbalances were random or preferential, we developed a method based on a hybrid of Bayesian and frequentist approaches. For this, each marker was analyzed separately in each individual, and individual data were subsequently combined for each marker. We then computed the ratio of the odds that imbalances occurred randomly rather than preferentially and then tested for significant departures from unity. We identified statistically significant allelic imbalance for 7 markers. Four of these 7 markers are located on chromosome 3. Our data suggest that a polymorphism is driving somatic copy number changes in tumors at this locus. The use of allele specific somatic alterations in tumors may provide a new means of identification of cancer susceptibility genes.

1983/F

Stratified analysis of RELN in autism using the Language Acquisition Discrepancy (LAD) score. *J. Jaworski[1], J. Brinkley[1], R.K. Abramson[2], H.H. Wright[2], J.L. Haines[3], J.R. Gilbert[1], M.A. Pericak-Vance[1], M.L. Cuccaro[1].* 1) MIHG, University of Miami, Miami, FL; 2) Dept. of Neuropsych, USC-SOM, Columbia, SC; 3) Center for Human Genetics, Vanderbilt University, Nashville, TN.

Several chromosome 7 candidate genes have shown association with autism (AUT) including RELN which is involved in neuronal migration and development. Previously, our group reported association for a repeat in the RELN 5'UTR in a Caucasian family dataset (N=327). However, this effect was not observed when the dataset was increased (N=471), most likely due to genetic heterogeneity. To deal with such heterogeneity, we have developed the Language Acquisition Discrepancy (LAD) score, based on the difference in age at first words and age at phrase speech as measured by the ADI-R. Clustering algorithms using age at first words [normal≤18 months <delayed] and LAD score [normal ≤15 months <delayed] yielded four clusters in our AUT dataset: DD = delayed first words/delayed LAD score; DN = delayed first words/normal LAD score; ND = normal first words/delayed LAD score; NN = normal first words/normal LAD score. Using the pedigree disequilibrium test (PDT) we tested for association to seven SNPs in RELN in our overall dataset (436 Caucasian families) and the cluster defined strata. Hcv2632989 showed association to AUT in two clusters (p=0.012 in NN and p=0.023 in the ND) both of which are characterized by normal age at first words. In addition, rs144525 showed association only in the DN cluster (p=0.032). These findings reveal associations to RELN that were apparent only in the language defined strata. While it does not appear that LAD influenced the association results, the findings support a potential role for RELN in a language based AUT subphenotype. This is consistent with previous work identifying age at first words as a QTL chromosome 7q. In sum, RELN continues to be a gene of interest in understanding genetic risk for AUT. ≤≤≤≤≤.

1984/F

Genetic association between interferon regulatory factor 5 (IRF5) and systemic lupus erythematosus in minority populations. *J.A. Kelly[1], J.C. Edberg[2], K.M. Kaufman[1,3,4], J. Kilpatrick[1], G.R. Bruner[1], J.T. Merrill[1,4], J.A. James[1,4], M.C. Marion[5], C.D. Langefeld[5], M.A. Petri[6], J.D. Reveille[7], R. Ramsey-Goldman[8], L.M. Vilá[9], G.S. Alarcón[2], R.P. Kimberly[2], J.B. Harley[1,3,4].* 1) Oklahoma Medical Research Foundation, OKC, OK; 2) Univ of Alabama at Birmingham, Birmingham, AL; 3) US Dept of Veterans Affairs Medical Center; 4) Univ of Oklahoma Health Sciences Center, OKC, OK; 5) Wake Forest Univ, Winston-Salem, NC; 6) Johns Hopkins Univ School of Medicine, Baltimore, MD; 7) Univ of Texas Health Science Center, Houston, TX; 8) Northwestern Univ School of Medicine, Chicago, IL; 9) Univ of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico.

Reports demonstrate genetic association between systemic lupus erythematosus (SLE) and the interferon regulatory factor 5 (IRF5) gene in European-Americans (EA). We evaluated up to seven polymorphic loci within or near IRF5 in a large collection of SLE cases and controls from minority (African-Americans (AA), Hispanics (HI), and Hispanics from Puerto Rico (HI-PR)) and EA samples. A total of 6020 samples from two independent collections (2683 cases and 3337 controls) were evaluated. Case-control association tests were evaluated using Pearson chi-square statistics and conditional haplotype analyses were conducted using WHAP. Genetic associations (p≤0.0002) were observed with SLE and rs2004640 and rs3807306 in the AA and HI collections. Both loci also demonstrated strong association with SLE in the EA collection ($p \leq 5.7 \times 10^{-13}$). Suggestive association between rs3807306 and SLE in the HI-PR collection was also observed. We identified a 5-marker risk haplotype in the AA and HI collections, with rs3807306 accounting for the majority of the observed statistical effect. The risk haplotype was again observed in the EA collection, and though its effect was not significantly different than a 3-marker haplotype previously reported, haplotypes containing the common A risk allele at rs3807306 were predictive of SLE risk (LR χ^2=38.55, $p=5.4 \times 10^{-10}$). In summary, we establish association with SLE and IRF5 in AA and HI, providing evidence that IRF5 is likely to be a crucial component in lupus pathogenesis in multiple ethnic groups.

1985/F

***PPARG* Gene Variants Are Associated with Diabetes Risk in the Women's Health Initiative - Observational Study.** *T. Niu[1,2], Y.-H. Hsu[3,4], Y. Song[1], L. Tinker[5], J. Hsia[6], S. Liu[4,7].* 1) Div of Prev Med, Dept of Med, BWH, Harvard Med School, Boston, MA; 2) Pgm Mol & Genet Epi, Dept of Epidemiology, HSPH, Boston, MA 02115; 3) Mol and Integ Physiol Sci Pgm, HSPH, Boston, MA 02115; 4) Pgm on Genomics & Nutr, Dept of Epidemiology, UCLA SPH, Los Angeles, CA 90095; 5) FHCRC, Seattle, WA 98109; 6) Dept of Med, George Washington Univ, Washington, DC 20037; 7) Dept of Med, UCLA David Geffen School of Med, Los Angeles, CA 90095.

The association of the *PPARG* gene with risk of diabetes mellitus (DM) was studied in the Women's Health Initiative (WHI) Observational Study (OS) using a two-stage approach. First, we genotyped 105 *PPARG* single nucleotide polymorphisms (SNPs) in a developing panel (N_d = 244) where we identified 24 haplotype-tagging SNPs (htSNPs). Second, we genotyped the 24 htSNPs in 1543 DM cases during a median follow-up period of 5.9 years and 2132 controls matched by age, ethnicity, clinical center, time of blood draw, and length of follow-up. In single-SNP analyses, compared with the *Pro12* allele, *Ala12* allele was associated with a significantly lower risk of DM [odds ratio (OR) = 0.53, 95% confidence interval (CI): 0.32-0.86, P = 0.0112]. Under the dominant genetic model, compared with the *Pro12*/*Pro12* genotype, *Ala12* carrier genotypes were associated with a significantly reduced DM risk (OR = 0.56, 95% CI: 0.35-0.89, P = 0.0137); in the meta-analysis of 21118 cases and 28142 controls (k = 54 studies), the summary OR of the *Ala12* carrier genotypes was 0.81 (95% CI: 0.74-0.87, P =1.45×10^{-7}) for the random effects model, given significant evidence of heterogeneity (I^2 = 43%, P = 0.0005). The sliding window (window width=3) haplotype-based analysis identified that haplotypes formed by *Pro12Ala-rs1373640-rs2972162* were significantly associated with the risk of DM (LRT χ^2 = 15.308, df = 5, P = 0.009). Our study with the most comprehensive assessment of the *PPARG* gene in the prospective WHI-OS strongly supports a significant relationship between the *PPARG Pro12Ala* polymorphism and DM risk.

1986/F

Association of Vascular Endothelial Growth Factor (VEGF) Polymorphisms with Childhood Asthma and Airway-Remodeling Phenotypes. *S. Sharma[1], B. Raby[1], A. Murphy[1], M. Soto-Quiros[2], L. Avila[2], B. Klanderman[1], J. Sylvia[1], A. Patel[1], J. Celedon[1], S. Weiss[1].* 1) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Division of Pediatric Pulmonology, Hospital Nacional de Niños, San Jose, Costa Rica.

Rationale: Asthma is a chronic inflammatory disease associated with airway remodeling and subsequent long-term decline in lung function. Expression of VEGF, an angiogenic factor implicated in airway remodeling, correlates with the severity of airflow obstruction. We hypothesized that VEGF gene polymorphisms are associated with asthma and airway-remodeling phenotypes. **Methods**: We genotyped 17 VEGF single nucleotide polymorphisms (SNPs) in 471 white (non-Hispanic) trios participating in the Childhood Asthma Management Program (CAMP). Family-based association tests were performed using PBAT under additive and dominant genetic models. We assessed asthma and three pulmonary function phenotypes: post bronchodilator FEV1, FVC, and FEV1/FVC ratio (FF). Haplotype block analysis was performed in FBAT. Repeated measures analysis of FF was conducted with FBAT-PC. We tested for evidence of replication in 439 asthmatic children and their parents from the Central Valley of Costa Rica. **Results**: In CAMP, one SNP was associated with asthma (p=0.01), and three others with FVC and FF (p=0.01-0.03). Most notably, rs4711750 was associated with FF in CAMP (p=0.01) and Costa Rica (p=0.02). In the Costa Rican trios, one SNP was associated with asthma (p=0.01) and two others were associated with FEV1 and FF (p=0.006-0.02). Haplotype block analysis confirmed the association with FF in both cohorts. Repeated-measures analysis also demonstrated an association between rs4711750 and FF over time. **Conclusions**: VEGF polymorphisms are associated with childhood asthma and airway remodeling phenotypes in two ethnically distinct populations. Our analysis suggests that variants in VEGF influence airway remodeling, which is a critical long-term outcome of asthma. **Funding**: Grants HL65899, HLO7427, HL04370, HL66289, and HL74193 from the National Institutes of Health.

1987/F

Four most Frequent PXE Mutations in the ABCC6 Gene are not Associated with an Increased Prevalence of Coronary Artery Disease in the Ludwigshafen Risk and Cardiovascular Health Study. *B. Struk[1], W. Renner[2], K. Lindpaintner[3], B.R. Winkelmann[4], B.O. Boehm[5], W. Maerz[2].* 1) Helios-Clinic, Charité and Max-Delbrueck-Centrum, Berlin, Germany; 2) Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University, Graz, Austria; 3) Hoffmann-La Roche Ltd, Roche Genetics, Pharmaceuticals Division, Basel, Switzerland; 4) Cooperation Unit Pharmacogenomics, Applied Genomics, University of Heidelberg, Heidelberg, Germany; 5) Division of Endocrinology and Diabetes, University of Ulm, Ulm, Germany.

Background: Mutations in *ABCC6* cause Pseudoxanthoma elasticum (PXE), a mendelian disorder that affects the elastic tissue in skin, eye and the cardiovascular system and leads to premature cardiovascular disease. Recently, the most frequent mutation, *R1141X,* was shown to be associated with a strong increase in the prevalence of premature coronary artery disease (CAD) in a Dutch case control study. However, since *R1141X* has distinct founder haplotypes and allele frequencies in different populations, it is possible that the association found is based on a specific founder effect of *R1141X* within the Dutch population. **Methods:** To further evaluate potential associations of *ABCC6* mutations and single nucleotide polymorphisms (SNP) with cardiovascular disease in the general population, we genotyped the four most frequent PXE mutations (*R1141X*, e23-29 deletion, *R1164X*, *Q378X*) and two SNP (*V614A, R1268Q*) in a total of 3316 participants of the LURIC study, a prospective cohort study. **Results:** We identified 14 carriers (0.4%) of PXE mutations in the study population (n=3290), that were equally distributed among cases (11/2564 = 0.4%) and controls (3/726 = 0.4%). Furthermore, there was no allelic association of *V614A* and *R1268Q* genotypes with the CAD phenotype in cases versus controls. **Conclusions:** Contrary to previous results, our data do not demonstrate an increased risk of premature CAD in the general population in heterozygous carriers of PXE mutations in *ABCC6.* Additionally, two common missense SNPs are not associated with CAD. Therefore, common and PXE-specific variants of *ABCC6* do not represent susceptibility markers for common CAD risk.

1988/F

Maternal transmission effects of the RUNX2 and TCOF1 genes among cleft case-parent trios from Four Populations. *J.W. Sull[1], K.Y. Liang[1], J.B. Hetmanski[1], M.D. Fallin[1], R.G. Ingersoll[1,2], J. Park[3], Y.H. Wu-Chou[4], P.K. Chen[4], S.S. Chong[5], F. Cheah[5], V. Yeow[6], B.Y. Park[7], S.H. Jee[1,7], E.W. Jabs[2], R. Redett[2], A.F. Scott[2], T.H. Beaty[1].* 1) Johns Hopkins School of Public Health, Baltimore, MD; 2) Johns Hopkins School of Medicine, Baltimore, MD; 3) Sungkyunkwan University, Korea; 4) Chang Gung Memorial Hospital, Taiwan; 5) National University of Singapore, Singapore; 6) KK Women and children Hospital, Singapore; 7) Yonsei University, Korea.

Oral clefts (cleft palate or CP; and cleft lip ± palate or CL/P) are among the most common human birth defects. RUNX2 and TCOF1 have been suggested as candidate genes for oral clefts. This study examines the association between markers in RUNX2 and TCOF1 and isolated, non-syndromic CP and CL/P, considering parent-of-origin effects. Case-parent trios from four populations (386 trios) were genotyped for 35 single nucleotide polymorphisms (SNPs) in the RUNX2 and TCOF1 genes. We performed the transmission disequilibrium test (TDT) and the transmission asymmetry test (TAT) on individual SNPs. Parent-of-origin effects were assessed using the parent-of-origin likelihood ratio test (PO-LRT) for both SNPs and haplotypes. For RUNX2, TAT revealed a block of 11 SNPs showing excess maternal transmission statistically significant at the p=0.01 level when all CL/P trios were combined. For these 11 SNPs, odds ratios (OR) of being transmitted to the case from the mother ranged from 3.0 to 4.0. For TCOF1, when all CP trios were combined, the OR (transmission) was statistically significant for SNP rs15251 (OR=2.88, p=0.007), as well as rs2255796 and rs2569062 (OR= 2.08, p=0.03; OR=2.43, p=0.041) when parent-of-origin was not considered. TAT also revealed 1 SNP (rs15251) that showed excess maternal transmission statistically significant at the p= 0.005 level (OR=6.50), however, the PO-LRT was only marginally significant for this SNP. Analysis of haplotypes of two SNPs (rs2255796 and rs15251) in TCOF1 also yielded possible evidence of a maternal transmission effect. RUNX2 and TCOF1 genes appear to influence risk of CL/P and CP, respectively, through a parent-of-origin effect.

1989/F

Aging in Neurofibromatotis 1 (NF1): Survival and Comorbidity According to US Death Certificates. *B.A. Carnes, J.J. Mulvihill, T.A. Teasdale, M.A. Grim, J.L. Mester.* Geriatric Medicine, Oklahoma University Helath Sciences Center, Oklahoma City, OK.

To gain insight into issues of aging in neurofibromatosis 1 (NF1), US multiple cause of death files for 1988-1998 (24.2 million death certificates) were examined in order to compare survival and morbidity characteristics between young and old NF1 decedents and between these groups and their age-matched non-NF1 counterparts. Median age at death for NF1 decedents was 54 for males and 60 for females. Although males with NF1 as a concomitant disorder died 7 years earlier than their female counterparts, the gender gap was eliminated for those dying from NF1 as the cause of death. Recursive partitioning revealed that decedents dying with NF1 at older ages had less cancer (connective tissue tumor and brain neoplasm) and congestive heart failure than those dying at younger ages; whereas those dying because of NF1 at older ages had less cardiovascular (cerebrovascular, chronic ischemic and congestive heart) disease and pulmonary disease (COPD). Logistic regression analyses, stratified by sex, confirmed that young and old NF1 decedents can be distinguished (area under the ROC = 0.77) purely on the basis of their comorbidity profiles (array of ICD codes). Conditional logistic regression and standardized death rate ratios revealed that younger NF1 decedents have a greater risk of intrinsic (non-accidental) death than older NF1 decedents, relative to their age- and sex-matched non-NF1 counterparts. These findings suggest that younger NF1 decedents may have a more severe form of the comorbidities associated with this single gene disorder. Parallel analyses are underway for Canadian and Danish death certificates, as part of a large project to identify geriatric issues in this common Mendelian disorder. (Funded in part with DOD-US Army Neurofibromatosis Program grant W81XWH-06-1-0465).

1990/F

The Pompe Registry: Centralized Data Collection to Track the Natural Course of Pompe Disease. *L. Case[1], P. Kishnani[2], A. van der Ploeg[3].* 1) Community and Family Medicine, Duke University Medical Center, Durham, NC; 2) Genetics, Duke University Medical Center, Durham, NC; 3) Genetics and Metabolism, Erasmus Medical Center, Rotterdam, NL.

Background: Pompe disease is a rare, progressive, and often fatal muscle disease due to a deficiency of lysosomal acid-α-glucosidase. The disease manifests as a clinical spectrum that varies with respect to age at onset, rate of disease progression, and extent of organ involvement. **Methods**: A global, observational Registry was developed to collect anonymous, longitudinal data on Pompe patients. **Preliminary Results**: As of March 9, 2007, 305 patients from 18 countries have been enrolled. The majority (75.4 %) are Caucasian. 20.3% (62/305) are infants, with rapidly progressive cardiorespiratory disease and death by one year; median age of diagnosis was 5.6 months. 68.9% (210/305) are older, typically with progressive skeletal/respiratory muscle weakness and longer survival; median age of diagnosis was 33.1 years. Age of onset was unspecified in 10.8% of patients. The median age at first recorded symptom was 3.5 months for infants and 26.4 years for adults. Of the patients genotyped, 60.6 (63/104) expressed the IVS1-13T>G mutation. 99 patients are currently reported as receiving enzyme replacement therapy. **Conclusion**: Preliminary Registry data show that the (median) range of time from symptom onset to diagnosis represents a significant lag as consistent with published literature, suggesting the need for greater disease awareness The overall objective of the Pompe Registry is to increase disease understanding across patient phenotypes/genotypes, medical disciplines and regional disease management norms and monitor the impact of treatment and other disease support methods over time.

1991/F

The Million Minds Approach: Community Annotation and Discovery in A Wiki for Professionals. *B. Mons[1,2,3], P.B. 't Hoen[1], M. Schuemie[2], E. van Mulligen[2,3], C. Chichester[1,3], J. den Dunnen[1], R. Jelier[1,2], H. van Haagen[1], A. Botelho bovo[1], Knewco Inc, Open Progress.* 1) Human Genetics , Leiden University Medical Center, Leiden, ZH, Netherlands; 2) Erasmus Medical Center Rotterdam, Netherlands; 3) Knewco Inc. Rockville, MD USA.

The scientific literature contains an exploding number of factual relationships between concepts that are pertinent. A growing subset of these relevant facts, such as the confirmed function of proteins have been curated in databases and these have become indispensable tools for biological research. However, the exponential growth of discovery renders complete annotation of the literature for facts by any central team of experts an unachievable goal. Here we describe a system to use the power of 'a million minds' to counter the information overload. We coin the process with the term Community Annotation, which is an interactive, collaborative process, of immediate added value to the day-to-day core activities of the collaborating Scientists. Web 2.0 technologies have been developed to generate a first version of a Community Annotation System. The approach is based on a relational Wiki-environment, supported by a new way to summarize knowledge about concepts and their interactions in a dynamic ontological structure called Knowlets. Knowlets contain multiple relationships between concepts that are both qualitative and quantitative. A knowlet space can be created based on mining with different technologies and approaches. The beta-system currently contains over 1 million Knowlets of concepts in Medline and a potential number of 1 million Author Knowlets. The system was recently featured in a Nature editorial (Nature 445, 691 (Feb 2007). Recent results provide proof of concept for the discovery of implicit knowledge from the literature using our methods. Scientists that currently struggle with the under representation of their favourite genes and proteins in established central data bases will be able to use the open source system to annotate their own part of the Knowlet space and will generate an interactive in silico discovery environment for themselves in the process.

1992/F

Ethnicity, gender and the incidence of congenital heart defects: a report from the National Down Syndrome Project. *A.E. Locke[1], L.H. Bean[1], E.G. Allen[1], S.W. Tinker[1], C. Druschel[2], C.A. Hobbs[3], L.A. O'Leary[4], P.A. Romitti[5], M.H. Royle[6], C.P. Torfs[7], K.J. Dooley[8], S.L. Sherman[1], S.B. Freeman[1].* 1) Dept. of Human Genetics, Emory University, Atlanta, GA; 2) New York State Department of Health, Troy, NY; 3) College of Medicine, Dept. of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR; 4) National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA; 5) Dept. of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA; 6) New Jersey Department of Health and Senior Services, Trenton, NJ; 7) Public Health Institute, Birth Defects Studies, Emeryville, CA; 8) Sibley Heart Center Cardiology, Children's Healthcare of Atlanta, Atlanta, GA.

The population-based National Down Syndrome Project used a combination of epidemiological and molecular methods to study congenital heart defects (CHDs) in infants with Down syndrome (DS). Between 2000 and 2004, six sites, representing approximately 11% of annual DS births in the US, identified 1469 eligible infants with DS. 44.2% had a major cardiac defect including atrioventricular septal defect (AVSD, 17.2%), secundum ASD (ASDII, 18.6%), ventricular septal defect (VSD, 19.2%), and/or tetralogy of Fallot (TOF, 2.7%). Infants with AVSD showed significant gender and ethnicity differences. There were twice as many affected females as males (OR: 2.19 (95% CI 1.48-3.24)). Compared to whites, black infants were twice as likely to have an AVSD (adj. OR 2.09 (95% CI 1.20-3.64)) while Hispanics were half as likely (adj. OR 0.50 (95% CI 0.28-0.92)). The increased AVSD risk among black infants and the decreased risk among Hispanics were more evident among those whose mothers were born outside the US. These findings suggested that black infants with AVSD would have a higher proportion of African-derived alleles than those without AVSD. Using ancestry informative markers (AIMs), we confirmed a higher proportion of Sub-Saharan Africa-derived alleles in those affected with AVSD ($p = .029$). We conclude that gender and ethnic differences exist for AVSD and, at least among blacks, the ethnic differences could be due to genetic risk factors.

1993/F

Factors associated with telomere length in a bi-racial population of older adults: The Health ABC Study. *O.T. Njajou[1], W-C. Hsueh[1], P. Holvoet[2], F. Harris[1], E.H. Blackburn[1], T.B. Harris[3], E. Simonsick[3], A. Newman[4], R. Li[5], J. Zmuda[4], P-Y. Kwok[1], N. Schork[6], S.R. Cummings[7], R.M. Cawthon[8], for the Health ABC Study[1].* 1) University of California, San Francisco, CA; 2) Katholieke Universiteit Leuven, Belgium; 3) NIH, Bethesda, MD; 4) University of Pittsburg, PA; 5) University of Tenessee at MEMPHIS, TN; 6) University of California, San Diego, CA; 7) California Pacific Medical Center, San Francisco, CA; 8) University of Utah, Salt Lake City, UT.

Telomeres are DNA capping structures at the ends of chromosomes, which shorten at each somatic cell division in humans. Previous studies suggest that telomere shortening may contribute to aging and poorer survival in humans. Oxidative stress accelerates telomere attrition in cultured cells, leading to cellular senescence. However, it is not yet clear what factors may affect telomere length (TL) in humans. We used data from 2,721 participants of the Health ABC study (age range: 68 to 80 years, 51% female, 1,605 white and 1,116 black participants) to address this question. We tested the association of several environmental and host factors with TL, including sex, oxidative stress, race, smoking, alcohol use, levels of physical activity, and socio-economic status (SES). TL in leukocytes was measured using a validated quantitative PCR method. Levels of ox-LDL (a marker of oxidative stress) were measured using a standard ELISA in the blood plasma. As observed previously, TL was negatively correlated with age ($r = -0.7$, $p < 0.001$) and was shorter in men (4,697bp ± 34bp) compared to women (5,124bp ± 34bp) ($p < 0.001$). TL was shorter in smokers, alcohol drinkers and people with high ox-LDL (all $p < 0.005$). Interestingly, after adjusting for age and sex, ox-LDL levels were negatively associated with telomere length ($\beta = -171 \pm 34$bp, $p < 0.001$), even after adjustment for LDL-cholesterol. Race, physical activity, and SES were not related to TL. In summary, we found that oxidative stress and some modifiable factors are associated with telomere length. Both biological and environmental factors can influence TL in humans, and perhaps consequently, aging.

1994/F

High carrier frequency of the GJB2 mutation (35delG) in the north of Iran. *M. Shahrani[1], M. Hashemzadeh Chaleshtori[1], E. Farrokhi[1], M. Dolati[2], L. Hoghooghi rad[2], H. Pour jafari[3], K. Ghatreh Samani[4], A. Crosby[5].* 1) Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran; 2) Department of Genetics,School of Medical Sciences,Qom University of Medical sciences,Qom,Iran; 3) Department of Genetics,School of Medicine, Hamadan University of Medicil Sciences, Hamadan, Iran; 4) Department of Clinical Chemistry, Tabriz University of Medical Sciences, Tabriz, Iran; 5) Medical Genetics Unit, St Georges Hospital Medical School, University of London, London, United Kingdom.

Objective: Mutations in the GJB2 gene are a major cause of autosomal recessive and sporadic nonsyndromic hearing loss in many populations. A single mutation of this gene (35delG) accounts for approximately 70% of mutations in white populations with a carrier frequency of 2%-4% in Europe. This study describes the screening of 35delG mutation in 550 unaffected unrelated subjects from 4 provinces of Iran and aims to determine the rate of 35delG carrier frequency in those regions. Methods: Genomic DNA was extracted from 0.5ml peripheral blood following the standard phenol chloroform procedure. The one base pair deletion (35delG) was analysed using a nested PCR procedure; 35delG mutation carriers were subsequently confirmed by sequence analysis. Results: Altogether the 35delG carrier frequency was found to be 1.8% in the populations studied. Of the 4 populations studied, we found a high carrier frequency of 2.8% in Gilan province in the north of Iran. This high rate of carrier frequency is of great importance in genetic counselling and medical care to control deafness in this region of Iran.

1995/F

A genetic instrumental variables analysis of the effects of maternal smoking on oral cleft risks. *G.L. Wehby, A. Marcinow, X. Quin, M.A. Mansilla, J.C. Murray.* Pediatrics, University of Iowa, Iowa City, IA.

Background: The effects of maternal smoking on oral cleft (OC) risks have been estimated without accounting for maternal self-selection into smoking based on her expectations of pregnancy risks. Since risk expectations are typically unobserved, self-selection cannot be addressed by classical analyses which would result in biased estimates. Objectives: This study aimed at estimating the effects of maternal smoking prior to or during the first trimester of pregnancy on OC risks accounting for self-selection using an instrumental variables (IV) model with smoking genetic variants as instruments. Methods: 15 SNPs in DBH, DDC, CCK, GABAB2, CHRNA4 and TPH genes previously shown to be related to smoking were typed in 212 OC cases and 170 controls from Iowa. The IV model ex-post randomizes the sample into groups that are comparable on "unobservable" self-selection characteristics and provides unbiased estimates under the assumptions that the instruments are significant predictors of smoking and that they are only related to OC through smoking. The IV model was fit using Two-Stage Least Squares regression adjusting for maternal education, alcohol and multivitamin use. Instruments were based on SNP variants and haplotype probabilities. Results: Five SNP variants in DBH, DDC, GABAB2 and CHRNA4 significantly increased smoking probability (RRs=2.0-3.0) in a multivariate regression (satisfying IV assumption 1). Smoking had no effect on OC under a classical model (RR=1.2, insignificant), but increased OC risks under the IV model (RR= 1.5-3.9 using various instrument combinations; larger RRs significant at $p<0.05$). The hypothesis that the genetic instruments were unrelated to OC except through smoking could not be rejected (IV assumption 2 satisfied). Conclusions: Results suggest that the contribution of smoking to OC risks has been underestimated perhaps by up to three times due to women at higher OC risks being less likely to smoke. The study has important counseling implications and provides a novel approach to study smoking-gene interactions and the effects of other behavioral and health factors with genetic components on perinatal outcomes.

1996/F

Simplification of methodology for multi-channel Bayesian analysis in complex sibship risk assessment. *R. Best, A.C. Edens.* Dept Obstetrics/Gynecology, Univ South Carolina Sch Med, Columbia, SC.

The use of Bayes' theorem in genetic counseling for X-linked recessive disorders allows for the integration of family history information across multiple generations as well as laboratory test studies, resulting in patient-specific risk estimates that include as much relevant information as possible. We present a clinical practice case involving Duchenne muscular dystrophy that includes a proband and three maternal aunts, each with relevant conditional information. We discuss the practical difficulties of increasing the number of channels of calculation with multiple generations and complex sibships and offer some simplifications to make the calculations more manageable. For complex sibships, the calculation table can be split into additional channels to accommodate multiple siblings with conditional information. To reduce the total number of calculations, channels can be eliminated when the mutations rate (?) enters the channel more than once. This approach has practical future applications, as it allows clinicians to be more efficient when performing Bayesian calculations.

1997/F

A Genome-Wide Search for Linkage to Chronic Kidney Disease (CKD) Phenotypes in a Community-based Sample. *N. Arar[1], S. Voruganti[2], S. Nath[1], F. Thameem[1], S. Cole[2], J. Blangero[2], J. MacCluer[2], C. Comuzzie[2], H. Abboud[1].* 1) Dept Medicine, Div Nephrology, Univ Texas Health Sci Ctr, San Antonio, TX; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

CKD phenotypes such as elevated urine albumin creatinine ratio (ACR), serum creatinine and/or decreased glomerular filtration rate (GFR) have been associated with several cardiovascular disease (CVD) risk factors. However, their role as independent risk factors for CVD in a community-based sample has not fully investigated. We conducted a genome-wide search to identify chromosomal regions linked to CKD phenotypes in Mexican Americans families enrolled in the San Antonio Family Heart Study (SAFHS). We used variance components decomposition, as implemented in the program SOLAR, to perform linkage analysis on 848 participants from 26 multiplex families of the SAFHS. A total of 417 microsatellite markers were genotyped at an average interval of 10 cM spanning 22 autosomal chromosomes. The mean age was 47.8 ± 14.8 years, 37% male. 22 % of the subjects had T2DM, 52% were hypertensive. Mean urine ACR was 0.06 ± 0.38 mg/mg, the mean serum creatinine was 0.85 ± 0.72 mg/dl and the mean GFR was 99.18 ± 25.69 ml/min. Urinary ACR showed suggestive linkage to chromosomes 20 (marker D20S107, LOD score of 2.93, p= 0.0020), 14 (marker D14S742, LOD score of 2.63, p = 0.00025), and 13 (marker D13S317, LOD score of 1.66, P= 0.0028). Serum creatinine showed suggestive linkage to chromosomes 9 (marker D9S922, LOD score of 2.61, p = 0.00026), 15 (marker D15S642, LOD score of 2.21, p = 0.00069), and 6 (marker D6S1056, LOD score of 2.19, p = 0.00073). GFR showed higher logarithm of odds (LOD) score than the other 2 phenotypes. Serum creatinine and GFR were both linked to chromosomes 9 and 15. Linkage to GFR was observed on chromosomes 9 (marker D9S1122, LOD score of 3.87, p = 0.00005), 15 (D15S642, LOD score of 1.61, p = 0.0018), and 2 (D2S1780, LOD score of 1.53, p =0.0092. Identifying gene(s) of complex diseases may lead to the development of novel therapeutic strategies targeted at high risk individuals.

1998/F

Multivariate dependence functions for genetic analysis of developmental disorders. *L.E.M. Sucheston[1,2], B.A. Lewis[3], C.M Stein[4], L.A. Freebairn[3], A.J. Hansen[3], S.K. Iyengar[4].* 1) Dept of Biostatistics, SUNY-Buffalo, Buffalo, NY; 2) Dept of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY; 3) Behavioral Pediatrics and Psychology, Rainbow Babies and Children's Hospital, Cleveland, OH; 4) Dept of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

We have developed an approach for the genetic analysis of longitudinal measures of developmental disorders, with specific application to a longitudinal pedigree study of children with Speech Sound Disorder(SSD). Analysis of this cohort is complicated by non-normal trait distributions and a potentially non-linear cognitive developmental trajectory. As an alternative to longitudinal analysis, we use multivariate dependence functions, called copulas, to develop a cross-sectional model to test for a polygenic age interaction. These functions separate a multivariate joint distribution into two parts: the correlation and the margins. Using these functions for analysis simultaneously addresses the non-normality problem, as the margins can be modeled with a wide variety of parametric probability distributions, and the developmental trajectory question, as we can incorporate age into the analysis through the use of a correlation function. The parameter estimate of this correlation function can be tested for significant deviation from zero, indicating a gene age interaction, specifically testing if development is linear over time. The psychometric measures administered to all participants assessed speech sound production, language comprehension and expression skills, and reading and phonological abilities. Of the 13 traits analyzed the likelihood ratio test statistic indicates that the polygene age copula model provided a significantly better fit than the polygene copula model for 4 tests: Nonsense Word Repetition (p=.02), Digit Span (p=.03), Digit Symbol-Coding (p=.0008), and Test of Written Spelling-Unpredictable Words (p=.04). Short term memory plays an important role in each of these tests, thus providing preliminary evidence that the genetic contribution to phenotypic variance of tasks involving memory is not stationary in children 6-18.

1999/F

Strong Evidence for a Genetic Component to Multiple Myeloma and Pleiotropy with Other Hematologic Malignancies. *N.J. Camp, T.L. Werner, L.A. Cannon-Albright.* University of Utah School of Medicine, USA.

A familial component of Multiple Myeloma (MM) has been suggested. However, only 1st degree familial relative risks (FRRs) have so far been reported. Analyzing beyond 1st degree is important because shared environment decreases for distant relatives and familiality can more readily be interpreted as evidence for a genetic component. Here we have performed FRRs for 1st, 2nd and 3rd degree relatives and genealogical index of familiality (GIF) analyses using data on all relatives. We investigated subgroups of MM based on sex, diagnosis age and survival to identify highly familial subtypes, and also the relationship between MM and other hematological and solid cancers to identify potential overlapping etiologies (pleiotropy). We used the Utah Population Database (UPDB) for our analyses. The UPDB is a unique resource, including genealogical data and Utah Cancer Registry data for all cancers diagnosed in Utah since 1966. We used UPDB data for the approximately 2 million individuals with genealogical data (3-10 generations) available. Strong evidence for familiality was found for MM, with significant excess risk apparent from both the GIF analysis (p<0.001) and in the FRR analyses out to 3rd degree relatives (FRR 2.69, 1.51 and 1.21 for 1st, 2nd and 3rd, respectively). No evidence for highly familial MM subtypes was found. Investigating other hematologic malignancies, chronic lymphocytic leukemia (CLL) was found in significant excess in the 1st and 2nd degree relatives of MM, and Non-Hodgkin Lymphoma (particularly B cell type, NHLB) in 1st. In complement, MM was found in excess in the 1st degree relatives of individuals with CLL and NHLB. Further, CLL was in excess in the relatives of NHLB. For solid cancers, prostate cancer and melanoma were significantly increased in 1st, 2nd and 3rd degree relatives of MM and in the cases themselves. Prostate cancer was observed in excess in NHLB and CLL, too. It is of note that characteristics such as diagnosis age, gender distribution and survival are also similar for these three malignancies. Our results indicate a strong genetic component to MM and suggest a potential for pleiotropic genes involved in MM, CLL and NHLB.

2000/F

Genetic analysis of 50 quantitative traits (QT) in 9 isolated villages. *M.P. Concas[1], L. Portas[1], F. Murgia[1], S. Milia[1], N. Pirastu[2], G. Maninchedda[2], M. Cosso[2], A. Picciau[2], M. Whalen[2], M. Pirastu[1,2].* 1) Institute Population Genetics, Alghero, Sassari, Italy; 2) Shardna Lifesciences, Cagliari, Italy.

Isolated founder population, which exhibit great genetic and environmental homogeneity, provide an attractive setting for the study of complex traits. Our project is focused on 9 isolated villages of the secluded Ogliastra region, all of them characterised by few founders, distinct genetic makeup and a different distribution of common diseases. The study aim was to investigate through heritability if QT associated to these diseases present a different genetic component in the 9 villages. First we estimated the heritability, using SOLAR, of 50 normalised traits related to blood phenotypes and anthropometric measures in a total of 15521 individuals clustered in 471 pedigrees of 3-5 generations. We constructed a dendrogram based on the correlations between traits: the QT which belong to the same biological pathways do indeed cluster together. High heritability was observed for mostly of the traits (70% with heritability between 0.3 - 0.9) with no striking differences among the villages. Similar results were observed either using larger families or pooling together the 9 village datasets.To identify the genetic factors that underlie QT we carried out GWS in one of the villages (Talana) with 1000 microsatellites in 77 families (1107 members) using the variance component approach. In addition a GWS with 16K SNPs (average of 150kb) was performed with MERLIN. Linkage analyses using microsatellites revealed 158 loci with LOD>1.3, 50 loci with LOD>2 and 15 loci with LOD>3. SNP linkage analyses confirmed 42% of loci with LOD>2 and 73% of loci with LOD>3 in addition to several new loci: 61 with LOD>2 and 8 with LOD>3. Several related traits shared identical loci. A dendrogram based on linkage results shows that some clusters match the phenotypic correlation while other unexpected clustering could reflect some common unknown genetic bases. Several loci, even some with low LOD score, contained genes that appear to be involved in the physiological pathways of related traits. For example leptin levels are associated to 3 loci which contain known genes involved in appetite control.

2001/F

The use of focus groups to increase participation when conducting epidemiologic studies that include a genetic component. *D.M. Williamson[1], L. Wagner[2], J. Henry[3].* 1) Division of Reproductive Health, CDC, Atlanta, GA; 2) Texas Department of State Health Services, Austin, TX; 3) Texas Department of Family and Protective Services, Austin, TX.

Participation in epidemiologic studies can be enhanced or diminished based on the study participants' perception, knowledge and understanding of genetic testing. To address these issues, focus groups were convened to gain an understanding of risk perceptions and identify potential barriers to study participation in a case-control study examining the joint role of environmental exposures and candidate genes as potential risk factors of multiple sclerosis (MS). Individuals with MS, identified in a previous cluster study, were invited to participate in the focus group discussions. Participants were asked to provide comments regarding the proposed case-control study goals, recruitment methods, questionnaire topics, specimen collection and dissemination of results. A total of 9 individuals participated in the focus group discussion. The majority of participants (n=8, 89%) were female. Focus group participants expressed interest in participating in the case-control study. Participants stated they would be willing to provide a blood sample for the study. Participants expressed concern about the confidentiality of the results from the genetic testing and that the results would not be provided to them. Comments received from focus group participants were incorporated into the study protocol including study materials, questionnaire and obtaining a blood sample. Revised documents were sent to those participants who agreed to pilot test the materials. The proposed questionnaire was administered over the phone, additional areas needing improvement were noted, and the questionnaire modified accordingly. Focus groups can provide investigators with an opportunity to pilot their study protocol and materials before going into the field. This allows problems to be identified and corrected before recruitment begins. It also allows investigators the opportunity to modify/adjust protocols and materials to effectively meet the needs of potential study participants and promote participation.

2002/F

Genetic disease in offspring of survivors of childhood and adolescent cancer. *J.J. Mulvihill[1], H. Munro[2], J.A. Whitton[3], D.M. Green[4], A.C. Mertens[5], R. Weathers[6], M. Stovall[6], L.C. Strong[6], L.L. Robison[7], The Childhood Cancer Survivors Study.* 1) Pediatrics, University of Oklahoma, Oklahoma City, OK; 2) International Epidemiology Institute, Rockville, MD; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Roswell Park Cancer Institute, Buffalo, NY; 5) Emory University, Atlanta, GA; 6) UT MD Anderson Cancer Center, Houston, TX; 7) St. Jude Children's Research Hospital, Memphis, TN.

No environmental agent has been proved to cause human germ cell mutation seen as genetic disease in offspring. Cancer survivors often receive intensive chemotherapy and radiotherapy that cause human and experimental somatic mutations and animal germline mutations. To study environmental germline mutagenesis, we used the Childhood Cancer Survivor Study, a retrospective cohort of 14,054 children diagnosed with cancer before age 21 years and surviving at least 5 years, at 26 US and Canadian institutions (*Med Pediatr Oncol* 2002;38:229). Participants were 54% male, 87% white, and 64% between ages of 20 and 39 years at follow-up; 68% received radiotherapy and 74% chemotherapy. Radiation doses to gonads were calculated from original records and phantoms to estimate dose-response and doubling dose; mean doses were 126 cGy to ovaries and 46 cGyto testes. Genetic diseases in patients, families, and offspring were ascertained by self-administered questionnaires; verification was by medical records and consensus rules for inclusion were by a 3-person panel. Genetic and congenital diseases occurred in 157 (2.6%) of 6129 offspring of survivors, compared with 111 (3.6%) of 3101 offspring of sibling controls; there were no apparent differences in the proportion of offspring with cytogenetic syndromes (7 in case offspring, 6 in sibling offspring), single-gene defects (14 and 8), or simple malformations (136 and 97). These preliminary results provide reassurance that cancer treatment using modern protocols does not carry a large risk of genetic disease in offspring conceived many years after treatment. (NIH-NCI grants U24CA55727 and 5R01CA104666).

2003/F

Linkage study in Puerto Rican families with Endometriosis. *E.M. Ledet[1], R. Thouta[2], J.E. Bailey-Wilson[3], I. Flores[4], D. Mandal[1].* 1) Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2) Department of Pathology, Louisiana State University Health Sciences Center, New Orleans, LA; 3) NHGRI/NIH, Baltimore, MD; 4) Department of Microbiology, Ponce School of Medicine, Ponce, PR.

Endometriosis is a disease which has affected millions of women; yet, much is still unclear about this often misunderstood condition. Endometriosis is defined by the growth of endometrial tissue, both endometrial stroma and endometrial glands, outside of the uterine cavity. Currently, the exact number of women suffering with endometriosis is unknown, but some, epidemiological studies have indicated a prevalence of 5-20% in women of reproductive age.

Our previous linkage studies on 39 Puerto Rican families produced a LOD score of 1.75 at one of the candidate regions on chromosome 10. For this study, 41 Puerto Rican families with two or more patients with surgically diagnosed endometriosis were recruited; blood samples and patient histories were obtained. Marker genotypes were obtained on chromosomes 1, 3, 7, 8 and 10. Specifically, Mendelian inconsistencies were screened and cleaned from the data set using Sib-Pair and PedCheck, and allele frequencies were calculated utilizing Sib-Pair. Significant allelic association was revealed with an empiric p value of 0.0095 at one of the candidate regions. The marker allele frequencies have been estimated from the data though Sib-Pair. The data would be further utilized to do linkage analysis to identify any susceptibility loci. Additionally, utilizing patient histories, the presence and incidence of other conditions, namely ovarian, lymphoma, breast, and prostate cancers, within the families with respect to endometriosis will be assessed and analyzed. In this study we intend to identify any markers associated with endometriosis on chromosomes 1, 3, 7, 8, or 10, identify and document any correlation, especially with relation to cancer, between family disease history and endometriosis, and, in general, characterize the histories and disease symptoms within this Puerto Rican population.

2004/F

Using the MFG Test to Assess ABO Maternal Fetal Incompatibility as a Risk Factor for Schizophrenia. *E.J. Lockwood[1], J.A. Turunen[2], C.G.S Palmer[1], J.A. Woodward[3], J. Lonnqvist[2], L. Peltonen[2,4,5], J.S. Sinsheimer[1].* 1) UCLA, Los Angeles, CA; 2) Natl Publ Hlth Inst, Helsinki, Finland; 3) UC Merced, Merced, CA; 4) Univ Helsinki, Helsinki, Finland; 5) Broad Institute, MIT, Cambridge, MA.

Maternal-fetal genotype (MFG) incompatibility arises from maternal-fetal genotype combinations that adversely affect the developing fetus by inducing a maternal immunological attack, and thereby increasing disease susceptibility. Previous studies have found RHD incompatibility is a risk factor for schizophrenia (e.g. Palmer et al 2002). This study sought to determine if MFG incompatibility originating at another blood group locus, ABO, is also a risk factor for schizophrenia. Since the effect of RHD incompatibility on schizophrenia risk appears to be limited to males, we also hypothesized that the effects of ABO incompatibility on schizophrenia may differ by gender. We analyzed 282 independent nuclear Finnish families with at least one ABO genotyped parent and affected child (296 affected male offspring, 207 affected female offspring) to test for MFG incompatibility. Our hypotheses were tested using the extension of the MFG test (Sinsheimer et al. 2003) proposed by Kraft et al. (2004) that allows for multiple siblings. We adapted the multiple sibling MFG test to include gender specific MFG incompatibility effects and offspring allelic effects. We did not find a significant effect of ABO incompatibility as a risk factor for schizophrenia (RR to incompatible offspring=1.05, p=0.67). We did not find a significant gender effect on ABO incompatibility (RR to incompatible males and incompatible females respectively is 1.21 and 0.85, p=0.19). There is no evidence for offspring allelic effects (RR of having one O allele or two O alleles 1.02 and 1.22, p=0.38). Power calculations show that the sample size was sufficient to detect moderate effect sizes if they were present. Our results are qualitatively consistent with findings by an independent investigation of ABO incompatibility as a schizophrenia risk factor (Insel et al. 2005). Our study demonstrates that the MFG test is an easily implemented and flexible method for examining maternal-fetal genotype combinations in the context of potential covariates.

2005/F

The dual role of HLA-DRB1*13 in ACPA positive and ACPA negative Rheumatoid Arthritis. *E. Lundström[1], H. Källberg[2], J. Rönnelid[3], L. Alfredsson[2,4], L. Klareskog[1], L. Padyukov[1].* 1) Department of Medicin, Karolinska Institutet and Hospital, Stockholm, Sweden; 2) Environmental Medicin, Karolinska Institutet, Stockholm, Sweden; 3) Oncology, Radiology and Clinical Immunology, Uppsala University, Sweden; 4) Stockholm Center for Public Health, Sweden.

Objective. Since the discovery of the importance of HLA-DRB1 alleles as risk factors for development of rheumatoid arthritis (RA), major interest to shared epitope (SE) alleles remains dominant and very little has been studied regarding influence on RA from non-SE HLA-DRB1 alleles. In this study we investigate the impact of several different DRB1 alleles in two major subgroups of RA defined by the presence or absence of anti-citrullinated protein antibodies (ACPA). Methods. HLA typing was performed by SSP-PCR for 1352 patients (820 anti-CP positive and 532 anti-CP negative) and 922 controls from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) material. Odds ratios (ORs) for HLA-DRB1 allele frequencies were calculated with 95% confidence intervals (95% CI) and interpreted as relative risks (RR), since the study was population-based. Results. We show that DRB1*13 is protective against anti-CP positive (RR 0.57, 95% CI 0.37-0.88) but not anti-CP negative RA (RR 1.10, 95% CI 0.81-1.50) when using dominant model. Further we confirm that DRB1*03 contributes to anti-CP negative (RR 1.37, 95% CI 1.01-1.87) but not anti-CP positive RA (RR 0.94, 95% CI 0.63-1.40). However, this was not true when we analyzed DRB1*03 using recessive model (RR: 1.60 95% CI 0.81-3.14). In opposite, we show that DRB1*03 together with DRB1*13 reveal significant risk for anti-CP negative disease, (RR: 1.75 95% CI: 1.04-2.94) suggesting a possible interplay between these two alleles. Conclusion. Our data show complex relations between different DRB1 alleles in development of RA. DRB1*13 is playing a dual role, being protective against anti-CP positive but increasing risk of anti-CP negative arthritis in combination with DRB1*03. Interestingly, DRB1*13 might be involved in protecting against virus infections, which may support the hypothesis concerning the viral etiology of RA.

2006/F

Meta-analysis of the MHC2TA -168A/G polymorphism and rheumatoid arthritis. *P.G. Bronson[1], L.A. Criswell[2], L.F. Barcellos[1,3].* 1) Division of Epidemiology, School of Public Health, University of California, Berkeley, CA; 2) Rosalind Russell Medical Research Center for Arthritis, Department of Medicine, University of California, San Francisco, CA; 3) Kaiser Permanente Division of Research, Oakland, CA.

Background: An association between major histocompatibility complex (MHC) genes, particularly those within the class II HLA region, and rheumatoid arthritis (RA) is well established, and accounts for an estimated 30% of the genetic component in RA. The MHC class II transactivator gene (MHC2TA) on chromosome 16p13 has recently emerged as the most important transcription factor regulating genes required for class II MHC-restricted antigen presentation. Previous studies of a promoter region polymorphism (-168A/G, rs3087456) in the MHC2TA gene and RA have yielded conflicting results.

Objective: To assess the association of the MHC2TA -168A/G polymorphism and risk for RA by meta-analysis.

Methods: Meta-analysis was performed for 6,861 RA patients and 9,270 controls from ten case-control studies. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each study. Summary ORs and 95% CIs were calculated in random effects models.

Results: No effect was observed for the G risk allele (OR 1.02, 95% CI 0.93-1.12, P=0.70) or the GG risk genotype (OR 1.14, 95% CI 0.95-1.36, P=0.16).

Conclusions: Our results indicate that the MHC2TA -168A/G polymorphism is not associated with RA yet underscore the importance of including shared epitope alleles, secondary phenotypes and more complete characterization of MHC2TA variation in future studies.

2007/F

Genetic contribution to vitamin D status in Hispanic and African Americans: the IRAS Family Study. *C.D. Engelman[1], T.E. Fingerlin[2], C.D. Langefeld[3], D.W. Bowden[3], P.J. Hicks[3], L.E. Wagenknecht[3], J.M. Norris[2].* 1) University of Wisconsin School of Medicine and Public Health; 2) University of Colorado at Denver and Health Sciences Center School of Medicine; 3) Wake Forest University School of Medicine.

The role of vitamin D sufficiency in optimal health is increasingly evident, making the high prevalence of vitamin D deficiency of public health concern. Vitamin D deficiency is associated with bone disease, cancer, multiple sclerosis and diabetes. Although much is known about non-genetic factors of vitamin D status, little has been reported on genetic factors. Two forms of vitamin D, 25-hydroxyvitamin D (25[OH]D), the precursor and predominant circulating form, and 1,25-dihydroxyvitamin D (1,25$[OH]_2$D), the more biologically active form, were measured in the plasma of 507 Hispanic Americans from San Antonio, Texas (SA), 505 Hispanic Americans from the San Luis Valley, Colorado (SLV) and 515 African Americans from Los Angeles, California (LA) from 60, 30 and 42 families, respectively, recruited in the Insulin Resistance Atherosclerosis (IRAS) Family Study. In addition, 30 SNPs (average spacing of 4.5 kb) in the vitamin D receptor (VDR), vitamin D 1α-hydroxylase (CYP27B1), and vitamin D-binding protein (GC) genes were genotyped. Variance components analysis was conducted using SOLAR software. The heritability of 25[OH]D adjusting for gender, age, clinic site, season, and body mass index was 0.33±0.06 ($P < 0.0001$) and of 1,25$[OH]_2$D adjusting for gender, age, clinic site and 25[OH]D was 0.31±0.06 ($P < 0.0001$). Adjusting for gender and age, two non-synonymous SNPs in high LD within the GC gene were associated with 25[OH]D ($P = 0.005$, 0.0003, 0.016 for rs4588 [Thr420Lys] in SA, SLV and LA, respectively and 0.008, 0.001 and 0.054 for rs7041 [Asp416Glu] in SA, SLV and LA, respectively). Rs4588, was also associated with 1,25$[OH]_2$D ($P = 0.007$, 0.025, 0.122 in SA, SLV and LA, respectively). We posit that these variants may decrease the affinity of circulating vitamin D binding protein in blood for 25[OH]D and 1,25$[OH]_2$D, resulting in lower levels of these forms of vitamin D being transported in blood and vitamin D deficiency.

2008/F

Dinucleotide polymorphism upstream SNCA gene determines susceptibility to Parkinson's disease. *Y. Fang[1], P. Rizzu[1], D. Sondervan[1], D.J.H. Deeg[2], B. Post[3], J.J. van Hilten[4], P. Heutink[1].* 1) clinical genetics, Free University Medical Center Amsterdam; 2) Institute for Research in Extramural Medicine (EMGO institute)), VUMC; 3) Dept. Of Neurology, Academic Medical Center, University of Amsterdam; 4) Department of Neurology, Leiden University Medial Center, Leiden, The Netherlands.

Parkinson's disease(PD) is a complex genetic disorder for which several genetic risk factors have been proposed. Mutations in the SNCA gene are responsible for PD in some families with Mendelian inheritance. Two meta-analyses reported that a dinucleotide repeat(REP1) upstream SNCA gene was associated with PD. The expression of the SNCA gene was found to be correlated with the length of the REP1 repeat providing a explanation of the biological effect of the risk factor. To replicate the association in our population, we tested the REP1 polymorphism in 2 PD cohorts from the Netherlands (total 498 patients), 317 cases from a clinimetric research cohort, Scales for Outcome in PD(SCOPA),and 181 from a clinical PD cohort from the Academic Medical Center Amsterdam(AMC). As control cohort we used the Longitudinal Aging Study Amsterdam(LASA,n=1693). 5 alleles(264,266,268,270 and 272bp) were found, 3(266,268 and 270bp) of them were common in our populations (covering 99% genotypes). The 266-allele was underrepresented and 270-allele was overrepresented in PD cases(p=2.4x10-7 for SCOPA,and 0.003 for AMC cohorts, respectively), compared to the LASA cohort. The association was present in both early (<50 yrs)and later (>50 yrs) onset PD case of SCOPA (p=0.0014 and 0.0024, respectively). Adjusting for age and gender, the 270/270 was found to significantly increase the risk of PD comparing to 266/266 with OR(95%CI) of 15.0(3.8-59.1). The age distribution in LASA cohort was significantly different by the genotype of common alleles (p=0.01), the 266-allele was associated with old age. While the PD-risk allele, 270-allele, was associated with young age. We concluded that the REP1 polymorphism increases susceptibility to PD in our population. Further studies are warranted to determine the survival effect of the polymorphism in the LASA population-based cohort.

2009/F

Replicable evidence that increased parental consanguinity confers substantial risk to Bipolar 1 Disorder in Egypt. *H. Mansour[1, 2], M. Talkowski[2], K. Chowdari[1], J. Wood[1], N. Ibrahim[1], W. Fathi[2], A. Eissa[2], A. Yassin[2], H. Salah[2], S. Tobar[2], H. El-Boraie[2], M. El-Hadidy[2], E. Hussein[2], V. Nimgaonkar[1,3].* 1) Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; 2) Department of Psychiatry, Mansoura University Hospitals, Mansoura, Egypt; 3) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

Background: Prior studies have suggested increased consanguinity rates may exist among families of patients with psychoses in certain Middle Eastern populations. Using a retrospective medical record survey, we found that parental consanguinity rates among patients with bipolar I disorder (BP1) were more than double the rates among several sets of independent Egyptian controls. We report here follow-up analyses using a prospective systematic approach to estimate rates of parental consanguinity among (BP1) patients and controls. Methods: Two independent studies were conducted. A community-based epidemiological study involved 2000 participants was conducted through defined geographical areas in Dakahlia Governorate, Egypt. This is followed by a prospective case-control study at Mansoura University Hospital, Egypt. Finally, DNA analysis followed to confirm findings from the prior two studies. Results: The epidemiological study revealed that parental consanguinity rates were higher among BP1 patients compared to the control group (BP1 cases, n = 35; controls; OR = 5.17, 95% confidence intervals, CI: 2.38, 11.23; Chi square = 19.7, $p < 0.0001$). The differences between patients and controls were confirmed during the current prospective case-control study (BP1 cases, n = 93; controls, n = 90, OR = 2.66, 95% CI: 1.34 to 5.29; Chi square = 8.125, p = 0.004). Intial DNA analysis reveals increased homozygosity rates among cases with BP1. Conclusion: Parental consanguinity rates are elevated among Egyptian BP1 patients in the Nile delta region, compared with a range of controls. Recessive inheritance or other factors can explain the results. The replicable results raise public health concerns.

2010/F

Rasch-based genomic scale construction and estimation of personalized relative risk. *N.J. Markward.* Pennington Biomedical Research Center, Baton Rouge, LA.

This project outlines the basic axioms underlying the Rasch measurement framework and, drawing on data generated by the NINDS genome wide association (GWA) study of Parkinson's disease, demonstrates how the Rasch family of measurement models can be employed to develop multi-SNP "genometric" scales that facilitate 1) evaluation and interpretation of person-specific genomic variation and 2) estimation of personalized relative risks (PRR) that integrate information on genetic background and epistatic interactions. The Rasch-based measures of association are then compared and contrasted to sample-level risk indices used in human genome epidemiology, highlighting inferential discontinuities that may preclude the use of population-based summary statistics and p-values as the sole foundation of genome-based diagnostic development and medical decision-making. Of particular interest is the finding that the associative effect of a particular locus--on disease susceptibility, treatment response, or a combination thereof--may depend intimately on the context in which a hypothesized causal variant resides. Indeed, the results indicate that a given allele or genotype can generate both an independent (positive or negative) effect at the population level and a distinctive interactive effect that depends on the placement of individuals relative to each SNP on the Rasch scale.

2011/F

Variance components analysis provide indirect proof of environmental homogeneity in an isolated population. *F. Marroni[1], D. Grazio[1], C. Pattaro[1], M. Devoto[2], P.P. Pramstaller[1,3,4].* 1) Institute of Genetic Medicine, European Academy, Bolzano, Italy; 2) The Children's Hospital of Philadelphia, Division of Human Genetics, and CCEB, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; Dipartimento di Medicina Sperimentale, Universita' La Sapienza, Roma, Italy; 3) Department of Neurology, University of Lübeck, Lübeck, Germany; 4) Department of Neurology, General Regional Hospital, Bolzano, Italy.

We measured 43 quantitative traits in 1,138 subjects living in three isolated villages in South Tyrol (Italy). We used variance components (VCs) to estimate narrow-sense heritability, individual-specific environmental effects, and shared environmental effects. Estimates of narrow-sense heritability were in good agreement with previous findings. Household effects (Vh/V) were significant for only a few traits, and after correcting for multiple testing no trait showed significant household effect. When a VC (in our case, Vh/V) has a very low value this could be due to the fact that: a) the variance of the studied traits is not influenced by the considered component; or b) the variability of the component in the study sample is reduced, thus the VC is estimated to be low as well. Previous studies have shown that shared environment can significantly affect QTs. This was not confirmed by other studies performed subjects who shared the same environment in the most comprehensive terms (i.e., neighbourhood, lifestyle and habits). It is thus possible that the low estimates of the effects of shared environment in our population are not due to a real lack of its contribution to the studied traits, but rather to its limited variability, which is caused by reduced inter-individual differences in environmental factors. This could explain why our heritability estimates are in good agreement with previous studies, while the estimates of shared environment effects are sensibly lower. We suggest that the low shared environment contribution is indeed an indirect proof of the reduction of environmental heterogeneity in the studied villages.

2012/F

Use of haplotype analysis to locate Breast Cancer Susceptibility Loci in a genome-wide association study. *P. Smith[1], K. Pooley[2], P.D.P. Pharoah[2], A.M. Dunning[2], D.R. Cox[3], D. Ballinger[3], D. Thompson[1], D.G. Evans[4], D. Eccles[5], N. Rahman[6], M.R. Stratton[6], J. Peto[7], O. Fletcher[8], B.A.J. Ponder[2], D.F. Easton[1].* 1) Public Health and Primary Care, University of Cambridge, UK; 2) Department of Oncology, University of Cambridge, UK; 3) Perlegen Sciences, Inc., USA; 4) Regional Genetic Service, St. Mary's Hospital, UK; 5) Wessex Clinical Genetics Service, Princess Ann Hospital, UK; 6) Institute of Cancer Research, UK; 7) London School of Hygiene and Tropical Medicine and Institute of Cancer Research, UK; 8) Breakthrough Breast Cancer Research Centre, UK.

Multi-marker haplotype analysis provides an alternative to single SNP analysis in association studies. Haplotype analyses are more powerful if either a rare variant has recently arisen, or if there is a cis-interaction between alleles.

We applied three methods of analysing haplotype data to a genome-wide study of breast cancer[1] 195,479 genotyped SNPs with a minor allele frequency >5%;, genotyped on 408 high-risk breast cancer cases and 400 controls from the UK in the stage I of this study, were analysed. The median distance between SNPs was 6.1kb. The main analysis used a sliding window approach, using all possible windows of two to eight SNPs, implemented in the Haplostats programs.

Strong evidence for two additional loci was found: a 2-SNP haplotype, p-value=$3x10^{-10}$, and a 3-SNP haplotype, p-value=$7x10^{-8}$.

Further follow-up in additional case-control studies will be required to determine whether these associations can be replicated, and whether the association is due to linkage disequilibrium with an untyped allele or a true haplotype effect.

[1]Easton *et al*, Nature, 2007.

2013/F

Genetic Components of Variance for Common Latent Components of Obesity-related Traits in African-Americans. *B. Tayo[1], D. Kan[1], R. Harders[1], A. Luke[1], X. Zhu[2], R. Cooper[1].* 1) Department of Preventive Medicine and Epidemiology, Loyola University, Chicago, Maywood, IL; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University,Cleveland, OH.

Objective: To identify significant common latent components or factors which account for observed covariation of obesity-related traits, and to estimate their genetic components of variance in African-Americans. Methods: We used the maximum likelihood factor analysis method to both determine the number of, and extract scores of significant common factors of selected obesity-related measures on 1775 subjects from 599 African-American families. The obesity-related measures include body mass index, body surface area, fat mass, percent body fat mass, resting metabolic rate, waist circumference and hip circumference. Variance-component analysis was performed to estimate the environmental and polygenic variance components of the common latent factors. Results: Two significant common latent factors were identified. The proportions of covariation of the obesity-related traits accounted for by the first and second factors are 0.862 and 0.136, respectively. The estimated sex and age-adjusted genetic components of variance for the two common latent factors are 0.374 and 0.433, with heritability estimates equal to 51.37% and 53.27%, respectively. Conclusions: The results of our analysis provide support for the existence of common genetic influence on obesity-related traits. Linkage or association analysis of common latent components of obesity-related traits can be useful in mapping pleiotropic loci for these traits.

2014/F

A comprehensive association analysis of Alzheimer's disease candidate genes reveals a risk haplotype in ACE. *E.S. Torstenson[1], T.L. Edwards[1], M. Pericak-Vance[2], J. Gilbert[3], E.R. Martin[2], M.D. Ritchie[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Genetic Epidemiology and Statistical Genetics, Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 3) Center for Genome Technology, Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL.

Alzheimer's disease (AD) is the most common form of progressive dementia in the elderly. It is a neurodegenerative disorder characterized by the neuropathologic findings of intracellular neurofibrillary tangles and extracellular amyloid plaques that accumulate in vulnerable brain regions. In this study, we examined single locus and haplotype association with late-onset Alzheimer's susceptibility in 738 Caucasian families and an independent case-control dataset exploring 12 candidate genes using traditional analytic approaches. The Multifactor Dimensionality Reduction Pedigree Disequilibrium Test (MDR-PDT) was used to explore single-locus effects as well as 2-locus and 3-locus gene-gene interactions associated with AD in the family data. We observed significant haplotype effects in ACE in both family and case-control samples. ACE also was part of a significant 2-locus and 3-locus MDR-PDT joint effects model with Alpha-2-Macroglobulin (A2M), which mediates the clearance of amyloid beta (Aβ), and Leucine-Rich Repeat Transmembrane 3 (LRRTM3), a nested gene in Alpha-3 Catenin (VR22) which binds Presenilin 1. Significant main effects for these models were confirmed using conditional logistic regression, but no evidence of effect modification was detected. These three genes are all related to Aβ clearance and thus this constellation of effects might constitute an axis of susceptibility for late-onset Alzheimer's disease. The consistency of the results between independent datasets, family data and unrelated cases and controls, is strong evidence in favor of the hypothesis asserting ACE as a susceptibility locus for Alzheimer's disease.

2015/F

Graphical browsing for whole-genome association studies of global gene expression. *W. Chen[1], L. Liang[1], M. Lathrop[2], W.O.C Cookson[3], G.R. Abecasis[1].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI, U.S; 2) Centre National de Genotypage, Evry, France; 3) Imperial College, London, U.K.

We describe an interactive package that provides graphical overviews of whole-genome association studies of datasets with very rich phenotypic information, such as global surveys of gene expression. The software incorporates a generic eQTL database and provides a graphic interface for browsing association between transcript levels and SNPs. For each transcript, our browser can tabulate and plot association test statistics, estimates of effect size and allele information across the genome. The browser automatically links results to the UCSC genome browser where users can examine each transcript in its genomic context. In addition to browsing the results by transcript or by position, results can be searched for information on specific SNPs. LD and tag information is provided for SNPs not in our database but evaluated by the International Hapmap Consortium. To illustrate the utility of our approach, we show how our database can be used to browse results of an association study of global gene expression. This study genotyped 408,298 SNPs to identify eQTLs associated with levels of 54,675 transcripts representing 20,599 known genes in EBV-transformed lymphoblastoid cell lines in ~400 children. Using their data, we constructed a database to summarize association results between transcripts and individual SNPs. The browser facilitates integration of the results with other gene mapping projects. For example, in a recent GWA association scan, a series of SNPs in an intergenic region on chromosome 5p were associated with Crohn's Disease (Libioulle, C. et al. 2007). The SNPs are more than 200 Kb away from the nearest annotated gene. Our database shows that these SNPs regulate expression of PTGER4 (e.g. association with rs4495224 can explain 4.7% of the variance in PTGER4 levels, $p < 7*10^{-5}$). In the future, the software has a potential to be scalable tool to browse even larger gene-expression genome-wide scans. The software can be downloaded at http://www.sph.umich.edu/csg/liang/asthma/.

2016/F

Integrative genomics and genome-wide association using family-based designs. *J.H. Degnan[1], J.A. Lasky-Su[2,3], B.A. Raby[3], M. Xu[3], C.M. Molony[4], E.E. Schadt[4], C. Lange[1,3].* 1) Biostatistics, Harvard School of Public Health, Boston, MA; 2) Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, NY; 3) Harvard Medical School, Channing Laboratory, Boston, MA; 4) Genetics, Rosetta Inpharmatics, Seattle WA.

Expression QTL mapping by integrating genome-wide gene expression and genotype data is a promising approach for identifying functional genetic variation, but is hampered by the large number of multiple comparisons inherent to such studies. A novel approach for overcoming multiple testing problems in genome-wide family-based association studies is screening candidate markers using heritability or conditional power. We apply these methods for the setting in which microarray gene expression data are used as phenotypes, screening for SNPs near the expressed genes. We perform association analyses for phenotypes using a univariate approach using CEPH data. Simulations were also performed on trios with large numbers of causal SNPs to determine the optimal number of markers to use in a screen. We demonstrate that our family-based screening approach performs well in the analysis of integrative genomic datasets and that it was able to find several associations that were genome-wide significant after correction for multiple comparisons. We also find that screening using either by heritability or conditional power had similar performance, both in the simulation and in the analysis of the CEPH data.

2017/F

The use of genome-wide eQTL associations to identify novel genetic pathways involved in complex traits. *J.L. Min[1], J.M. Taylor[1], T. Watts[2], K.R. Ahmadi[3], J.B. Richards[3], J. Broxholme[1], F. Pettersson[1], I. Ragoussis[2], T.D. Spector[3], K.T. Zondervan[1], L.R. Cardon[1].* 1) Bioinformatics & Statistical Genetics, Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; 2) Genomics Laboratory, Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; 3) Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital Campus, King's College London, United Kingdom.

Despite recent successes of genome-wide association studies in several complex traits, many associations between clinical phenotypes and genetic variants will remain difficult to uncover because of phenotypic heterogeneity. In such cases, the use of downstream biological phenotypes may provide a more powerful approach. Gene expression levels are highly variable and heritable, and are known to be strongly associated with genetic variants. This study investigates the association between a range of quantitative metabolic phenotypes and gene expression levels in twins, followed up by targeted SNP association analysis. Expression profiling was conducted in lymphoblastoid cell lines from 119 monozygotic pairs and 60 dizygotic pairs from the St. Thomas' UK adult twin registry, and 60 unrelated CEU HapMap individuals, using the Illumina Sentrix Human-6 version 2 BeadChip. Out of the 46,713 transcripts measured, the 5,070 most variable probes in twins, and 4,918 probes in the HapMap individuals, were selected. To find SNPs associated with expression levels, we performed a genome-wide SNP analysis in the HapMap individuals between 946,479 non-redundant SNPs (HapMap Phase II) and 4,918 probes. Significance was assessed through permutation; consistency of the associations is being investigated through comparison with other published genome-wide eQTL analyses. Of the 5,070 selected probes in the twins, 1648 (32%) showed at least moderate heritability. These heritable probes are being examined for association with metabolic traits. Those that show significant association are cross-referenced with the genome-wide association HapMap results for identification of associated SNPs. These SNPs will be genotyped in the present twins, and a replicate sample, to test the association.

2018/F

Whole genome based estimation of the probability to develop a complex disorder and application to Crohn's disease. *S. Hansoul[1], C. Sandor[1], V. Botta[2], L. Wehenkel[2], T. Meuwissen[3], M. Georges[1].* 1) Department of Animal Genomics, University of Liège, Liège, Belgium; 2) Department of Bioinformatics, University of Liège, Liège, Belgium; 3) Institute for Animal Science and Aquaculture, As, Norway.

Thanks to the HapMap project and to recent improvements in genotyping techniques, an increasingly large number of whole genome association studies of complex disorders are currently conducted. Some of them already released new insights about the genetic architecture of the studied disorder, but generally have limited power. Only the biggest effects are currently identified and they are by essence overestimated. Prediction of the genetic susceptibility to develop a complex disease based on these loci only remains inaccurate.

With a sufficiently dense marker map (>100K SNPs), one might expect most of the true disease signals to be captured in the study, whereas not necessarily associated to a significant p-value. In order to predict a probability of disease outcome, one must take into account all the information contained in the sample. To that aim, we investigated three different methods. One of them is a data mining technique based on decision trees and the two other ones work within a Bayesian framework.

These approaches have been applied to a data set consisting in 527 Crohn's Disease patients and 928 healthy controls, all of Caucasian origin. This cohort has been genotyped for more than 300K SNPs using the Illumina HumanHap300 Genotyping BeadChip. With all methods, the correlation between disease status and the estimated probability to develop the disease increases as more SNPs (ordered by p-value) are included in the model. This hints that there is valuable information contained in markers that classical methods wouldn't pick out.

.

2019/F

Genetic and other factors affecting individual variation in gene expression. *M.A. Rivas[1,2,4], M.J. Daly[2,3], I. Pe'er[4].* 1) Department of Mathematics, Massachusetts Institute of Technology, Cambridge, MA; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Center for Human Genetics Research, Massachusetts General Hospital, Boston, MA; 4) Department of Computer Science, Columbia University, New York, NY.

Genetic genomics holds the promise to dissect the heritable factors that regulate all expression-mediated processes in the cell. Indeed, coupled whole genome genotype and expression data for human lymphoblastoid cell lines have recently been analyzed by classical methods to map numerous expression QTLs.

We sought to elucidate the different factors driving individual levels of gene expression, including genetic variation, age and sex. We consider public data from CEPH extended pedigrees including expression profiles as well as microsatellite and SNP genotypes. We report 951 and 302 transcripts levels that are associated with age and sex respectively. We perform more elaborate genetic analysis of these data, that increases power to detect association. We thus detect 125 transcripts with strong signals (p-value < 1e-8) of association to a SNP in cis, most of which were not reported by simpler analyses of the same data. We further report several previously unrecognized significant signals (p-value < 1e-12) of association to trans SNPs.

We follow up these clues with analysis of functional annotation to expose meaningful trends. Examples include: (i) Enrichment of immune-related genes whose expression increases for older individuals - a phenomenon that replicates across datasets, tissues and organisms (ii) A cluster of co-regulated transcripts enriched for associations to neurodegenerative disorders, that show effects of both age-association as well as genetic regulation between a SNP in LRP1B and the expression of its ligand, APOE.

Last, we systematically investigate age X genotype interaction. We use elastic net regularization to infer age of all typed samples. This significantly increases power to detect age-mediated genetic association signals, achieving a genomewide compendium of such effects.

2020/F

Association of haplotype of the signal transducer and activator of transcription gene (STAT4) with RA in the Korean population - Asian and Caucasian populations share common risk haplotype. *H.S. Lee[1, 3], E.F. Remmers[2], J.M Le[2], D.L. Kastner[2], D.H. Yoo[3], S.C. Bae[3], P.K. Gregersen[1].* 1) The Feinstein Institute for Medical Research, Manhasset, NY; 2) NIAMS, Bethesda, MD; 3) Hanyang University of College of Medicine and the Hospital for Rheumatic Diseases, Seoul, Republic of Korea.

Recently, a study in North American Caucasians has documented the association of a common STAT4 haplotype with risk for rheumatoid arthritis and systemic lupus erythematosus (Remmers et al., manuscript; 2007 ASHG abstract). In order to replicate this finding in the Korean population, we performed a case-control association study. Sixty seven SNPs within STAT4 were genotyped in 1123 Korean patients with RA and 1008 ethnicity-matched controls. The association of the risk genotype/haplotype with RA, anti-cyclic citrullinated peptide(AntiCCP), earlier-onset age, and radiographic severity were analyzed. Attributable proportions (AP) were also calculated as a means to measure interaction between shared epitopes (SE) of HLA-DRB1 and a STAT4-risk haplotype for RA. The most significant four risk SNPs (rs11889341, rs7574865, rs8179673, and rs10181656 located within the third intron of STAT4) among 67 SNPs are identical with those in the North American Caucasian study. All 4 SNPs have the modest risk for RA susceptibility (odds ratio 1.21 - 1.27). The same haplotype (TTCG) as the Caucasian study shows the significant risk for RA [34% versus 28%, P=0.0027, ORs (95% CI) 1.33 (1.10 - 1.60)]. These significant associations of STAT4 were observed in both antiCCP+ and antiCCP- RA groups. In the analysis for interaction with SE, the risk haplotype showed significantly increased RA risk by interaction with SE alleles (AP=0.227, 95% CI 0.044-0.410). In the logistic regression analysis, this haplotype is an independent risk factor in addition to SE for RA. Unlike several other risk genes such as PTPN22, PADI4, and FCRL3 for RA, a haplotype of the STAT4 gene shows consistent association with RA susceptibility across the Asian and Caucasian racial groups.

2021/F

When mean and variance are related: improved conditional t test for identifying differentially expressed genes. *Z. Luo, J. Cabrera.* Departmetn of Statistics, Rutgers,the State Univeristy of New Jersey, Piscataway, NJ.

Amaratunga and Cabrera (2004) proposed a conditional t suite of tests for identifying differentially expressed genes in a microarray experiment with little replications. Now we adjust conditional t test to take consideration of the situation that the mean and the variance are correlated. It is widely known that the correlation between the mean and the variance of gene expressions is very strong in raw data. Although in many cases, the relationship is greatly reduced after taking transformation, it may still exist. Target to it, we present the improved conditional t test. Our simulation studies show that when the mean and the variance are independent, improved conditional t test give us similar results as conditional t test. While the mean and the variance are correlated, improved conditional t test is much better than conditional t test in the sense that it gains more power and identifies more significantly differentially expressed genes. Both conditional t test and improved conditional t test are implemented in DNAMR, which is a collection of R and Splus programs. This package is freely available at http://www.rci.rutgers.edu/~cabrera/DNAMR.

2022/F

Analysis of familial recurrence patterns of nonsyndromic oral clefts in Denmark: a registry study with 6,811 probands. *C. Chevrier[1,2], D. Grosen[1], C. Bille[1,3], J.C. Murray[4], K. Christensen[1].* 1) Epidemiology, Institute of Public Health, Univ Southern, Odense, Denmark; 2) Inserm, U625, GERHM, Univ Rennes I, IFR 140, Rennes, France; 3) Plastic Surgery Dep, Odense University Hospital, Odense, Denmark; 4) Paediatrics Dep, Univ Iowa, Iowa, USA.

Oral clefts (congenital anomalies that affect 1/700 live births) are genetically complex. This study provides an analysis of their familial recurrence patterns from Danish registries. Two groups are distinguished: cleft lip with/without cleft palate (CL/P) and cleft palate only (CP). Based on the theory developed by Risch (1990), we compute familial risk ratios defined as the risk to a type of relative of an affected individual divided by the population prevalence and we compare the observed values with the predicted values under various genetic models. We also use the method of Schliekelman and Slatkin (2002) for estimating the number of susceptibility loci involved. From 4,685 CL/P probands and their 37,749 relatives, we observe the following risk ratios to 1st, 2nd and 3rd degree relatives: respectively, 22.3 (confidence interval: 20.0-24.6), 4.5 (3.6-5.5) and 2.7 (1.9-3.7). From 2,126 CP probands and 16,744 relatives, the observed risk ratios are: 41.3 (34.5-48.6), 6.6 (4.3-9.4) and 1.9 (0.6-4.0). These results exclude single-locus inheritance of both conditions CL/P and CP, rejecting also models assuming multiple additive loci and multiple independent loci. We observe that both conditions are likely to be determined by several loci acting in multiplicative fashion. Numerous models are plausible but no single locus appears to account for more than a threefold increase in risk to 1st-degree relatives of CL/P proband and the maximum effect of the CP susceptibility loci is to increase the risk to 1st-degree relatives by a factor of six. We estimate a number of loci between 1 and 5 for CL/P and between 1 and 9 for CP. These results benefit from a well-defined population, high-quality data and accurate estimates of the population prevalence in Denmark. They provide the most accurate indication of the mode of inheritance of nonsyndromic oral clefts. Such analyses are now needed on other single geographic populations.

2023/F

Application of Bayesian Classification in an Association Study of Impaired Glucose Tolerance versus Impaired Fasting Glucose. *S. Kwon[1], M.O. Goodarzi[1], K.D. Taylor[1], J. Cui[1], B. Hildalgo[1], J.I. Rotter[1], W. Hsueh[2], X. Guo[1].* 1) Cedars-Sinai Medical Center, Los Angeles, CA; 2) UCLA, Los Angeles,CA.

Conventional statistical approaches face challenges when dealing with a large number of single nucleotide polymorphisms (SNPs) (p) with a relatively small sample size (n). We extended the Bayesian classification with binary responses to multinomial ordinal responses using singular value decomposition (SVD). We developed a Markov Chain Monte Carlo based computation algorithm to realize the Bayesian classification with SVD method (BCSVD). Using simulated data with 3 ordinal responses, we demonstrated that the BCSVD method can be reliably used to analyze large scale association data when p>>n. We applied the method to evaluate candidate genes for impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) in a subsample of subjects recruited through a coronary artery disease proband in the Mexican-American Coronary Artery Disease Project. 449 adult offspring and offspring spouses went through detailed phenotyping, including fasting and 2-hr glucose levels; all were genotyped for 91 SNPs from 16 genes selected for a prior relationship to insulin physiology: resistance (R: LPL, CAPN10, PRKAG3, CRP, C5, IL4, IL4R, IL6, NOS3, NPPA, SCNN1A, ADRB2), secretion (S: CAPN10, TCF7L2, SORCS1), and clearance (C: AMPD2, PRKAA2). An individual was defined as IFG if his/her fasting glucose level was between 100 and 125; and defined as IGT if a 2-hr glucose level was between 140 and 199. We generated two sets of data, each having 3 disease stages with 20 samples in each stage (n=60). The first data (V1) has 3 stages: both glucoses normal (N/N), fasting glucose normal and IGT (N/IGT), IGT and IFG (IFT/IGT); the second (V2) was classified as: N/N, IFG/N, and IFG/IGT. Assuming a dominant genetic model, we found that SNPs in 5 R genes (LPL, PRKAG3, C5, IL4R, IL6) and 1 S gene (SORCS1) were associated with both V1 and V2; SNPs in CAPN10 (S) gene, were associated with V1 only; while SNPs in PRKAA2 (C), 2 R genes (NOS3, SCNN1A), and TCF7L2 (S) were associated with V2 only. These results suggest that IGT and IFG may indicate different pathways to diabetes, with different genetic determinants.

2024/F

Accommodating longitudinal unstructured clinical information in genetics studies. *R.M. Salem[1,2], N.J. Schork[1,3].* 1) Dept Family and Preventive Medicine, UCSD, La Jolla, CA; 2) Graduate School of Public Health, SDSU, San Diego, CA; 3) Scripps Genomic Medicine, TSRI, La Jolla, CA.

Dynamic complex traits, quantitative phenotypes measured over time, are influenced by the interplay of multiple genetic and environmental factors. Analysis of such traits offers insights into disease processes, progression, and temporality, in contrast to the single dichotomous outcome in the more commonly utilized case-control design. The case-control study design is problematic in that it suffers from bias, uses limited data, and provides no such insights. Unfortunately, many of the existing statistical methods perform poorly or do not fully utilized available data. We introduce a novel statistical framework to model and analyze dynamic complex traits. The first step, involves modeling the dynamic trait using non-parametric functions (curves) fitted to all available data. The dissimilarity (or "distance") between a set of individuals functions is calculated and related to genetic and environmental factors via a Multivariate distance matrix regression (MDMR) method. This approach accounts for uncertainty of fitted functions, can accommodate weighting factors, and is easily extended to a multivaraite analysis settings. We compare our approach with standard quantitative longitudinal statistical methods with data from three clinical studies. Across the studies, measurement of blood pressure (BP) varies from highly structured clinical studies to unstructured medical care data. One study, containing medical records represents a valuable and unique perspective for studying BP in a clinical setting. Use of medical data in research poses considerable challenges and has been labeled the Longitudinal Unstructured Clinical Information (LUCI) Problem. Advancement on these problems has direct applications to the study of disease occurrence, progression, and drug response. The proposed method is very flexible, accommodates a wide range of complex and high-dimensional longitudinal clinical datasets, and utilizes all available data. In conclusion, our method anticipates a shift from use of case-control to longitudinal cohort study designs in genetics research.

2025/F

Time-varying ACE components in random effects growth modeling of longitudinal cohort data for twins. *B. Muthén, S.L. Clark.* Education, UCLA, Los Angeles, CA.

Longitudinal twin data are often collected using cohorts that differ widely in their ages. This has the advantage of studying development over wide age ranges, but has the disadvantage that typical modeling assumptions are strained. Random effects growth modeling of longitudinal twin data has the limiting assumption that the A, C, and E variance components of the standard twin model are constant across time. With widely different ages, however, it is quite conceivable that for example the E variance component varies substantially, and it is also of interest to allow for variation in the A variance component across ages where genetic effects emerge. Methods to alleviate the limiting assumptions are to include age as a covariate and/or perform a separate analysis for different cohorts. This paper presents a more satisfactory method that explicitly allows for changes across age in the variance components by applying parameter constraints where variances are expressed as functions of age. The method is applied to a study of the development of loneliness in Dutch twins across ages 10-60. It is found that the variance components deviate from constant values, instead showing substantial increasing and decreasing trends at different age ranges.

2026/F

Characterizing the Factor V Leiden (FVL) thrombophilia phenotype: A model to tackle the genetic architecture of complex diseases. *F. Gagnon[1], D.E. Bulman[2], P.S. Wells[2].* 1) Public Health Sciences, University of Toronto, Toronto, ON, Canada; 2) Ottawa Health Research Institute, Ottawa, ON, Canada.

Venous thromboembolism (VTE) is a common complex disease with known environmental risk factors and a well-characterized major gene variant, FVL. FVL thrombophilia is associated to a single point mutation in the factor V gene leading to the Activated Protein C Resistance phenotype, which is associated to an increased risk of VTE. This disorder has an autosomal dominant inheritance and a population frequency of 2-15%, and up to 60% in VTE cases. The predictive clinical value of FVL is limited since only 20-50% of heterozygous individuals develop VTE despite accounting for other known risk factors. Experimental evidence suggests that this variability is more likely due to modifier genes than unknown environmental factors. Several genetically determined hemostatic- and lipid-related quantitative traits (QT) have been associated to VTE but their distributions in specific thrombophilias are unknown. Thus, we are taking advantage of the well-characterized FVL variant to identify genes implicated in VTE. Our major objective is to identify the modifier genes in FVL thrombophilia, and our main strategy is to capitalize on the several QT associated to VTE. We have recruited 7 large French-Canadian families (n=306) through simplex ascertainment of probands with both VTE and FVL. Over 30 QT from the hemostatic and lipid pathways, as well as several putative environmental covariates (e.g. hormonal therapy, smoking), have been collected on all family members. The specific aims of this paper are to phenotypically characterize FVL thrombophilia based on generalized linear models accounting for familial dependences; and to present results from Bayesian Markov chain Monte Carlo-based oligogenic segregation analyses; e.g. In a large meta-analysis, we recently reported that a factor XIII A-subunit variant has a protective effect against VTE. Here, we report that plasma factor XIII activity is significantly higher in FVL carriers vs. non-carriers, and that it is correlated to several lipid-related QT (e.g. plasma cholesterol), as well as other hemostatic QT including plasma levels of factor V.

2027/F

Comparison of gene expression filters in the eQTL study. *L. Chen[1], T. Metha[1], G.P. Page[1], R. Feng[1], X. Cui[1,2].* 1) Biostatistics, University of Alabama at Birmingham, Birmingham, AL; 2) Department of Medicine, Division of Genetic and translational Medicine, University of Alabama at Birmingham, AL.

Filtering gene expression data is a common practice in eQTL studies to reduce dimensionality. In this paper, we examined the effect of three filters on the CEPH lymphoblastoid cell expression data (Affymetrix Human Focus arrays).

First we evaluated two gene level filtering strategies, the variance-based filter used by Morley et al. and a P/A call based filter. We found that the genes selected by the two filters largely overlap. However, substantial differences are also observed. For the 5541 transcripts selected by the P/A call, 3331(60.1%) probe sets were also selected by the variance-based filter. We then applied the linkage mapping for the selected transcripts and comparing the linkage peaks between these two filtering methods. Most of the peaks identified by the variance filter were also identified by the P/A call filter.

We also examined the effect of a probe-level filter for removing the SNP-containing probes from the probe sets. We then do the linkage mapping on the two set of the transcript measurement with/without filtering the SNP-containing probes. We found that overall less than 67% of the significant peaks agreed between the two analysis regardless of the choice of significance threshold. The agreement decreases as the number of SNP-containing probes in a probe set increases. The comparison of correlation coefficients between the probe set summaries before and after filtering showed that removing the SNP-containing probes did reduce the correlation coefficient more than randomly removing equal numbers of probes from a probe set in some genes.

At probe set level, both variance-based filter and P/A call filter were able to select probe sets with high signal/noise ratio, increased the power of downstream analysis and reduced the number of hypotheses. At probe level, filtering out the SNP-containing probes showed some impact on the downstream analysis. The impact partially comes from the reduced probe affinity due to presence of SNPs in the targeted sequences.

2028/F

Vascular endothelial growth factor gene polymorphisms in thyroid cancer. *M.Y. Lu, P.J. Hsiao, F.Y. Chiang, S.J. Shin, Y.D. Tai, S.H. Juo.* Kaohsiung Medical University, Kaohsiung, Taiwan.

Objective: Vascular endothelial growth factor (VEGF) is a potent stimulator for angiogenesis. It has been implicated in growth and metastasis of thyroid cancer. Three functional single nucleotide polymorphisms (SNPs) of VEGF (-2578C/A, -634G/C and +936C/T) are known to be related with VEGF expression. Methods: We conducted a case-control study to evaluate the genetic effects of these three functional SNPs on the development of thyroid cancer and lymph node metastasis. A total of 332 cases and 261 controls were recruited for this study. The genotypes were determined by the TaqMan 5' nuclease assay. Hardy-Weinberg equilibrium (HWE) was tested for each SNP, and genetic effects were evaluated by the x2- test and multiple logistic regression. Results: All three SNPs were in HWE. The A allele of -2578C/A (i.e. SNP rs699947) significantly increased a risk for thyroid cancer (adjusted OR =1.36, 95% C.I = 1.02~1.81, P=0.039). Haplotype analysis yielded a less significant result (an empirical p value of 0.07). There was a tendency of increasing the frequency of the risk allele from controls, patients without lymph node metastasis to patients with lymph node metastasis (P trend = 0.019). The genetic effect was only in men (adjusted OR=1.97, 95% C.I = 1.16 ~ 3.37, P =0.013) but not in women (adjusted OR=1.15, 95% C.I = 0.81~1.62, P =0.437). The other two SNPs did not show significant results. Conclusion: The A allele of the SNP rs699947 increased the risk of thyroid cancer development and regional lymph node metastasis in men.

2029/F

Using the optimal ROC curve to design a predictive genetic test. *Q. Lu, R.C. Elston.* Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Current extensive genetic research into common complex diseases, especially with the completion of genome-wide association studies, is bringing to light many novel genetic risk loci. These new discoveries, along with previously known genetic risk variants, offer an important opportunity to improve health care. For researchers who are interested in applying these new finding into clinical practice soon, we introduce a powerful tool to help design a new predictive genetic test. By utilizing the information from any previous association study, the method can provide an estimate of its classification accuracy and the required sample size to verify this accuracy. The proposed predictive test is asymptotically more powerful than tests built on any other existing method and can easily be extended to scenarios where loci are linked or interact. We illustrate the approach for the case of Type 2 diabetes. For a general population, we incorporate recently discovered risk factors into the proposed test and find a potentially better predictive genetic test. The area under the ROC curve (AUC) of the proposed test has a higher value (AUC=0.669) than that of the existing test (AUC=0.580). We also investigate a predictive genetic test for subjects at high risk of diabetes. Based on 44 single nucleotide polymorphisms (SNPs), the discriminative ability of this test for high risk individuals could reach an even higher level of accuracy (AUC=0.855).

2030/F

Risk factors for migraine taking into account family structure in a sample of Portuguese families. *C. Lemos[1,2], M.J. Castro[1,2], I. Alonso[1,2], J. Barros[3], J. Sequeiros[1,2], J. Pereira-Monteiro[2,3], D. Mendonça[2], A. Sousa[1,2].* 1) UnIGENe, IBMC, Porto, Portugal; 2) ICBAS, Univ.Porto, Portugal; 3) Serviço Neurologia, HGSA, Porto, Portugal.

Migraine is a primary, chronic headache and a highly prevalent disease. An increased risk for relatives of migraineurs suggests that genetic factors may be implicated in the most common forms of this disease. In a previous study, we validated the use of family history to classify relatives as migraine sufferers and we found that probands were able to correctly identify their affected relatives although migraine in familial members was underestimated, but not overestimated. In that sample of Portuguese families, we also found evidence of familial aggregation in first-degree relatives of probands. In the present study, we evaluated relative's age at observation and gender as risk factors for migraine, since this is an age and gender-dependent trait. We also included proband's age at onset in the model to test if this variable was associated with relative's affection status in our families. A total of 131 Portuguese families were selected for this study. Among 492 first-degree relatives of probands with migraine, 317 were affected and 175 were healthy. Proband's age at onset was used as dichotomous variable (<16, 16+ years) and relatives were divided in two groups according to their age at observation (<40, 40+ years). We performed a logistic regression analysis and general estimating equations (GEE) were used to account for residual correlation among members from the same family. After adjusting for the remaining variables, gender was found to be a risk factor for migraine (OR=3.22; 95% CI= 2.13- 4.86), with females at higher risk than males. Proband's age at onset and relative's age at observation were not associated with the outcome. No significant interactions were found between these variables. In a previous study, a lower age at onset in probands was associated with relative's affection status. In our study, the proportion of affected relatives was independent of proband's age at onset. Our findings showed that, as expected, gender is a risk factor for migraine.

2031/F

Disease prediction with multiple common variants. *E. Ziv, D. Hu, L. Fejerman.* Dept Medicine, Institute for Human Genetics, Comprehensive Cancer Center, Univ California, San Francisco, San Francisco, CA.

In the era of whole genome association studies, numerous common variants are being identified as risk factors for complex traits such as cancer, diabetes mellitus, and autoimmune diseases. One of the potential benefits of these discoveries is the identification of enough risk factors in the population to predict disease risk in pre-symptomatic individuals who can then receive preventive interventions. The effectiveness of such risk prediction depends on the predictive power of the combination of variants identified. We develop a framework to consider the risk prediction of a combination of common variants. We use the C-statistic, a commonly used measure of model discrimination, as our measure of predictive power of a multi-gene test. The C-statistic can be interpreted as the probability that an individual with disease is considered higher risk based on their risk score compared to an individual without disease. The C-statistic ranges between 0.5 (equivalent to chance) and 1 (perfect predictive power). It is equivalent to the area under the receiving operator characteristic (ROC) curve. We model a combination of common variants (allele frequency >3%) with moderate susceptibility (multiplicative RR: 1.1 - 2) each of which leads to a set value of the population attributable risk. We consider how, starting with the same population attributable risk, the number of variants, their frequency and their relative risk affects the C-statistic. We find that for a simple 1 gene model, the optimal C-statistic for any given population attributable risk is usually when the high risk allele is at a frequency of 0.1 - 0.2. We find that even for genes with high population attributable risk (0.5), the C-statistic is often low (<0.6). The framework that we develop can be used to judge the predictive utility of single gene and multi-gene tests for risk prediction.

2032/W

Smarter clustering methods for high-throughput SNP genotype calling. *E. Feingold[1,2], Y. Lin[2], G. Tseng[2], L.J.H. Bean[3], S.L. Sherman[3].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Biostatistics, Univ Pittsburgh, Pittsburgh PA; 3) Dept Human Genetics, Emory University, Atlanta GA.

Many different SNP genotyping technologies are now in common use. Most use clustering methods to "call" the SNP genotypes, but standard clustering methods are not optimal in distinguishing the genotype clusters of a SNP because they do not take advantage of a number of specific features of the genotype calling problem. In particular, prior information about the distribution of the measurements for each cluster can be used to choose an appropriate model-based clustering method and can significantly improve the genotype calls. Furthermore, when family data are available, pedigree information can be used to call all genotypes for a family together. We propose two new methods to call genotypes using family data. The first method is a modification of the K-means method. The second is a likelihood-based method that combines a Gaussian or beta mixture model with a pedigree likelihood. We compare the performance of these methods using simulation studies and demonstrate them on real data. We show that incorporation of external information can improve genotype calls even for "good" data. We also demonstrate the extension of our algorithm to calling genotypes for trisomic individuals.

2033/W

On Summarizing and Modeling Higher Order Linkage Disequilibrium Patterns. *S. Feng[1], Z-B. Zeng[2], B. Weir[3].* 1) Biostatistics & Bioinformatics, Duke University, Durham, NC; 2) Bioinformatics, North Carolina State University, Raleigh, NC; 3) Biostatistics, University of Washington, Seattle, WA.

Studies on multi-loci linkage disequilibrium (LD) patterns are important for population genetic research and gene-disease association mapping. A novel statistical method is developed to measure the complexity of higher order LD structures among multiple single nucleotide polymorphism (SNP) markers on a dense map. Derived from a multi-order Markov Chain model, this method uses the order of Markov Chain as a quantity to estimate the order of LD and summarize general LD structures along chromosomes. Based on the new method, complicated LD structures can be decomposed into multiple constraints, with each interpreted exactly as functions of conventional LD parameters. As a by-product of the novel approach, a new three-locus LD measure emerges naturally. It is defined similarly to the widely used three-locus LD measure, but sensitive to the order of the three loci on the chromosome. Some statistical properties are investigated by simulation studies. To illustrate the power and effectiveness, the proposed method is applied to re-analyze two published data sets.

2034/W

Visual Analytics: A Novel Approach for Mining Inbred Population Pedigrees. *C. Fuchsberger, C. Pattaro, P.P. Pramstaller.* Institute of Genetic Medicine, Bolzano, Italy.

To study inbred populations is a promising approach to identify disease susceptibility genes. In such populations, mining the very complex genealogies is a major challenge. Existing approaches keep statistical analysis and visualization step separate and, for computational issues, often focus only on sub-pedigrees. Visual Analytics (VA) is a technique combining Human's outstanding visual capabilities with the power of analytical methods to support the knowledge discovery process. We developed a novel, VA-based approach consisting of four steps: 1-ANALYSE FIRST. Application of pre-processing steps, such as, normalization and clustering. To preserve the hierarchical structure results of the clustering step were integrated into the pedigree drawing algorithm. 2-SHOW THE IMPORTANT. The inclusion of qualitative/quantitative information depends on the study goals. By using distortion techniques like Fish-Eyes, user focuses on details by preserving the global structure. 3-ZOOM, FILTER AND RE-ANALYSE. Since identifying family clustered diseases, risk factors and heritability patterns is an exploratory process (EP), dynamic queries were integrated. 4-DETAILS ON DEMAND. During the EP additional information is required: static data is retrieved from a data repository; dynamic information, such as the connection path between individuals, is calculated on the fly. We used the novel approach on 3 Italian population isolates (whole genealogy including 50,037 subjects; ~960 qualitative/quantitative traits available for 1175 subjects) to assess which disease combinations were common and tend to group in families. We discovered clusters at the population level. Identification of nuclear family disease clusters required deeper pedigree explorations. Several paths between different clusters were identified during the EP. Working with the whole genealogy missing and incomplete phenotypical and genealogical data could be partially inferred by human perception. VA does not quantify numerically the influence of the various factors; however, it allows the exploration of several hypotheses.

2035/W

Choosing relatives for missing person identification by DNA typing. *J. Ge[1,2], R. Chakraborty[1].* 1) Dept Biomed Eng, Univ Cincinnati, Cincinnati, OH; 2) Ctr Genome Information, Dept Environmental HIth, Univ Cincinnati, Cincinnati, OH.

Over the past two decades use of DNA forensics in criminal and civil investigations established it as a reliable tool for personal identification. Concerns have recently shifted on developing an infrastructure of DNA-based identification of war victims in mass graves, missing soldiers or military personnel from past wars, missing person from mass disasters caused natural catastrophes or terrorism acts, etc. When direct reference samples (i.e., antemortem samples) from missing individuals are not available, identifications are based on ranking of likelihood ratios constructed from comparison of DNA profiles of remains of presumed missing person with reference sample of family members, one or multiple of them at a time. A novel method based on the classical Elston-Stewart algorithm is developed for personal identification with autosomal markers. This method jointly considers DNA profiles from all available family members and missing persons are identified by ranking the pedigree likelihood ratios with alternative hypotheses (e.g., the missing person is unrelated to the family members of the pedigree) for all putative pedigrees. In general, the more relatives are typed, the better precision is obtained in identification. However, to reduce cost and increase efficiency, it is more economical to sample and type the most informative relatives. To determine which and how many relatives should be typed, we selected the most informative relatives (e.g. parent, child, full sib, etc.), and ranked the information content of different combinations of relatives (e.g. both parents, two children, two full sibs, etc.) to make recommendations on the type and number of relatives for identification. Simulation study shows that, with single reference sample, informativeness of relationship increase with coefficient of kinship; but single second-degree relative reference sample (e.g. half sib and grandchild) is not reliable for identification. If two reference samples were to be chosen, with both parents typed the likelihood ratio generally ranks the highest. References with at least one parent or child typed are recommended. (Research supported by grant NIH-GM-41399 (to RC).

2036/W

Estimation of allelic frequencies and inbreeding coefficient. *R. He, R. Chakraborty, M. Rao.* Center for Genome Information, University of Cincinnati, Cincinnati, OH.

Estimating allele frequencies and inbreeding coefficient in the case of a bi-allelic gene is simple and fairly routine. When we move away from the bi-allelic case to multi-allelic case, formidable problems crop up. In this presentation, we will outline how a multi-allelic problem can be solved by focusing on solving a series of associated bi-allelic problems. Special emphasis will be placed on the tri-allelic case. Tests are developed that the inbreeding coefficient model is valid for the problem on hand.

2037/W

Comparing the power of discordant sib pairs study and case-control association study. *Q. Long[1], Q. Zhang[1], J. Ott[1,2].* 1) Chinese Academy of Sciences, Beijing Institute of Genomics, Beijing, Beijing, China; 2) Laboratory of Statistical Genetics, Rockefeller University, NY, USA.

Population-based association studies are commonly used to map genes. The statistical analysis may be susceptible to false positive results because of population stratification. Several methods that use family-based controls have been proposed, e.g., the transmission-disequilibrium test, discordant sib pairs and affected family-based controls. Such tests have fewer false-positive results produced by population stratification. However, the power of such methods may be lower than ordinary case-control studies. To quantitatively describe power differences of these approaches, we simulate affected and unaffected data to calculate the p-values of both case-control study and discordant sib pares (DSP) study. In each round of the simulation, we first fix the model (dominant, recessive, additive) and parameters of the population prevalence and penetrance. Then we perform the analysis to find the significance level (p-value) of identifying the corresponding gene. By repeating the process of data simulation and analysis many times, we compare the power of DSP and traditional case-control study. We found results as follows: When we fix parameters but let the penetrance of the genotype DD (D = disease allele) change, in the dominant model, the power of DSP is slightly smaller than case-control; but in additive and recessive models, the difference increases markedly as the penetrance decreases.

2038/W

PCAtag: Software for Selecting Tagging-SNPs using Principal Component Analysis. *N. Naiman[1], G.B. Christensen[1], J. Wong[1], C. Teerlink[1], A. Thomas[1], B.D. Horne[1,2], N.J. Camp[1].* 1) Genetic Epidemiology Division, Department of Biomedical Informatics, University of Utah, Salt Lake City, UT; 2) Cardiovascular Department, LDS Hospital, Salt Lake City, UT.

To be able to comprehensively test the role of candidate genes in association studies the selection of informative SNPs is paramount. Specifically, it is important to select tagging-SNPs (tSNPs) that represent a large portion (>90%) of the genetic variation of a gene. Here we describe a new software tool, PCAtag, that performs tSNP selection using principal component analysis (PCA) as described in Horne and Camp (2004). The Horne-Camp method has two steps. In step 1, linkage disequilibrium (LD) groups are identified. In step 2, tSNPs are selected. The advantage of PCA analysis for tSNP selection is that LD groups do not need to be contiguous and can be overlapping. This flexible framework does not impose over-simplified assumptions on the genetic architecture structure, and likely fits reality much better. PCAtag is written in JAVA and is freely available. The input is genotype, and, optionally phenotype, data. The tagging process can be performed using the genotype data (assuming an additive model for alleles) or haplotype data. For the haplotype option, phases are estimated from the genotype data using expectation-maximization (EM), and haplotypes are then used in the tagging process. The software GCHAP (Thomas 2003) is used to perform the EM procedure. The PCA procedures within PCAtag are performed in R. If phenotype data are entered, the user can opt that the tagging is performed in the cases and controls separately, as well as together. This is an important and novel feature. If the genomic structure in diseased individuals is significantly different to the general population -as is likely the case for some underlying modes of inheritance- tSNPs chosen from cases and controls may differ substantially. This must be known at the selection stage to select appropriate SNPs. The output from PCAtag includes both the LD groups and the tSNPs suggested, with additional tabulated data, such as factor loadings. The accuracy of PCAtag was compared to PCA analyses performed by-hand using SAS and SPSS.

2039/W

Identifying maximally unrelated individuals in population isolates using simulated annealing. *C.I. Sandefur[1], J.D. Douglas[1,2].* 1) Bioinformatics, University of Michigan; 2) Department of Human Genetics, University of Michigan.

When making genetic inferences in population isolates, e.g., testing levels of linkage disequilibrium in the context of a family-based study, it is often useful to identify a maximal set of unrelated individuals. This is a combinatorial optimization problem that belongs to the class of NP-complete problems. Such problems have deterministic solutions that are conjectured to increase in complexity at an exponential rate in n. If n is the number of individuals, then identifying a maximal set of unrelated individuals requires examining 2n subsets. Although Martin et al. (2003) proposed some reduction techniques to address this specific problem, their approach does not address the related problem of identifying a set of maximally unrelated individuals. Because individuals from population isolates typically share one or more recent, common ancestors, the kinship coefficient between any two individuals is usually non-zero, i.e., no two individuals are unrelated. To identify a set of maximally unrelated individuals, we implemented and evaluated simulated annealing (Kirkpatrick et al. 1983 and Cerny 1985). Simulated annealing is a general-purpose algorithm for solving difficult combinatorial optimization problems and is especially appropriate for finding the global minimum of a cost function that may possess several local optima. It works by emulating the physical process whereby a solid is slowly cooled in stages until it reaches a minimal energy configuration. In the current context, we define and examine several cost functions, including the average and maximum kinship coefficient conditional on the known genealogy connecting a set of individuals. We evaluate our method for a variety of parameters, including the initial temperature, cooling schedule, and stopping condition, and neighborhood structures. Finally, we illustrate our method on data from a genetic study in the Old Order Amish of Lancaster County Pennsylvania, a population isolate derived from a modest number of founders. Preliminary data suggest that our implementation of simulated annealing performs reasonably well.

2040/W

MendelPro: Software for Genetic Datasets to Simplify Project Management, Pedigree Drawing, and the Interface to Statistical Analysis & Graphing. *R. Sripracha, D.H. Alexander, E.M. Sobel, J.C. Papp.* Human Genetics, University of California, Los Angeles, Los Angeles, CA.

MendelPro is a new genetic project management tool with a novel, fast pedigree drawing algorithm. MendelPro's graphical tools are designed to streamline statistical genetic analysis. MendelPro includes an embedded database designed to handle very large genetic datasets, including dense genome-wide SNP datasets. The program can be used to create data either through a graphical interface or spreadsheet format. Data can also be imported and exported in standard formats. In addition to its utility as a data repository and project management tool, MendelPro is a front-end to the Mendel statistical analysis package. Mendel can perform all standard, and many unique, statistical genetic analyses, currently including 22 analysis categories. The results of Mendel's analyses are stored in the database, associated with the underlying data and models.

MendelPro's pedigree drawing procedure uses extensions of the Sugiyama layout heuristics from the graph-drawing branch of computer science. These extensions, designed to handle pedigree-specific issues, help optimize the pedigree-drawing layout. Additional procedures are used to improve the aesthetics of the drawing. The entire process is low order quadratic in the number of individuals. The final drawing does not use duplicated individuals, even for inbred pedigrees. The drawing can be viewed in standard or mating-node form, and follows the Pedigree Standardization Task Force nomenclature. Traits and phenotypes can be displayed within the drawing. Haplotypes determined by Mendel, or other software, can also be displayed with the pedigrees. These haplotypes are color optimized for visualization, and may be printed or exported for presentation or publication.

MendelPro currently runs under Windows. MacOS and Linux versions will be available. MendelPro is designed as a multi-user system for laboratory-wide consistency and access. Enterprise-level systems, with an external database, are under construction. Flash-based demonstration videos and example output can be viewed at http://mendel.genetics.ucla.edu.

2041/W

A genotype calling algorithm base on Dual Gaussian Mixture Modeling and Hardy-Weinberg Law. *Y. Wang[1], W. Fu[1], L. Jin[1,2].* 1) Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, China; 2) CAS-MPG Partner Institute for Computational Biology, SIBS, CAS, Shanghai, China.

Automated genotype calling is important in high-through genotyping. Though different genotyping system generate different types of raw data, it is possible to transform these raw data to one informative classification variable respectively. We show that Gaussian distribution which is used by many current algorithms for automated genotype calling does not work well enough. We develop an EM algorithm for genotype calling using Dual Gaussian Mixture Model for estimating probability density. Our algorithm also integrates Hardy-Weinberg Law which is useful for fine tuning the classification. We apply our algorithm on 5 problematic datasets from most-used genotyping platforms using sequencing data as the gold standard. Our result is approximately consistent with the sequencing result and outperforms the calls using the software provided by the venders of these genotyping platforms.

2042/W

Haplotype Inference for Tightly Linked Markers from Large Pedigrees in the Presence of Recombinants. *K. Zhang, Y. Yoo.* Dept Biostatistics, Univ Alabama at Birmingham, Birmingham, AL.

Haplotype inference plays an important role in association studies because haplotype based analysis can provide additional power for gene mapping and haplotypes of diploid individuals cannot easily be acquired. The availability of a large number of tightly linked markers and the presence of missing data pose daunting challenges for haplotype inference from pedigrees. Many methods have been developed for haplotype inference from pedigrees, but they are not suitable for tightly linked markers in the presence of recombinants. Some likelihood-based methods, such as HAPLORE and ZAPLO, assume that no recombination occurs within a pedigree. The other likelihood-based methods, such as GENEHUNTER, Merlin, and Simwalk2, allow for the recombinants but assume linkage equilibrium between alleles among adjacent loci or blocks, which is inappropriate for tightly linked markers. Several rule-based methods have proposed to identify all compatible haplotype configurations with the minimum number of recombinants but most of them fail to provide reliable estimation of haplotype. We propose an EM algorithm incorporating the rule-based algorithm and the haplotype elimination algorithm for haplotype inference in general pedigrees. The algorithm does not assume the linkage equilibrium among markers and can handle pedigrees with recombinants. It can be outlined as the follows: 1) Apply a set of logic rules to identify compatible haplotypes; 2) Perform the haplotype elimination algorithm and only keep the haplotype configurations with the minimum number of recombinants; and 3) Perform the EM algorithm to estimate the haplotype frequencies based on haplotype configurations identified in step 2; only haplotypes with the frequency greater than a threshold will be retained. The partition-ligation technique is implemented to handle large number of markers. We evaluate its performance and compare it with several available methods for haplotype inference from general pedigrees through simulated haplotypes of tightly linked markers based on real pedigrees. Our results indicate that our method outperforms other methods.

2043/W

Combining the effects of IBD and association in genetic case-control studies. *Q. Zhang[1], Q. Long[1], J. Ott[1, 2].* 1) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, Beijing, China; 2) Laboratory of Statistical Genetics, Rockefeller University, NY, USA.

Lander and Botstein's homozygosity mapping is a well accepted method in pedigree linkage analysis. As an extension of it, Broman and Weber proposed a statistical analysis that discriminates between autozygosity and allozygosity. As recent studies show, the human founder population was relatively small so that even in outbred populations we can find long segments of homozygosity. Here we propose to us this approach in case-control studies by focusing on genome-wide sets of SNPs. We derive statistically appealing criteria for the length of stretches of autozygosity by connecting scan statistics with homozygosity mapping. Previous approaches either looked only at homozygosity without paying attention to IBD or worked with fixed lengths of windows of markers, for which average homozygosity was determined. Our test statistic appears more powerful than the conventional genotype and allele tests.

2044/W

Low correlation among association tests for quantitative traits. *J.E. Herrera-Galeano[1,2], R.A. Mathias[2], H. Sung[2], N. Faraday[1], D.M. Becker[1], L.C. Becker[1], A.F. Wilson[2].* 1) Dept of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD; 2) Genometrics Section, NHGRI, NIH, Baltimore, MD.

Background: Recently, there has been a dramatic increase in the amount of genotyping data available for testing for association with quantitative traits. Several different methods of testing for associations are available; these methods use different kinds of information and have different strengths and weaknesses with respect to their statistical properties. **Objective:** To determine the pair-wise correlations among the following methods: ASSOC (SAGE v4.6.1), FBAT v1.7.3, GEE (SAS v8.0) and ROMP v0.2. **Methods:** Levels of 24 traits related to platelet aggregation were measured before and after 2 weeks of daily doses of 81 mgs of aspirin (ASA) in 541 African Americans and 955 Caucasians, in 155 and 264 families, respectively. Genotypes were determined for 2638 SNPs in 191 candidate genes using the Illumina Golden Gate platform. Tests of association were performed with each of the 4 methods in each ethnic group for each trait (506,496 total tests). Pair-wise Pearson product moment correlations were calculated, as were McNemar chi-squares categorizing p-values as significant ($p \leq 0.001$) or not significant. **Results:** Pair-wise correlations between the methods were evaluated only on those tests that returned a result for all four methods (57,018). The Pearson correlations were <0.14 for all pair-wise comparisons of these four methods. Furthermore, the pair-wise comparisons of the methods with the McNemar chi square tests were significant $p \leq 0.001$ for all pairs except ROMP-FBAT. **Conclusions:** Our results indicate that there is little correlation between the four tests of association for quantitative traits. In the absence of a consensus across association methods, the method that uses the most information should be given the greatest weight. In this case, a test of association in two and three generation family data, ASSOC, a likelihood based method that includes phenotype and genotyping information on all family members makes the fullest use of the available information.

2045/W

PRESTO: Rapid calculation of order statistic distributions and multiple-testing adjusted p-values via permutation for one- and two-stage genetic association studies. *B.L. Browning.* Nutrigenomics New Zealand and Department of Statistics, The University of Auckland, Auckland, New Zealand.

Genome-wide association studies are now being performed with hundreds of thousands of markers genotyped on thousands of individuals, yet disease-associated variants with sufficiently low frequency and/or modest effects may still remain undetected by these large-scale studies. When there are multiple independent weakly-associated variants there may be significantly more markers with p-values below some threshold than expected by chance, even when no single p-value is significant after adjusting for multiple testing.

The k-th order statistic is the k-th largest test statistic, and the distributions of order statistics can be used to test whether the top ranked markers have lower p-values than expected by chance. PRESTO uses permutation of the trait status to calculate the empirical distribution of order statistics for one- or two-stage genotyping designs under the null hypothesis of no disease-associated markers. These distributions can be used to calculate the statistical significance of any statistic that is a function of order statistics (e.g. rank-truncated products [1]), and can be used to determine the number of top-ranked markers to test in a second-stage experiment.

PRESTO can analyze a large whole-genome association study in a few hours of computing time, can perform any combination of allelic tests and genotypic tests (recessive, dominant, or overdominant), and can test both single markers and haplotype clusters identified by BEAGLE [2]. PRESTO is well-documented, easy-to-use, and freely available at http://stat.auckland.ac.nz/~browning/presto/presto.html.

[1] Dudbridge F, Koeleman BP. Rank truncated product of P-values, with application to genomewide association scans. Genet Epidemiol 2003;25:360-6.

[2] Browning BL, Browning SR. Efficient multilocus association mapping for whole genome association studies using localized haplotype clustering. Genetic Epidemiology 2007. In Press.

2046/W

Missing call bias in genome-wide association studies. *W. Fu[1], Y. Wang[1], L. Jin[1,2].* 1) Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, China; 2) CAS-MPG Partner Institute for Computational Biology, SIB, CAS, Shanghai, China.

The advent of high-throughput and cost-effective genotyping platforms have allowed genome-wide association studies a reality. While the primary focus has been invested upon the improvement of reducing genotyping error, the problems associated with missing calls are largely overlooked. To probe into the effect of missing calls on genome-wide association studies, we first show that missing call bias is a universal and important problem in genome-wide association studies using four technologies (Affymetrix 500K SNP array, SNPstream, Taqman, and Illumina Beadlab). We will show that missing call bias changes the allele frequency and leads to false conclusions. In particular, the influence of missing call bias is more serious than genotyping error, especially when alleles are relatively rare.

2047/W

Log-multiplicative models of haplotype-haplotype interaction in case-only studies. *T.S. Price.* ITMAT, University of Pennsylvania School of Medicine, Philadelphia, PA.

Case-only designs provide a powerful means to detect departures from multiplicative risk deriving from independent genetic or environmental factors. The results of case-only studies, however, are typically interpreted in terms of odds ratios for binary risk factors even when the measured risk factors have more than 2 categories. In particular, methods for detecting interactive effects between multilocus genotypes have not yet been implemented. I present a simple method using log-multiplicative models to assess the nonindependence of nominal or ordered risk factors. Log-multiplicative models have greater power than standard likelihood ratio or goodness of fit chi-square tests of independence when the contingency data approximate a latent class model with two classes, as is the case for unlinked haplotypes indexing a true epistatic interaction. Simulations are presented that attest to the power of the method across a range of parameters. The method generalizes to stratified analyses and cross-classifications of dimension greater than 2.

2048/W

Methods to Impute Missing Genotypes for Population Data. *Z. Yu[1], D.J. Schaid[2].* 1) Dept Statistics, University of Irvine, Irvine, CA; 2) Division of Biostatistics, Mayo Clinic, Rochester, MN.

For large scale genotyping studies, it is common for most subjects to have some missing genetic markers, even if the missing rate per marker is low. This compromises association analyses, with varying numbers of subjects contributing to analyses when performing single-marker or multi-marker analyses. In this paper, we consider eight methods to infer missing genotypes, including two haplotype reconstruction methods (local expectation maximization-EM, and fastPHASE), two k-nearest neighbor methods (original k-nearest neighbor, KNN, and a weighted k-nearest neighbor, wtKNN), three linear regression methods (backward variable selection, LM.back, least angle regression, LM.lars, and singular value decomposition, LM.svd), and regression tree, Rtree. Their accuracies were evaluated under a variety of conditions and parameters. Our results indicate that LM.lars had the lowest error rates across different samples. LM.back and fastPHASE gave slightly less accurate estimate of missing genotypes than LM.lars, but both had better performance than the other methods. Our results suggest that either fastPHASE or LM.lars should be used to impute missing marker genotypes.

2049/W

Effects of parameters of microsatellite loci on the distribution of the imbalance index for detecting past demographic changes of population size. *R. Chakraborty, R. Deka.* Ctr. Genome Information, Univ Cincinnati, Cincinnati, OH.

With multiple segregating alleles at any microsatellite locus, designated by repeat sizes, several statistics provide information about the same composite parameter that dictates the pattern of their allele frequency distributions. For example, under the stepwise mutation model, expectation of gene diversity and allele size variance at such loci are both functions of the same parameter, $\theta = 4Nv$, where N is the effective population size (constant over generations) and v, the mutation rate. The ratio of the estimate of θ from allele size variance, divided by that from gene diversity, is defined as the imbalance index (β). A deviation from the constancy of population size makes the expectation of β different from 1 (i.e., expected $\ln\beta \neq 0$). While estimation of imbalance index has been worked out, here we performed empirical evaluations of the null distribution of $\ln\beta$ to examine how it is influenced by number of loci (L), mutation rate (v), and sample size (n). Coalescence-based simulations were used to generate allele frequencies of a number (L) of microsatellite loci, each from a population under mutation-drift equilibrium (with constant effective size of N, and mutation rate v). The number of alleles sampled (n) was another simulation parameter. Of the various parameters affecting the null distribution of $\ln\beta$, the number of loci (L) appears to have the largest effect. Imbalance index, estimated from a small number of loci, produces asymmetry in the distribution of $\ln\beta$, making observed values of β less than 1, or $\ln\beta < 0$, more frequent, under the constant population size model. For detecting signatures of past bottleneck or recent population expansion, 50 to 100 loci are recommended. In contrast, variations in sample size (n) and/or mutation rate (albeit, θ) have little effects on $\ln\beta$, particularly when $n > 50$, and/or $\theta \geq 1$. A computer routine for generating the confidence interval for the null distribution of $\ln\beta$ is freely available in our web-site (www.cgi.edu). As an example, we obtained the confidence interval of $\ln\beta$ in the Samoan population to detect evidence of recent expansion of this population. (Supported by NIH grant GM41399).

2050/W

Robust methods for QTL linkage analysis in nuclear families. *S. Bhattacharjee[1], C. Kuo[2], N. Mukhopadhyay[1], G.N. Brock[3], D.E. Weeks[1,2], E. Feingold[1,2].* 1) Dept of Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Dept of Biostatistics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 3) Dept of Bioinformatics and Biostatistics, Univ of Louisville, Louisville, KY.

Variance component (VC) based score statistics for linkage mapping of quantitative traits have been proposed by a number of authors. Apart from being computationally simple, these methods offer robustness to ascertained sampling and non-normality of traits, while preserving the power of the traditional likelihood ratio based VC approach. As a result, they have received substantial theoretical attention and different variations and extensions have been proposed. However, these methods have not been applied to real data as frequently as they should be, mostly due to the fact that some practical implementation issues have not been addressed adequately. In this study we summarized and classified the existing score statistic variants based on their theoretical properties and proposed some new variants that are theoretically expected to have improved performance. We also compared the statistics in terms of robustness of type I error and power using comprehensive simulations. We addressed various practical issues such as choice of denominators, effect of selection, effect of incorporating dominance and sensitivity to mis-specified trait parameters. Our study included standard regression-based statistics such as that implemented in the software *merlin-regress* (Sham *et al*, 2002), as well as the recently proposed GEE-based higher moment statistics (Chen *et al*, 2005). In some cases, our proposed variants of these statistics gave significant improvements over the original versions. Based on our simulation study, we formulated guidelines for choosing powerful and robust score statistic variants in different practical scenarios.

2051/W

Incorporating Quantitative Covariates into Multipoint Linkage Mapping Using Affected Relative Pairs. *Y. Chiu[1], J. Chiou[2], C. Lee[1].* 1) Biostatistics & Bioinformatics, National Health Research Inst, Zhunan, Miaoli, Taiwan; 2) Institute of Statistical Science, Academia Sinica, Taiwan, R.O.C.

Many dichotomous traits for complex diseases are often associated with quantitative covariates or traits. Incorporating these quantitative variables into linkage mapping could greatly improve the efficiency and precision in disease locus localization. Previously, we proposed a robust multipoint Identity-by-Descent (IBD) approach to estimate a disease locus using affected sib pairs with incorporation of a quantitative covariate (Chiou et al., 2005). In the present study, we studied the relative efficiency of estimating a disease locus with and without incorporating a quantitative covariate through a systematic simulation study. The quantitative covariate could be a quantitative trait associated with the disease through different genetic mechanisms. The information about the relative efficiency under different genetic models is also helpful to elucidate the relationship between a quantitative trait and the disease. We examined the relative efficiency of estimating a disease locus under a variety of pleiotropy and co-incident models of a quantitative trait. Further, we extended this approach to different types of affected relative pairs (ARPs). This extension allows us to account for heterogeneity in risk ratios for different ARPs when conducting linkage mapping. Different types of ARPs may provide some insight to the underlying genetic model of a disease. The collaborative study on the genetics of alcoholism (COGA) data released for GAW14 was used to illustrate the application of this extended method. We showed the efficiency was enhanced by using affected relative pairs than using affected sib pairs after incorporating the quantitative variable "maximum number of drinks in a 24 hour period" into the linkage mapping. This approach could also be applied to incorporate an additional categorical covariate using affected relative pairs. By applying this method to the same data set, we demonstrated the efficiency improvement in estimating the disease locus by incorporating "smoking status" into the linkage mapping using affected relative pairs.

2052/W

Ignoring intermarker linkage disequilibrium induces false-positive evidence of linkage for consanguineous pedigrees when genotype data is missing for any pedigree member. *S.M. Leal, B. Li.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Missing genotype data can increase false-positive evidence for linkage when either parametric or nonparametric analysis is carried out ignoring intermarker linkage disequilibrium (LD). Previously it was demonstrated by Huang et al (2005) that no bias occurs in this situation for affected sib-pairs with unrelated parents when either both parents are genotyped or genotype data is available for two additional unaffected siblings when parental genotypes are missing. However, this is not the case for consanguineous pedigrees, where missing genotype data for any pedigree member within a consanguinity loop can increase false-positive evidence of linkage. The false-positive evidence for linkage is further increased when cryptic consanguinity is present. The amount of false-positive evidence for linkage and which family members aid in the reduction of false-positive evidence of linkage is highly dependent on which family members are genotyped. When parental genotype data is available, the false-positive evidence for linkage is usually not as strong as when parental genotype data is unavailable. For a pedigree with an affected proband whose first-cousin parents have been genotyped, further reduction in the false-positive evidence of linkage can be obtained by including genotype data from additional affected siblings of the proband or genotype data from the proband's sibling-grandparents. For the situation when parental genotypes are unavailable, false-positive evidence for linkage can be reduced by including in the analysis genotype data from either unaffected siblings of the proband or the proband's married-in-grandparents.

2053/W

Power of measured genotype-based association analysis, conditional on a variance components-based linkage model, in related individuals. *H.H.H. Goring, J.W. Kent Jr., V.P. Diego, T.D. Dyer, J. Blangero.* Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Linkage analysis is often the initial step for gross localization of genetic factors influencing complex traits. Once a linkage region has been identified using a sample of families, it is generally advantageous, for a variety of reasons, to pursue fine mapping by LD analysis in the same sample, rather than to collect a new sample of unrelated individuals for this step. A complication in any family-based LD analysis method is that the relatedness of individuals must be properly modeled. In penetrance model-based analysis, it is conceptually straightforward to do this and conduct LD analysis conditional on the existence of linkage. In popular alternative statistical approaches to gene mapping, such as variance components-based analysis, it is much more difficult to separate the linkage and LD components. We have previously shown (Kent Jr. *et al.* 2007 Genet Epidemiol 31:173) that measured genotype analysis within variance components-based pedigree analysis is a valid approach to test for association in related individuals, as long as a QTL linkage component is included in the model to account for the non-independence of family members at that point in the genome. Here, we have used analytical and simulation-based methods to examine the power of such an approach as a function of the family size/structure, the strength of evidence of linkage, the degree of linkage disequilibrium and the effect size of a functional variant. We observe that the power to detect association is diminished in a sample of related individuals versus a sample of unrelated individuals of the same size. However, using all individuals in the association analysis while simultaneously accounting for their overall and pointwise genetic similarity is much more powerful than using only unrelated individuals in the association analysis. The general implication is that the power to detect association in a sample of related individuals previously used for linkage analysis can be substantial, even if the number of unrelated individuals embedded in the families is small.

2054/W

Models, Test Statistics, and Designs for Genetic Association Studies with Pooled Genotyping. *S.Y. Cheong[1], E. Feingold[2,1].* 1) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

Although most genetic association studies use individual genotyping, it is possible that many studies can be performed more efficiently using pooled DNA, particularly at the initial screening stage. Pooled studies are challenging, however, because there are unresolved issues of how to incorporate pooling error into the designs and test statistics. We develop several models for the bias and variance introduced by pooling and then use those models to consider optimal case-control test statistics and designs. We consider several different case-control study designs with the same chip number for each cohort group, in order to find out which design is most powerful. In addition, we consider designs that incorporate covariate information. We investigate test statistics for each design theoretically, and also verify the results with simulation studies.

2055/W

Assessing tSNP selection: neutral versus disease-based discovery panels. *K. Curtin, N.J. Camp.* Genetic Epidemiology, Univ. of Utah, USA.

Discovery panels for tSNP selection are generally population-based, chosen without regard to disease status. However, a diseased population will contain susceptibility alleles at higher frequencies than the general population which may result in unique LD structure and haplotypes, and influence tSNP selection. Using simulation, we investigate the limitations of relying on neutral panels for tSNP selection. 100,000 chromosomes of 20kb sequence were generated under a coalescent model using the program ms (Hudson 2002), assuming a constant diploid population, per-base mutation rate of 10^{-8} and recombination of 1cM/Mb. A variant position was selected as the disease SNP (dSNP). Diploid individuals were created by random sampling with replacement and disease status was generated using a multiplicative genetic model allowing for sporadics. Three single locus models were examined: common, low-penetrance (MAF 0.20); less common, modest-penetrance (MAF 0.05); and rare, high-penetrance (MAF 0.01). Diseased individuals were randomly selected for disease panels and individuals independent of disease were selected for neutral panels of size 100, 50 or 25. From each panel, tSNPs were selected using a principal components method (Horne and Camp, 2004), and the highest correlation between tSNPs and the dSNP was determined using pairwise r^2. This was repeated 1000 times. Mean r^2 for the highest correlation in diseased and neutral panels did not significantly differ under models 1 and 2. However, for rare, high-penetrance disease, neutral panels exhibited significantly lower mean r^2 that declined rapidly with decreasing panel size (neutral vs. disease panel: 0.7 vs. 0.8, n=100; 0.4 vs. 0.7, n=50; 0.2 vs. 0.5, n=25). This preliminary investigation suggests that there are limitations to selecting tSNPs from neutral panels, particularly for rarer predisposition variants. The next step is to assess the subsequent decrease in power in association analyses using the lower correlated neutral tSNPs and to further determine the extent of these limitations for more sophisticated genetic models. The extent of any loss of power could have profound effects on the utility of resources that are based on neutral data (e.g. HapMap, NIEHS SNP Program).

2056/W

The Statistical Equivalent Of The Binary TDT For Quantitative Traits. *S. Ghosh.* Human Genetics Unit, Indian Statistical Institute, Kolkata, India.

The classical Transmission Disequilibrium Test (TDT) for binary traits proposed by Spielman et al. is a family-based alternative to population-based case-control studies and circumvents the problem of population stratification as it tests for allelic association in the presence of linkage. However, since the clinical end-point traits are often defined by quantitative precursors, it has been argued that it may be a more prudent strategy to analyze the quantitative phenotypes without dichotomizing them into binary traits. The paradigm of linkage disequilibrium in the context of quantitative traits is not straight forward and methods have generally considered the intuitive concept of differences in allelic frequencies between individuals having high values of the quantitative trait and those with low values of the trait as evidence of linkage disequilibrium between the marker locus and the QTL. Although some methods have been developed for testing transmission disequilibrium in the context of quantitative traits, these are not direct extensions of the classical TDT. We propose a simple logistic regression based test which can be analytically shown to be statistically equivalent to the TDT for binary traits, and hence is not susceptible to the presence of population stratification in the data. We perform Monte-Carlo simulations under a wide spectrum of disease models and varying parameter values of linkage disequilibrium to evaluate the power of the proposed procedure. We find that similar to the binary TDT, the power decreases with increase of dominance and decrease of heterozygosity at the QTL. We apply our method to analyze an alcoholism related phenotype from the Collaborative Study on the Genetics Of Alcohism (COGA) project. The endophenotype defined as the number of externalizing symptoms associated with anti-social behavior has exhibited significant evidence of linkage on Chromosome 4 in the alcohol dehydrogenase (*ADH*) gene cluster. We find significant association between the quantitative phenotype and a biallelic marker in the *ADH1C* gene.

2057/W

Impact of data synthesis on the power and stability of association analysis: joint analysis of family and case-control data with a moving window approach. *C. Gray-McGuire, R.C. Elston, Q. Lu.* Dept Genetic Epidemiology, Case Western Reserve Univ, Cleveland, OH.

As more and more genome-wide and candidate gene association studies are conducted, two primary challenges to the analysis and interpretation of such data have emerged: 1) how can we obtain the large sample sizes vital to the detection of the small effects likely to characterize complex disease and 2) will the analysis of a large number of single nucleotide polymorphisms (SNPs) individually be sufficient to detect these effects? Attempts to overcome the first challenge have focused primarily on the assembly of large case-control samples without making use of the many large collections of family data already available from previously conducted linkage studies. Strategies to address the second challenge often include estimation of haplotypes - which may lose power, particularly when evaluating genome scan data, because of the increase in degrees of freedom involved.

We present here an approach for the joint analysis of family samples, extended pedigrees and case-control data, offering a means by which to dramatically increase sample sizes, together with moving window approach to increase the power of high density SNP data. Within the context of a candidate region analysis, we demonstrate that by combining information from a family sample and population based controls, as well as by combining information from adjacent SNPs, we can obtain results that are both less significant in regions of spurious association (from p= 7.5 x 10-8 to p=0.5) and more significant in a region containing an independently identified disease susceptibility haplotype (from p=0.09 to p= 8 x 10-6). Further, we show that, even for a relatively small sample size, the Wald and likelihood ratio test (LRT) statistics are far more stable using a multilocus (Wald/LRT ratios ranging from 0.99 to 18) rather than a single locus (Wald/LRT ratios ranging from 0.95 to 56) model. Finally, we offer suggestions, based on our results, for finding the optimal window size.

2058/W

Statistical issues when multipoint linkage analysis is performed at only one position. *S.E. Hodge[1,2], L. Rodriguez-Murillo[1], L.J. Strug[1], D.A. Greenberg[1,2].* 1) Psychiatry & Biostatistics, Columbia University, New York, NY; 2) NY State Psychiatric Institute, New York, NY.

(1) Methods: Following Xing & Elston (2006; X&E), we performed simulations of multipoint linkage analysis at a single position (i.e., not maximized over position). We calculated both "lods" (i.e., analyzing the data under the correct or generating model, and "mods" (maximizing the multipoint lod scores over 18 models). These models were dominant (D) and recessive (R), with penetrances of 0.10, 0.20, ... 0.90, signified by D10, D20, ..., D90, R10, ..., R90. Data were generated under D20, D50, D80, and R20, R50, R80. Disease allele frequency was 0.01 for D models, 0.14 for R. Phenocopy rate was set to 0.001. Each simulation involved 1000 datasets; each dataset contained *n* four-child families, ascertained to have ≥2 affected children. Parental markers were fully informative, and parental affectedness was included in the analyses. *(2) For the lods:* We demonstrate by theoretical arguments, and also confirm via simulation, that as datasets become more informative, *type I error approaches zero* (unlike for usual statistical analyses). E.g., for D50 data, type I error dropped from .013 for *n*=10 to .000 for *n*=30; for R50 data, it dropped from .040 to .000. *(3) For the mods:* We demonstrate a similar pattern via simulation, though less straightforward for R than for D. For D50, type I error dropped from .045 to .007, for R50, from .040 to .024, as *n* increased from 10 to 30. We also investigate which models yielded the false positive mod scores, as did X&E, but we argue that this question is fundamentally irrelevant to analysis of complex data. *(4)* Finally, we argue that multipoint lods and mods evaluated at a single position presumably have different statistical properties than multipoint lods or mods that are maximized over position. Since most investigators maximize their multipoint scores over position, relying on results from single-position simulations is potentially misleading.

2059/W

Evaluation of the quality and quantity of DNA from buccal samples in the National Birth Defects Prevention Study. *M.M. Jenkins[1], M.L. Gallagher[1], S.A. Rasmussen[1], C. Sturchio[2], D.A. Koontz[1], P. Richter[1], S. Collier[1], M.A. Honein[1].* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Battelle Contractor to CDC, Columbus, OH.

Analysis of polymorphisms in genes encoding proteins involved in metabolism of tobacco smoke is planned as part of a study to identify gene-environment interactions in the etiology of gastroschisis and anorectal atresia. The planned study will use data from a multisite, population-based case-control study of major birth defects that includes a maternal interview and self-collection of buccal cells using cytobrushes for each mother, father, and infant. Thus far, we have performed pilot studies to better understand the quality and quantity of DNA from buccal samples collected for the multisite study of major birth defects. An initial pilot study included 41 DNA samples from the Atlanta study site. Genotyping was completed in duplicate for 20 variants from 6 *CYP* and 2 *NAT* genes using Pyrosequencing® technology. 11 of 41 samples (27%) had low DNA concentrations (<0.1ng/μl), as determined by a real-time PCR assay specific for human gDNA. Among these samples, unsuccessful PCR amplification and evidence of allele drop-out (ADO) were observed. Three variants were selected for a subsequent pilot study completed on 65 Atlanta samples. The same methodology was used except that samples were tested in quadruplicate. 25 of 65 samples (38%) had low DNA concentrations. Unsuccessful amplification and discordance between replicate results, consistent with the occurrence of ADO, were again seen almost exclusively in the low DNA concentration samples. DNA quantitation is complete on all 1,721 parent and infant samples selected for the study of anorectal atresia and gastroschisis. 255 of the 1,721 samples (15%) had low DNA concentrations and will be excluded from further study to reduce genotyping errors. The preliminary studies have contributed to a better understanding of the quality and quantity of DNA obtained from buccal samples and the correlation between DNA concentration and ADO in NBDPS samples and may be of value to other genetic epidemiology studies.

2060/W

Haplotype-based association in case-control studies - a latent variable approach. *T. Wang[1,2], H.J. Jacob[2], Z.B. Zeng[3].* 1) Div Biostatistics, Medical Col Wisconsin, Milwaukee, WI; 2) Human Molecular Genetics Center, Medical Col Wisconsin, Milwaukee, WI; 3) Bioinformatics Research Center, North Carolina State University, Raleigh, NC.

There is a growing utilization of high-dense genetic markers such as single nucleotide polymorphisms (SNPs) in genome-wide association studies to identify genetic risk factors of complex diseases. Haplotype-based association is attractive in reducing the data complexity especially for tightly linked SNPS. It may also increase the statistical power of detecting disease susceptible loci comparing with the single marker analysis under certain circumstances. However, haplotype analysis faces potential problems including the uncertainty in haplotype frequency estimates, adjustment for sampling ascertainment and multiple testing. Classical weighted logistic regression based on the prospective likelihood is flawed by the fact that it may no longer provide equivalent parameter estimates as the maximum likelihood estimates from a retrospective likelihood due to a constraint of Hardy-Weinberg equilibrium on the genotypic distribution of markers. A systematic testing procedure may also be required not only for haplotypes of the markers but also for haplotypes from a subset of the markers as well as for all marker alleles, since the status of the disease risk factor is often unknown. In the present study, we describe a latent variable method for association analysis between a disease and multiple closely linked markers with unphased genotype data from case-control studies. Based on a retrospective likelihood, the method can appropriately account for both the uncertainty in haplotype frequency estimates and the case-control sampling ascertainment. By introducing a latent variable into the genetic model to play a flexible role of a potential risk factor that may consists of a single allele or a haplotype from the whole set or a subset of the markers, the method also allows us to build a composite test for genetic association between the disease and the marker genotypes regardless of the composition of the risk factor. Simulation studies have been implemented to assess the performance of the method.

2061/W

Comparison of haplotype-tagging SNPs from two resequencing projects (HapMap and PopGen) for 70 human genes related to immune responses. *Y. Yoo[1], K. Zhang[1], J. Tang[3], A. Loraine[1], J. Edberg[4], R. Kaslow[2].* 1) Dept Biostatistics; 2) Dept Epidemiology; 3) Dept Medicine; 4) Dept Clinical Immunology & Rheumatology, Univ Alabama at Birmingham, Birmingham, AL.

SNP data from the International HapMap Project are being widely used as a reference panel for selecting SNPs for genotyping in population studies. For many genes, data obtained by re-sequencing serve as an alternative panel for haplotype-tagging SNP (htSNP) selection to provide denser coverage. We have compared the performance of htSNPs selected from HapMap and from the NIH/NIAID Population Genetics Analysis Program (PopGen) for 70 immune response genes independently re-sequenced at the SeattleSNPs facility. For each candidate gene region, we obtained SNPs with minor allele frequency (MAF > 1%); from HapMap and from PopGen and identified those SNPs that were genotyped in both projects. We compared the percent coverage ($r^2 > 0.80$) using all SNPs genotyped for both projects with the percent coverage using the htSNPs selected from all SNPs available from both projects. We also compared the number of htSNPs selected from each project and the number of htSNPs selected from both projects using MultiPop-TagSelect algorithm (Howie et al., 2006). For Caucasians, 789 SNPs were obtained from HapMap only, 2,210 SNPs from PopGen only, and 662 SNPs from both projects. For Africans, 960 SNPs were from HapMap only, 3,513 from PopGen only, and 826 from both sources. For HapMap, 87% and 92% SNPs were covered by SNPs genotyped in both projects for Caucasian and African populations, respectively. For PopGen, 54% and 40% SNPs were covered by SNPs genotyped in both projects for Caucasian and African populations, respectively. Using only htSNPs, 86% and 91% SNPs from HapMap were covered by SNPs genotyped in both projects, and 38% and 30% SNPs from PopGen were covered by SNPs genotyped in both projects, for Caucasian and African populations. These analyses indicate that the SNPs in HapMap do not capture nearly all of the htSNPs of MAF >1% in the 70 selected genes. They suggest that, when feasible, more complete coverage of htSNPs may be achieved by combining data from multiple sources.

2062/W

Detecting associations under models of complex traits prone to an effect reversal. *D.V. Zaykin[1], K. Shibata[1], L. Diatchenko[2].* 1) National Institute of Environmental Health Sciences, RTP, NC; 2) University of North Carolina at Chapel Hill, NC.

Failure to replicate a genetic association is a common problem. It has been observed that the direction of the effect in different studies may be reversed as well. Although an explanation for many of these cases is likely to be statistical in nature, it has been recently suggested that a reversal of effect (flip-flop) can be a consequence of a change in linkage disequilibrium (LD) between a causal and the observed variants. We develop a more general model, showing that a flip-flop phenomenon can be completely attributed to a change in LD only in situations when the studied variant is only a proxy marker for unobserved functional variation. More generally, a flip-flop can occur without a change in LD, or even when the LD is zero. We give specific conditions for the form of genetic effects that allow for such flip-flops. In this model, a flip-flop is driven by a shift in population haplotype or allele frequencies. Nevertheless, both the population prevalence and the allele frequency of the observed variant can be the same in two populations that exhibit a flip-flop. If all relevant variants are scored, a flip-flop can no longer take place, thus it is a consequence of partial knowledge. In the case of a quantitative trait, the unobserved variants induce a difference in the variance of the trait among individuals with different scored alleles. This suggests a statistical approach for discovering associations that is more robust to loss of power due to a genetic flip-flop.

2063/W

What's the best statistic for a simple test of genetic association in a case-control study? *C.L. Kuo[1], E. Feingold[1, 2].* 1) Dept Biostatistics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Large-scale genetic association studies genotype as many as 500,000 SNPs at a time, but analysis typically starts with univariate statistical tests (e.g. chi-squared tests, regression) of each marker individually. For example, for a case control study most people perform chi-squared tests in the initial association scan, but this can be an allele-based test or a genotype-based test. For a genotype-based test, one can use a 2 df test on the 2 x 3 table (3 genotypes), a 1 df trend test, or a 1 df test that combines the heterozygote class with the rarer homozygote class. Some studies use logistic regression instead of chi-squared tests so that covariates can be incorporated into the initial scan. This presents essentially the same options as the chi-squared test for modeling the genotype using 1 or 2 degrees of freedom, but in addition the model can involve gene-environment interactions if desired. Surprisingly little literature has compared the power of these different statistical procedures, and we have observed a huge variety of procedures used in real applications. The seminal paper by Sasieni (1997) compares the allele-based test with the genotype-based trend test, and concludes that the allele-based test is generally not recommended. However, Sasieni was for the most part considering an estimation question rather than the hypothesis-testing question that is probably more relevant to genome scans. In addition, newer ideas about what kinds of population genetic models are expected under both the null and the alternative may mean that Sasieni's conclusions should be reconsidered. In our work we review the options for a simple genetic association test in a case-control study and take a rigorous statistical approach to discovering which tests are most powerful for initial scans in genetic association studies. We consider the problem primarily in terms of which test has the highest power for a single test of association under different genetic models, but we also comment briefly on the issue of how the tests/procedures might be expected to perform when an entire genome is scanned.

2064/W

A Comparison of Single-Locus Measures of Association with Permutation Testing for Whole-Genome Association Studies. *W. Bush, S. Turner, T. Edwards, E. Torstenson, M. Ritchie.* Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN.

Whole-genome association studies present several statistical challenges. When conducting single locus analysis, correlation structures among genotypes and other multiple testing issues make controlling the experiment-level false positive rate difficult. In addition to the well-known Bonferroni correction and methods that control the false discovery rate(FDR), permutation testing is a viable technique for assessing false positives. Permutation testing randomly reassigns the affection status of individuals in a dataset to create a distribution under the null hypothesis of no association. Permutation testing was used in a recent whole-genome study of type II diabetes by Sladek et al. to allow multiple genetic model considerations for Cochran-Armitage trend test analysis (Nature 445, 881 - 885). When using permutation testing, the distributional assumptions of the statistical test used become irrelevant. In this context, we do not use the theoretical distribution of a test statistic to assign a significance value; we instead define significance using the empirical distribution generated by the permutation testing procedure. As such, alternate measures of association with uncharacterized statistical properties may prove to have higher power than traditional test statistics. In this study, we examine the computational feasibility and statistical power of multiple basic single-locus association measures in the context of permutation testing. We simulated whole-genome case-control data containing patterns of linkage disequilibrium using genomeSIMLA software, and evaluate and compare the chi-square test of association, likelihood ratio test, normalized mutual information measures, odds ratio, and the Cochran-Armitage trend test under a variety of genetic models. We will show which approaches yield improved power for genome-wide association studies.

2065/W

Increasing Power in Association Studies using Prior Information. *E. Eskin.* Dept Computer Sci, Univ California, Los Angeles, Los Angeles, CA.

The availability of whole genome human variation reference sets such as those provided by the International HapMap project provide an opportunity to incorporate various types of genomic data as prior information in genetic association studies. We present an approach for incorporating this information by revisiting how we perform multiple hypothesis correction. In a traditional association study, in order to correct for multiple hypothesis testing, the significance threshold at each marker, t, is set to control the total false positive rate. In our framework, we vary the threshold at each marker t_i and use these thresholds to incorporate prior information. We present a numerical procedure for solving for thresholds that maximize association study power using prior information. We present the results of benchmark simulation experiments using the HapMap data which demonstrate a significant increase in association study power under this framework. We provide a webserver for performing association studies using our method and provide thresholds optimized for the the Affymetrix 500k and Illumina HumanHap 550 chips.

2066/W

Recovering Unused Information in Genomewide Association Studies: A Revision of Quality Control Convention. *D. Fardo[1], C. Lange[1,2].* 1) Dept Biostatistics, Harvard School of Public Health, Boston, MA; 2) Channing Laboratory, Brigham & Women's Hospital, Boston, MA.

Although the rapid advancements in high-throughput genotyping technology have made genomewide association studies possible, these studies still remain an expensive undertaking, especially when considering the large sample sizes necessary to find the small-to-moderate effect sizes that define complex disease. It is therefore prudent to utilize all possible information contained in a genomewide scan. We propose a straightforward analytical approach to recover often unused information without sacrificing statistical validity.

Screening SNPs for Hardy-Weinberg Equilibrium (HWE) is a common quality control measure when performing genetic association studies (Gomes et al, 1999). There are at least two issues with testing for HWE in the context of genomewide association studies: the large number of tests being conducted increases the chance of observing large deviations from HWE and high-throughout genotype calling algorithms, oftentimes unsupervised, can be susceptible to miscalls, especially from true heterozygotes (Rabbee and Speed, 2006). Genomewide studies using HWE as a screening tool can effectively remove tens of thousands of SNPs from an analysis (Yeager et al, 2007).

We simulate heterozygote miscalls under a variety of models consistent with observed miscall rates and then conduct the standard Pearson chi-square test for departures from HWE. We find that true disease susceptibility loci subject to various levels of heterozygote miscalls can be largely out of HWE and, thus, be candidates for removal prior to association testing. We additionally show that miscalled null SNPs do not induce bias under certain ascertainment schema and suggest that HWE testing not be employed as a quality control measure when conducting genomewide association studies in these scenarios.

2067/W

A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *X. Gao[1], J. Starmer[2], E.R. Martin[1].* 1) Miami Inst Human Genomics, Univ Miami Miller Sch Medicine, Miami, FL; 2) University of North Carolina at Chapel Hill, Chapel Hill, NC.

Multiple testing is a challenging issue in genetic association studies using large numbers of single nucleotide polymorphism (SNP) markers, many of which exhibit linkage disequilibrium (LD). Failure to adjust for multiple testing appropriately may produce excess false positives or overlook true positive signals. The Bonferroni method of adjusting for multiple comparisons is easy to compute, but is well known to be conservative in the presence of LD. On the other hand, permutation-based corrections can correctly account for LD among SNPs, but are computationally intensive. Recent attempts to quickly and accurately adjust for multiple testing have lead to advances in both areas. However, given information about the degree of LD, these methods remain unnecessarily conservative or difficult to compute. In this work, we propose a novel multiple testing correction method for association studies using SNP markers. It is shown to be simpler and more accurate than the recently developed methods and is comparable to permutation-based corrections using both simulated and real data. It can also be applied to genome-wide association studies. The efficiency and accuracy of the proposed method make it an attractive choice for multiple testing adjustment when there is high intermarker LD in the SNP dataset.

2068/W

Power-based tag SNP selection using efficient power evaluation. *B. Han[1], H. Kang[1], E. Eskin[2].* 1) Dept Computer Sci, Univ California, San Diego, La Jolla, CA; 2) Dept Computer Sci, Univ California, Los Angeles, Los Angeles, CA.

Discovering statistical correlation between genetic variation and clinical traits through genetic association studies is an important method for identifying causal variation. Since fully resequencing is practically infeasible, genetic association studies take advantage of local correlation structure (or linkage disequilibrium) between single nucleotide polymorphisms (or SNPs) by selecting a subset of SNPs to be genotyped (tag SNPs). While many current association studies are performed using commercially available high-throughput genotyping products that define a set of tag SNPs, choosing tag SNPs remains as an important problem for both custom follow-up studies as well as designing the high-throughput genotyping products themselves. The most widely used tag SNP selection method uses a criteria based on r^2, or the correlation between SNPs. However, tag SNPs chosen based on a r^2 criterion are not necessarily the tags chosen to maximize the statistical power of an association study. We propose a flexible study design framework that chooses SNPs to maximize the statistical power of an association study. Our method both obtains a tag set as well as measures the statistical power of this tag set through empirical simulations for a wide range of individuals. Empirical simulations based on HapMap reference data support that our method gains considerable amount of power compared to the traditional r^2 based method, or significantly reduces the number of individuals given the desired power of the study. Using our method, we designed a 500k microarray, which has superior power to Affymetrix 500k or Illumina 550k microarray. In addition, our design framework provides an efficient method to empirically evaluate genome-wide power for a wide range of number of individuals. The implementation of our method is publicly available via web server.

2069/W

Choosing a platform and design for genomewide association studies: cost, sample size, and power trade-offs. *J.P. Lewinger[1], D.J. Duggan[2], D.M. Taverna[2], W.J. Gauderman[1], D.O. Stram[1], D.C. Thomas[1].* 1) Dept Preventive Medicine, Univ Southern California, Los Angeles, CA; 2) Translational Genomics Research Institute (TGen), Phoenix, AZ.

Several commercial genotyping platforms are available for genomewide association studies (GWAS), differing sometimes widely in cost and coverage of the human genome. For a case-control design, the most powerful GWAS is obtained by genotyping all available samples on the platform with the best overall coverage. However, this is usually unaffordable, thus it becomes unclear whether higher power would be achieved with a smaller sample on a high-coverage/high-cost platform or a larger sample on a lower-cost/lower-coverage platform. Further complexity is introduced when a two-stage design is being contemplated. In a two-stage design a fraction of the available samples is genotyped on all single nucleotide polymorphisms (SNPs) in the first stage, and only the 'promising' SNPs are genotyped on the remaining samples in the second stage. Two-stage studies are cheaper than one stage-studies and therefore a larger total sample size can be afforded. This larger sample size can translate into higher power than an equal cost one-stage design if the number of SNPs declared promising and the proportion of the samples allocated to the first and second stages are chosen carefully. We extensively investigated the trade-offs between cost/coverage, sample size, and study design for the Illumina 300K, 550K and 1M, and the Affymetrix 500K and 1M platforms. For each of these platforms we computed the average power as a function of the available budget and sample size for a one-stage and two-stage designs assuming each HapMap II SNP can be causal. To account for multiple testing we computed platform specific empirical null distributions by resampling haplotype pairs from the phased HapMapII genotypes. Although the specifics depend on the target effect size and the budget and sample size constraints faced by each study, the overarching conclusion is that sample size should not be traded-off for higher coverage, and that when budget is more limiting than available samples a two-stage design should be preferred.

2070/W

Whole-genome association quality assurance using probe intensity data. *L. Li, K.S. King, L.R. Budde, D.P. Yarnall, C.J. Cox, K.J. Davies, E.H. Lai, M.G. Ehm, M.R. Nelson.* Pharmacogenetics, GlaxoSmithKline.

Genetic association studies are increasingly carried out on a genome-wide scale, wherein up to a million single nucleotide polymorphisms (SNPs) may be genotyped and tested. Although the development of technology and calling algorithms have resulted in relatively high average genotyping accuracy, various sources of experimental variability can result in genotyping errors, which may lead to false positive associations. This is particularly true with small sample sizes, such as in many pharmacogenetic studies, in which many of the most significantly associated results may be enriched for genotyping errors. In three recent Affymetrix 500K whole-genome association studies of adverse drug reactions, we followed up the top association results by genotyping the selected SNPs with single base chain extension genotyping assays to confirm the microarray-based genotypes. We found that many of the strongest associations in each of the studies could be explained by genotyping inconsistencies between the two platforms: 9 of 10, 7 of 10, and 12 of 41. Although such confirmatory genotyping can help to eliminate associations due to genotyping errors, it can add a substantial amount of additional cost and time to a project. We present methods that use the microarray probe intensity data to assess the quality of genotypes for top associated markers and apply them to these three Affymetrix 500K-based studies. In most instances, the intensity data patterns identify the SNPs with genotyping errors and can eliminate them from further follow up. In addition to its application to post analysis confirmation, this method can also be applied as a pre-analysis filter to exclude low precision markers.

2071/W

***In Silico* Genotyping for Genome-Wide Association Studies.** *Y. Li, C.J. Willer, J. Ding, P. Scheet, G.R. Abecasis.* Center for Statistical Genetics, Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI.

Large scale genome-wide association (GWA) studies hold the promise of detecting the small genetic effects that underlie genetic susceptibility to complex diseases but pose a range of analytical and computational challenges. We propose a method that can rapidly impute several million SNPs genotyped by the HapMap consortium using genome-wide SNP genotyping data such as that provided by commercial genotyping platforms by Illumina, Affymetrix or Perlegen. The method uses a hidden Markov model to assemble mosaics of haplotypes observed in the appropriate HapMap reference population that match each of the sampled individuals. We illustrate our approach with real data sets studying age-related macular degeneration and type 2 diabetes. We demonstrate the capability of our method (1) to generate highly accurate genotypes along with correspondent measures of imputation uncertainty, (2) to improve coverage and gain power in association mapping, and (3) to facilitate meta-analysis across studies that use different commercial panels for genotyping. Our method is computationally efficient. For example, in our study of type 2 diabetes, we imputed several billion genotypes using genotypes from the Illumina 317K panel as input. The computation took less than two days for the largest chromosome and multiple chromosomes were conveniently run in parallel. The allelic concordance between imputed and true genotypes is ~98.5%, which can be further improved to over 99% by excluding the 5% of SNPs that are estimated to have lower quality imputed genotypes. Our method is implemented in C++, runs on Windows, Mac and Linux and is available at www.sph.umich.edu/csg/abecasis/mach/.

2072/W

A new multipoint method for genome-wide association studies via imputation of genotypes. *J. Marchini, B. Howie, S. Myers, G. McVean, P. Donnelly.* Dept Statistics, Oxford Univ, Oxford, United Kingdom.

Genome-wide association studies are set to become the method of choice for uncovering the genetic basis of human diseases. A central challenge in this area is the development of powerful multipoint methods that can detect causal variants that have not been directly genotyped. We propose a coherent analysis framework that treats the problem as one involving missing or uncertain genotypes. Central to our approach is a model-based imputation method for inferring genotypes at observed or unobserved SNPs, leading to improved power over existing methods for multipoint association mapping. Using real genome-wide association study data from the Wellcome Trust Case-Control Consortium, we show that our approach is accurate and well calibrated, provides detailed views of associated regions that facilitate follow-up studies, and can be used to validate and correct data at genotyped markers. An important future use of our method will be to boost power by combining data from genome-wide scans that use different SNP sets.

2073/W

A Bayesian Hierarchical Mixture Model for Genotype Calling in a Multi-Cohort study. *C. Spencer, J. Marchini, Y.Y. Teo, P. Donnelly.* Dept Statistics, Univ Oxford, Oxford, United Kingdom.

It is becoming well understood that artifacts from genotype-calling algorithms can lead to elevated false-positive rates in genome-wide association studies. This problem is as serious as the well-known confounding effect of unknown population structure but has received far less attention in the literature. We have developed a new genotype calling algorithm, "CHIAMO", implemented in a Bayesian statistical framework. The model underlying the algorithm is hierarchical, allowing the pooling of information across different collections and from external sources or other studies, with a prior structure that favours plausible configurations of the positions and shapes of intensity clusters. CHIAMO was used to call genotypes for the Wellcome Trust Case-Control Consortium multi-disease study. Using genotypes assayed on both Illumina and Affymetrix platforms we assessed performance of the algorithm in comparison to the widely used BRLMM algorithm. We found that CHIAMO approximately halved both the error rate (0.60% to 0.37%) and percentage of missing data (0.63% to 0.33%). A detailed analysis of calls at individual SNPs shows that BRLMM, CHIAMO and the Illumina platform are prone to different kinds of errors. We show how to adapt the algorithm for the next-generation genotyping chips.

2074/W

Association Tests for Real-World Studies. *T.M. Teslovich, D.J. Cutler.* Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Understanding the role a genetic variant plays in disease is an enormously complicated endeavor. Patient samples are difficult to acquire. Obtaining family members and/or appropriately matched controls may be even harder. After genotyping, data may be missing, and undetected genotyping error is nearly unavoidable. Finally, human populations exhibit variation in allele frequencies. Any of these factors may create false positive results or obscure true genetic effects.

Once these issues are addressed, the complex nature of genetics can frustrate a study. Many diseases exhibit sex-specific effects. Diseases may exhibit some epigenetic component. In the "simplest" of cases, alleles may be dominant, recessive, over-dominant or semi-dominant. Finally, if one performs enough different tests (at multiple loci, and multiple tests at each locus), sooner or later false positives will arise from the multiple testing problem.

We have created a single unified framework to solve all these challenges: Likelihood Association Tests (LATS). Using a likelihood approach, we explicitly model such things as genotyping error, missing data and population stratification, eliminating false positives due to these complications. Further, when a marker is associated with disease, we estimate the size of genetic effects in both males and females, allowing for parent of origin effects and arbitrary dominance relationships between alleles. Data from multiple trio and/or case-control datasets may be analyzed simultaneously. When many markers are typed, we combine data between SNPs, substantially increasing the power of case-control studies. Finally, we present a method of multiple test correction that can be performed instantaneously and yields p-values equivalent to those obtained by permutation testing.

By utilizing far more of the information inherent within genetic studies, this approach results in genetic tests that yield both fewer false positives and fewer false negatives than any previously described tests. We argue that LATS is the most complete package for analyzing genetic association data and the most powerful approach for real world designs.

2075/W

Genome Wide Significance and Locus Identification in Genome Wide Association Studies. *P. Van Eerdewegh, J. Segal, P. Croteau, T. Keith.* Genizon BioSciences, St-Laurent, QC, Canada.

GWAS have provided a paradigm shift in the identification of disease susceptibility loci for complex traits. Common genetic factors with moderate relative risks have now been identified in several GWAS. Although often only the top signal across the genome meets significance after conservative Bonferroni correction for multiple testing, many other true signals are present in the datasets. Identifying these additional loci is a critical challenge if we want to maximize the usefulness and fulfill the promises of GWAS. We have developed an iterative evaluation of genome wide significance that combines permutations and conditional inference and allows the identification of the number of significant signals at specified levels of genome wide significance. The approach does not require a-priori knowledge of the number of true signals or the specification of prior probabilities for a signal to be real. In contrast to other methods such as False Discovery Rate (FDR), the method is applicable to haplotype analyses as well as single SNPs. Since real signals will not necessarily always be among the top hits, we construct the null distributions of various order statistics by permutation of case and control status. The genome wide significance of the ranked nominal p-values is evaluated by comparing each p-value to its respective null order statistic. The conditional inference starts by identifying the highest p-value that meets genome wide significance and, assuming it is under the alternative, removing it from the list of observed values. The remaining observed p-values have their rank reduced by one and are compared again to the various order statistics for significance. The process terminates when no new p-value meets genome wide significance. The number of "peels" provides an estimate of the number of true signals and although order statistics are cumulative, localization of the real signals in the genome is obtained from the rank where the peel occurs. This conditional inference by peeling genome wide significant hits will be illustrated from one of Genizon's GWAS in the Quebec Founder Population and contrasted with FDR and Bonferroni correction.

2076/W

A simple approach for assessing the strength of evidence for association at the level of the whole gene. *A.E. Vine[1], D. Curtis[1], J. Knight[2].* 1) Centre for Psychiatry, Queen Mary's School of Medicine and Dentistry, London E1 1BB, UK; 2) Social Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK.

Introduction It is expected that different markers may show different patterns of association with different pathogenic variants within a given gene. It would be helpful to combine the evidence implicating association at the level of the whole gene rather than just for individual markers or haplotypes. Doing this is complicated by the fact that different markers do not represent independent sources of information. **Method** We propose combining the *p* values from single locus and/or multilocus analyses of different markers according to the formula of Fisher, $X=\Sigma(-2\ln(p_i))$, and then assessing the empirical significance of this statistic using permutation testing. We present an example application to 19 markers around the HTRA2 gene in a case-control study of Parkinson's disease. **Results** Applying our approach shows that, although some individual markers produce low p values, overall association at the level of the gene is not supported. **Discussion** Approaches such as this could be useful in assimilating the overall evidence supporting involvement of a gene in a particular disease. Information can be combined from biallelic and multiallelic markers and from single markers along with multimarker analyses. Single genes can be tested or results from groups of genes involved in the same pathway can be combined in order to test biologically relevant hypotheses.

2077/W

Genome-Wide Autozygosity Mapping in Human Populations. *S. Wang[1], C. Haynes[2], F. Barany[3], J. Ott[2].* 1) Dept Biostatistics, Columbia Univ, New York, NY; 2) Laboratory of Statistical Genetics, Rockefeller Univ, New York, NY; 3) Department of Microbiology, Weill Medical College of Cornell Univ, New York, NY.

Individuals are frequently observed to have long segments of uninterrupted sequences of homozygous markers. One of the major mechanisms that gives rise to such long homozygous segments is consanguineous marriages, where parents pass shared chromosomal segments to their child. Such chromosomal segments are also known as autozygous segments. The clinical evidence that progeny from inbred individuals may have reduced health and fitness because of homozygosity of recessive alleles is well-known. As the length of such homozygous segments depends on the degree of parental consanguinity, it would be logical to observe shorter homozygous segments in more outbred populations. However, a recent study identified long homozygous regions, thus likely to be autozygous segments in the HapMap populations. While an abundance of homozygous segments may significantly reduce the ability to fine map disease genes using association studies, detecting tracts of extended homozygosity related to disease status seems the natural next step in genome-wide association studies beyond allele, genotype and haplotype association analyses. In this study, we propose a new algorithm to map disease-related segments based on autozygosity using case-control data. The underlying rationale for the proposed method is that shared homozygous regions that differ between diseased and healthy individuals may harbor mutations underlying diseases. Specifically, our algorithm uses a sliding-window framework and employs a LOD score measure of autozygosity coupled with permutation-based methods to identify disease related regions. We illustrate the advantage of the algorithm with its application to a genome-wide association study on Parkinson's disease.

2078/W

Evaluating marker-based pairwise relatedness estimators for population-based genome-wide association study. *K.S. Wang[1], L.Y. Wu[2], M. Liu[1].* 1) Gen & Genomic Biol/TMDT East, Hosp Sick Children, Toronto, ON, Canada; 2) Dept statistics, Univ Waterloo, Canada.

The knowledge of relatedness between individuals is central to many studies in genetics. However, for natural populations detailed pedigree structure is usually unknown. A large number of SNPs are now available in humans as well as in other model organisms and provide one potential application in determination of pairwise genetic relationships in natural populations. Recently, a unified mixed linear model (MLM) method for association mapping accounting for multiple levels of relatedness was developed (Yu et al. Nature Genetics 2006). However, little is known about the relatedness estimators' ability to detect the relationship among individuals. The objective of our study is to evaluate six relatedness estimators using a large real data set: KL(Loiselle et al. 1995), KR(Ritland 1996), RLR(Lynch & Ritland 1999), RQG(Queller & Goodnight 1989), RW(Wang 2002) and RL(Li et al.1993). The evaluations are conducted using genotypes of 1940 heterogeneous stock mice and their relatives from 81 pedigrees derived from eight inbred strains (Valdar et al. Nature Genetics 2006). These pedigrees include 1470 parent-offspring (PO), 6581 full-sib (FS), 4026 avuncular (AV), 726 grandparent-grandchild (GG), 5104 first-cousin (FC) and 12090 unrelated founder (UR) pairs. The six estimators are calculated using 1099 unlinked SNPs via the program SPAGeDI. The KL estimator has the lowest the mean squared error (MSE) for PO, FS, GG and AV pairs while the KR estimator has the lowest MSE for FC and UR pairs but similar to those of KL. The regression coefficient of estimated relatedness on expected pedigree relatedness (β) was close to one for the estimators of KL and RQG. The correlation between the estimated and pedigree relatedness (ρ) ranged from 0.78 to 0.94, which are highly significant ($p<0.0001$). In conclusion, our results showed that the SNP-based estimators of KL and RQG approximate to the pedigree relatedness, especially the KL estimator also showed lower MSE in most cases, which are promising for population-based genome-wide association study. The KR estimator is another good choice.

2079/W

A unified association analysis approach for family and unrelated samples correcting for stratification. *X. Zhu[1], S. Li[2], R.C. Elston[1].* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Cleveland Clinic Foundation, Cleveland, OH.

There are two common designs for association mapping of complex diseases: case-control and family-based designs. A case-control sample is more powerful than a family -based sample that contains the same number of persons, although additional markers may be required to control for spurious association. When family and unrelated samples are available, statistical analyses are often performed in the family and unrelated samples separately, resulting in reduced power. In this report, we propose a unified approach allowing for both family and case-control samples and at the same time correcting for population stratification. We apply the principal components of a marker matrix to adjust for the effect of population stratification. This unified approach is more powerful than the analysis for unrelated and family samples separately, or meta-analysis performed by combing the results of separate analyses. This property is demonstrated in both simulations and real data. The proposed approach can be applied in the analysis of both qualitative and quantitative traits.

2080/W

Test of Association between quantitative traits and haplotypes using haplotype similarity. *H. Chen, Q. Sha, S. Zhang.* Dept Mathematical Sci, Michigan Technological Univ, Houghton, MI.

The association tests based on multi-marker haplotypes may be more powerful than those based on single markers. For case control design, the association tests based on multi-marker haploytpes include Pearson's chi-squared test which tests for the difference of haplotype distributions in cases and controls, and haplotype-similarity based methods which compare the average similarity among cases with that of the controls. Recently, a new association test proposed by Sha et al. (2007) compares the average similarities within cases and controls with the average similarities between cases and controls. They show that in most cases, their new proposed method is more powerful than both Pearson's chi-squared tests and other haplotype-similarity based methods. In this paper, we extend the approach in Sha et al. (2007) to obtain a new haplotype similarity tests that can deal with both quantitative trait data and case control study design. The new method can be applied to either phase-known or phase-unknown data. We have conducted several simulation studies and a real data analysis based on the new method.

2081/W

Haplotype analysis of time to event outcome with unphased genotypes in the presence of stratification. *N. Li[1], S. Basu[1], X. Kong[1], H. He[1], T. Rebbeck[2], A. Israni[3, 4].* 1) Division of Biostatistics, University of Minnesota, Minneapolis, MN; 2) Center for Clinical Epidemiology & Biostatistics, University of Pennsylvania, Philadelphia, PA; 3) Division of Nephrology, Hennepin County Medical Center, Minneapolis, MN; 4) Division of Epidemiology & Community Health, University of Minnesota, Minneapolis, MN.

For studying the association between traits and unphased genotype data, full maximum likelihood (ML) approaches have been developed that jointly maximize the likelihood with respect to haplotype (and covariate) effects parameters and haplotype frequencies. However special software is needed to perform such analysis and one is limited to certain statistical models. In contrast, the two-step expectation substitution method (ES, Zaykin et al, 2002) is very easy to implement for any desired analysis and has been shown to work fairly well for case-control studies. We used simulations to compare the performance of ML and ES methods in the context of semi-parametric analysis of time-to-event data. We were interested in the effect of model misspecification, especially stratification. The ES method was easily adopted to stratified samples by estimating haplotype frequencies separately for each stratum. We observed that the ML method as implemented in the program hapstat was fairly robust under model misspecification. The performance of the ES method was very close to that of the ML method under a variety of conditions. In our simulations, We also found that the effect of stratification was small even when it was ignored. In the analysis of haplotype effect on time to decline in renal function in a kidney transplant cohort study, some difference was noted when stratification was taken into account. For two related outcomes, the global p-values changed from 0.006 and 0.015 to 0.009 and 0.0025, respectively. In conclusion, the ES method can have some practical advantages over the ML method in situations requiring more complex statistical models, such as analysis of haplotype effect on time-to-event data in the presence of population stratification.

2082/W

Bayesian methods for quantitative traits. *J.S. Pereira-Gale, J. Marchini, P. Donnelly.* Department of Statistics, University of Oxford, Oxford, Oxfordshire, United Kingdom.

Genetic association studies focus on finding regions of the genome associated with particular diseases. While most genetic association studies focus on binary traits, the presence or absence of disease, there is potentially more information to be gained by studying continuous/quantitative traits. We have developed a Bayesian method for the analysis of single-SNP association for quantitative traits. We use a model formulated in terms of the trait population mean, additive and dominance effects and a within genotype variance parameter and show how these parameters are related to measures of trait heritability. We have found that this relationship is a useful guide when choosing priors for the parameters of our model. On simulated data we find that the Bayesian approach can be more powerful than a standard Frequentist association test (F-test) for quantitative traits. This work has been extended in two directions (a) we have developed a multi-point method for association testing that combines information from multiple SNPs to carry out tests at untyped variants, and (b) we have developed a model for multiple phenotypes. We illustrate both of these methods using simulated data.

2083/W

A Constrained Regression Approach for Studying Haplotype-Specific Association. *J.Y. Tzeng, H. Bondell.* Department of Statistics, North Carolina State University, Raleigh, NC.

In the haplotype analysis of refine-stage studies such as candidate-gene or candidate-region studies, the central goal is to understand the effects of specific haplotypes so to identify etiological variants and infer biological explanations. One common approach for studying haplotype-specific effect is to examine if haplotypes have significant regression coefficients. However, such analysis only reveals the effect of a haplotype with respect to the reference haplotype that is often arbitrarily defined. Consequently the results can be sensitive to the choice of the reference haplotypes, and little information is learned about the relationship among non-reference haplotypes. Another popular strategy is to create a binary variable that records "haplotype of interests" versus "the else", and use this variable to study the effect of the target haplotype. This strategy can be problematic especially when each of these "else" haplotypes has different or even opposite effects on the phenotypes. Ideally, a thorough haplotype-specific analysis should call for pair-wise comparisons among all haplotypes, same procedures as in the post-hoc analysis of ANOVA. However, this thorough pair-wise comparison may suffer from power loss due to the multiple comparison adjustment, and often yields a contradictory conclusion on which haplotypes share the same level of effects. To address these concerns, we consider a constrained-regression approach that performs the ANOVA-type of post-hoc analysis to study haplotype effects. The method uses constraints that encourage haplotypes with similar effect sizes to be estimated with exact equality, and transfers the post-hoc analysis from a multiple-comparison procedure to a variable-selection framework. Through simulation, we evaluate the performance of the proposed approach and illustrate how the output can be used to characterize the haplotype-specific associations.

2084/W

Comparing Methods for Association Test of Longitudinal or Multivariate Phenotypes. *H. Wu, Q. Yang.* Department of Biostatistics Boston University School of Public Health Boston MA.

Longitudinal or multivariate phenotype data may be frequently encountered in genetic studies. Such data contain more information than independent univariate phenotype data, but how to best analyze them is not always straightforward. We have evaluated three approaches to analyzing longitudinal or multivariate phenotype data in association studies: random effects models, creating a single summary measure of all traits and combining multiple test statistics from the test of each trait. Simulation studies were conducted to assess the validity and efficiencies among these strategies with 1000 replicates. Our results suggested that random effects models performed as good as using mean of multiple quantitative traits if these traits follow the same marginal distribution with exchangeable correlations among them. We are comparing these two strategies for traits that follow different marginal distribution with heterogeneous correlation structure, as well as to the third strategy, combining test statistics from individual univariate analysis. This study will provide insights on choosing strategies for analyzing longitudinal or multivariate phenotype data in association studies.

2085/W

Automatic Haplotype Construction in PedGenie. *R.P. Abo, J. Wong, N.J. Camp.* Biomedical Informatics, University of Utah, Salt Lake City, UT.

A popular design for candidate gene association analyses is to use tagging-SNPs (tSNPs). To maximize the potential for the tSNPs to detect signals from an underlying disease variant, these SNPs should be analyzed both independently and in multi-SNP combinations (as haplotypes or composite genotypes). These haplotypes need not span all SNPs nor be from contiguous SNP loci. Approaches to construct and test multiple SNPs are therefore required. The difficulty is in establishing which SNPs to consider and how to construct the association analysis (haplotypes/composite genotypes, monotype/diplotype tests). A common multi-SNP construction strategy is to add SNPs to extend haplotypes that exceed a significance threshold; however, with large numbers of SNPs this procedure can become very cumbersome. The approach proposed here consists of constructing and testing multi-SNP combinations for association through a forward-backward stepwise process. Rather than considering exhaustive SNP subsets, the stepwise approach narrows the subset considered through a sequence of steps. The user can define whether the contingency tables tested are for diplotype (individuals) or monotype (chromosomes) data. For diplotype tests, both haplotypes (phase important) and composite genotypes (phase ignored) are considered. The stepwise forward process begins by considering association between the disease and all single SNPs. Each subsequent forward step considers an additional SNP. A user-defined significance threshold determines which SNPs or group of SNPs move to the next step based on their statistical test results. The backward process is initiated if the third step ($\geq$3-SNP sets) is reached. A backwards step consists of testing all (n-1)-sized subsets that were not considered in the previous step. Chi-square and odds-ratio test results are noted at each step. The software implementation of this approach is an extension to PedGenie (Allen-Brady et al. 2006). By utilizing PedGenie, these multi-SNP construction analyses can be performed for independent individuals as well as related individuals in pedigrees of arbitrary size. PedGenie also provides the Monte Carlo framework for appropriately assessing statistical significance of the constructed multi-SNP sets.

2086/W

Direct testing of untyped SNPs using multimarker tags. *S. Griffiths, F. Dudbridge.* MRC Biostatistics Unit, Cambridge, United Kingdom.

In association studies it is generally too expensive to genotype all variants in all subjects. We can exploit linkage disequilibrium between SNPs to select a subset that captures the variation in a training data set obtained either through direct resequencing or a public resource such as the HapMap. These tag SNPs are then genotyped in the whole sample. Multimarker tagging is a more aggressive adaptation of pairwise tagging that allows for combinations of tag SNPs to predict an untyped SNP. Here we describe a new method for directly testing the association of an untyped SNP using a multimarker tag.

Previously, other investigators have suggested testing a specific tag haplotype, or performing a weighted analysis using weights derived from the training data. However these approaches do not properly account for the imperfect correlation between the tag haplotype and the untyped SNP. Here we describe a straightforward approach to testing untyped SNPs using a missing-data likelihood analysis, including the tag markers as nuisance parameters. The training data is stacked on top of the main body of genotype data so there is information on how the tag markers predict the genotype of the untyped SNP. The uncertainty in this prediction is automatically taken into account in the likelihood analysis.

We compare our approach with testing specific tag haplotypes and separately with WHAP, a method described recently by Zaitlen et al. We show that our approach yields more power than single haplotype imputation and similar power to WHAP, yet it has the advantages that it takes into account training set phenotypes and we may obtain an estimate of the odds ratio.
.

2087/W

Haplotype associations with quantitative traits in the presence of complex multilocus and heterogeneous effects. *K. Shibata[1], L. Diatchenko[2], D.V. Zaykin[1].* 1) National Institute of Environmental Health Sciences, National Institutes of Health; 2) Center for Neurosensory Disorders, University of North Carolina at Chapel Hill.

We extend earlier work on characterization of haplotype associations with quantitative traits by incorporating haplotype-specific variance parameters into the likelihood for un-phased data. The inference proceeds within the likelihood framework that involves simultaneous estimation of haplotypic effects and their frequencies. The addition of the haplotypic variance was found to improve power of detecting associations under complex models including those where only a subset of functional polymorphisms has been scored, as well as heterogeneity models where multiple mutations are linked to the haplotypes under study via linkage disequilibrium. We describe the association tests and estimation procedures for specifically haplotypic effects, as well as the inference based on entire un-phased diplotypes. An overall association test including all of the haplotypes at once is derived as well. The method was successful in finding a strong association of adrenergic receptor beta-2 (ADRB2) haplotypes with blood pressure.

2088/W

Modeling Genetic Imprinting of Quantitative Traits in Humans. *W. Hou[1], S. Wu[2], T. Liu[2], J. Yang[2], J. Yap[2], R. Wu[2].* 1) Epidemiology and Health Policy, University of Florida, Gainesville, FL; 2) Department of Statistics, University of Florida, Gainesville, FL.

Gene imprinting is thought to affect many developmental and disease traits in humans. With the availability of high-throughput single nucleotide polymorphisms (SNPs), there is a pressing need to quantify the effects of imprinting DNA sequence variants on complex traits. Here, we describe a general statistical strategy for testing and estimating imprinted quantitative trait nucleotides (iQTNs) that contribute to genetic variation in a natural population. This strategy was made by implementing parent-of-origin effects of iQTNs within a mixture model framework, thus allowing the test of imprinting genomic effects on the phenotypic value of a trait. We derived the closed forms for the EM algorithm to estimate haplotype frequencies, allele frequencies and linkage disequilibria, and additive, dominant and imprinting genetic effects. A series of hypotheses are formulated to test the genetic control mechanisms of trait variation. Simulation studies were performed to examine the statistical behavior of this model. The new model provides a standard procedure for genomic mapping of iQTNs involved in the genetic control of complex traits in human populations.

2089/W

Comparison of the performance of single- versus multi-SNP tags in an independent European sample. *S.P. Dickson[1], M.R. Nelson[2].* 1) Bioinformatics, North Carolina State University, Raleigh, NC; 2) Genetic Epidemiology and Analysis, GlaxoSmithKline, RTP, NC.

Current association study designs rely heavily on data from the International HapMap project to assist in the selection, application, and interpretation of genetic markers. These data have been particularly informative in the selection of population-specific tagging SNPs (tSNPs) and in the assessment of linkage disequilibrium (LD) between markers in fixed panels and common genetic variation. Recent methods have been proposed to increase the efficiency of the tag selection process and increase the information available from fixed panels by using haplotypes of typed SNPs (multi-SNP tags, or MSTs) to infer the allelic state of unmeasured variants. Multiple studies have been carried out to demonstrate that single SNP tags selected in the HapMap European sample (CEU) provide comparable genetic coverage in most other populations of European origin. Similarly, we set out to determine the relative value of MSTs by evaluating the performance of MSTs selected in the CEU sample from among SNPs on the Affymetrix 500K SNP panel in an independent sample of 203 individuals of European origin. MSTs are shown to have a larger selection bias than tSNPs, which appears to increase linearly from 2- to 3-SNP tagsthe median r^2 value in tSNPs was 0.09 lower in the independent sample than it was in CEU, while the median r^2 value decreased by 0.14 and 0.18 in the 2- and 3-SNP MSTs. Although the use of MSTs for aggressive tagging can reduce the number of SNPs typed by up to 20%, we find that this savings is partly offset by the corresponding reduction in genetic coverage. However, MST performance does support their application as an efficient and readily interpretable way to extend the coverage of fixed panels.

2090/W

Combined Linkage and Association Mapping of Quantitative Trait Loci with Missing Genotype Data. *R. Fan[1], L. Liu[1], J. Jung[2], M. Zhong[1].* 1) Dept Statistics, Texas A&M Univ, College Station, TX; 2) Department of Medical and Molecular Genetics, Indiana University, School of Medicine, IN.

In genetics study, the genotypes or phenotypes can be missing due to various reasons. In this paper, the impact of missing genotypes is investigated for high resolution combined linkage and association mapping of quantitative trait loci (QTL). We assume that the genotype data are missing completely at random. Two regression models, ``genotype effect model'' and ``additive effect model'', are proposed to model the association between the markers and the trait locus. If the marker genotype is not missing, the model is exactly the same as those of our previous study, i.e., the number of genotypes or alleles is used as weight to model the effect of the genotypes or alleles in single marker case. If the marker genotype is missing, the expected number of genotypes or alleles is used as weight to model the effect of the genotypes or alleles. By analytical formulae, we show that the ``genotype effect model'' can be used to model the additive and dominance effects simultaneously; the ``additive effect model'' only takes care of additive effect. Based on the two models, F-test statistics are proposed to test association between the QTL and markers. The non-centrality parameter approximations of F-test statistics are derived to make power calculation and comparison, which show that the power of the F-tests is reduced due to the missingness. By simulation study, we show that the two models have reasonable type I error rates for a dataset of moderate sample size. The method is applied to analyze the angiotensin-1 converting enzyme (ACE) data.

2091/W

Assessing Departure from Hardy-Weinberg Equilibrium in the Presence of Disease Association. *M. Li[1], C. Li[2].* 1) Department of Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN.

Assessing Hardy-Weinberg equilibrium (HWE) is often employed as an important initial step for genotype data quality checking in genetics studies. Since HWE is a population property, tests for HWE often assume the genotypes are randomly sampled from the general population. However, in many human genetics studies, subjects are ascertained through their disease status, and affected individuals (and their relatives in family-based studies) are often overly represented in the ascertained sample than in the general population. As a result, when a marker is associated with the disease, the marker genotypes may no longer be a random sample and this may lead to inflation of type I error rate in HWE tests. Here we develop a general likelihood framework that allows assessment of departure from HWE while taking into account potential association with the disease. The framework can be used for various data structures, including unrelated cases and controls, case-parents trios, and nuclear families with multiple affected offspring. We describe two HWE tests, which are based on likelihood ratio and goodness-of-fit statistics. The type I error rates of these two tests are under control for a broad range of genetic models. When the tested marker is not associated with the disease, our tests have comparable power as the traditional tests for rare diseases and are more powerful for common diseases. When disease association exists, our method can help differentiate departure from HWE caused by disease association from departure caused by other reasons, such as genotyping errors. For nuclear families with one or two offspring, our method can help identify genotyping errors that are not fully detectable by checking Mendelian inconsistencies. We believe our method will provide a valuable tool for researchers in genetics studies of complex diseases.

2092/W

Family-based association method for incorporating half-sib data. *Y.W. Li[1,2], Y.J. Li[2].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Dept Statistics, North Carolina State Univ, Raleigh, NC.

The current family-based association tests are based on pedigree structures with parent-offspring triads, parents and multiple affected full siblings, discordant full sibpairs of large size, or extended pedigrees including related nuclear families and sibships. Therefore, full siblings or parents have been the target of ascertainment in genetic research. In this study, we aimed in developing a family-based association method that can incorporate half-siblings as well. This method will benefit to understudied populations, especially when recruitment is difficult. The new method is based on the same framework of the pedigree disequilibrium test (PDT), in which we used inverse kinship coefficient to weight each marker transmission or sharing score obtained from each pair of samples. The type I error and statistical power were evaluated by extensive simulation studies. We evaluated scenarios for pedigrees containing concordant half sibpairs with parents, discordant half sibpairs without parents, and combination of full and half sibpairs without parents. Two existing methods, PDT and FBAT, were used for comparison. The simulation results demonstrated that our extended PDT (EPDT) method has correct type I error rates (0.045 to 0.052). For the data of 200 concordant half sibpairs with parents, our proposed EPDT method showed comparable statistical power to the PDT and FBAT method (79.8% in EPDT, 69.5% in PDT, and 75.2% in FBAT). We noted that PDT and FBAT cannot analyze certain types of data structure with half siblings such as the discordant half sibpairs without parents. The EPDT method is the only method to handle these types of family data. Thus, EPDT provides a valid test of linkage disequilibrium for family data including half siblings. This method can be extended further to distant related individuals in the future.

2093/W

Two approaches to Family Based Association Analysis. *Y. Song, R. Sinha, S. Won, C. Xing, C. Thompson, R. Goodloe, Y. Wang, C. Gray-McGuire.* Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

The study of complex diseases via genome-wide association analysis has gained increased attention with the availability of densely spaced SNPs. However, most methods developed and evaluated for these designs have been based on case-control data, leaving family data unused. We evaluate type I error and power of two family based association methods, one that conditions on parental genotype (FBAT) and one that does not (ASSOC). FBAT models the offsprings' transmitted allele as the dependent variable. ASSOC uses a regression model to obtain residuals that approximate normality, while at the same time allowing for the non-independence of family data. In addition, since ASSOC can utilize the unrelated control data, we evaluated the impact of including controls into the unconditional analysis. Analyses were done using densely spaced SNP data simulated as a part of the 15th Genetic Analysis Workshop. We chose 250 families and, additionally, in ASSOC, 125 controls. Significance of each of 17820 SNPs (average spacing of 9.6 kb) were evaluated after adjusting for covariates. Type I error was assessed using SNPs most telomeric to the two simulated disease genes, and power using each of 200 SNPs nearest the two disease loci. The distribution of the unconditional results is exactly as would be expected for an association test, with significance narrowing the region to about 0.5 cM from the disease locus and only to SNPs in LD with the disease at a D' > 0.6. The FBAT results are just as would be expected for a test of linkage in the presence of association, with significance sustaining to > 1.5 cM from the disease locus and to SNPs in LD with the disease at a D' as small as 0.2 . Combining family data and as few as 250 controls into the unconditional analysis did not change the type I error rate and resulted in a 5-11% increase in power. We note that these results highlight the differences between FBAT, a test for both linkage and association, and therefore best suited as a coarse filter of the genome, and ASSOC, a test for allelic association only, and best suited for more fine scale identification of disease loci.

2094/W

Power of affected-relative allele-sharing models in a small number of moderate-sized pedigrees. *C. Xing.* McDermott Ctr, Univ Texas SW Medical Ctr, Dallas, TX.

For some complex but less common diseases, doctors can usually only collect through probands a small number of moderate-sized pedigrees with multiple affected members. Linkage screen for allele sharing identical by descent (IBD) among affected relatives is routinely the first step toward identifying the disease-disposing genes. In this study we compared the power of three affected-relative allele-sharing models including the non-parametric linkage (NPL) method, the so-called Kong and Cox linear model, and the exponential model in a small number of moderate-sized pedigrees. The NPL method gives liberal p-values whereas the Kong and Cox linear model provides conservative p-values, which is different from their usual behavior that the former is more conservative while the later is more appropriate in case of incomplete inheritance information. The Kong and Cox exponential model gives proper significance levels. In summary, the exponential model should be advanced in case of a small number of moderate-sized pedigrees with multiple affected members. Moreover, the "odd" phenomenon of liberal NPL scores but conservative Kong and Cox linear scores at the same region may indicate excess allele sharing IBD among the affected.

2095/W

Haplotype reconstruction in pedigrees using the Cluster Variation Method. *C.A. Albers, H.J. Kappen.* Dept. Biophysics, Radboud University, Nijmegen, Netherlands.

Haplotyping is an important tool for mapping susceptibility genes of complex diseases. Application of exact likelihood-based methods is generally not feasible in complex pedigrees with many markers. We present a probabilistic approach for approximately optimal haplotype reconstruction in general pedigrees. We reconstruct the haplotypes by assigning in every iteration a fixed number of the ordered genotypes with the highest marginal probability, conditioned on the marker data and ordered genotypes assigned in previous iterations. We use the Cluster Variation Method (CVM) to estimate the marginal probabilities. The CVM is an analytical variational approximation method designed for efficient estimation of marginal probabilities in complex Bayesian probability models, through optimization of marginal distributions on overlapping subsets of variables (the clusters) for which exact probability calculus is feasible. In data sets simulated in a pedigree with 53 individuals, 5 SNPs and missing genotype rates of 30-70 percent where exact computation was feasible, the haplotyping accuracy, measured as the percentage of inferred ordered genotypes equal to the true simulated ordered genotype, was as high as that of the exact maximum likelihood haplotyping program Superlink. In data sets with 20 SNPs and 13 genotyped individuals where exact computation was not feasible, our approach was significantly more accurate than SimWalk2 when both methods assigned a subset of the alleles and equally accurate when both methods assigned all alleles. Computation time of our approach increased approximately linearly with the number of markers, while that of SimWalk2 increased faster than linear. In a real data set for a complex mouse pedigree of 331 individuals with 322 typed for 10 SNPs (Valdar et al.), our approach reconstructed haplotype configurations with on average (N=10) 2.5 percent higher log-likelihoods than SimWalk2, at respective mean computation times of 54 and 2481 minutes. Thus, our approach is at least as accurate as SimWalk2 and significantly faster for large problem instances, and provides more detailed information about uncertainty in the haplotype reconstruction.

2096/W

QTL-ALL: software for robust QTL linkage analysis in nuclear families. *N. Mukhopadhyay[1], S. Bhattacharjee[1], C-L. Kuo[1], B.H. Reck[2], D.E. Weeks[1], E. Feingold[1].* 1) Dept Human Genetics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA; 2) Genetics Analysis, GlaxoSmithKline, RTP, NC.

There has been extensive development of new statistics for quantitative trait locus (QTL) mapping over the last few years, but a number of the most promising new methods are not yet available in end-user software. This is particularly true of methods such as score statistics that are important for studies using selected (non-population) samples. Our new "QTL Analysis and Linkage Library" (QTL-ALL) is a software package designed to make as many as possible of these new statistics widely available. The list of statistics consists of many score statistics, some of which use higher moments of the trait distribution (skewness and kurtosis), and a number of other statistics for special designs. QTL-ALL contains several computational advances that dramatically accelerate the computations. QTL-ALL is highly automated: starting from input data files in a slightly modified LINKAGE format, it guides the user through a set of simple menus to select marker and trait loci for analysis, does error checking, lets the user select from a list of statistics appropriate for the pedigree structures in the data, and then computes the selected statistics on the data, providing readable, formatted text output as well as graphical plots of p-values. The current version can handle sibling pair and sibship data, including specialty designs such as discordant and concordant (affected) pairs. Here, we present the results of analyzing a part of the Rheumatoid Arthritis data provided to the GAW15 participants. We have included both microsatellite markers and SNPs in our analysis. The QTL-ALL package is available at http://watson.hgen.pitt.edu/register/.

2097/W

An efficient computational approach to making inferences in multivariate linkage analysis. *N. Morris, C. Stein, R. Elston, T. Wang.* Department of Epidemiology and Biostatistics, Case Western Reserve University.

In a world where complex multiple phenotype linkage data are abundant, there is a corresponding need of a method to analyze such data multivariately. Multivariate approaches can provide an effective way of controlling type I error, increasing power and disentangling pleiotropic effects. However, there is no simple way to characterize the distribution of currently available multivariate linkage statistics where tests are performed under complex one-sided constraints. As a result, ad hoc approximations of degrees of freedom or computationally intensive methods such as permutation tests must be used for making valid inferences. Recently, Wang and Elston proposed a robust score statistic for multivariate linkage analysis. Using a modified version of this statistic for illustration, we present a new Monte Carlo approach to calculating the asymptotic p-value under nonstandard conditions. This approach involves decomposing the parameter space into direction and length components, thus reducing the dimension of the parameter space. Theoretical limits to the computational gains attainable by this method and situations where it is efficient are discussed. A similar numerical integration.approach is also suggested. Type I error, power and run times under various simulated situations are presented.

2098/W

QRAT: A novel robust and powerful QTL association test for nuclear families. *X. Qin, E.R. Hauser, S. Silke.* Center for Human Genetics, Duke University, Durham, NC.

Risk models for complex human diseases often include traditional disease-associated covariates, such as body mass index or cholesterol levels, along with genetic susceptibility. It is a challenge to incorporate these types of covariates into gene discovery studies. Models for the relationship between covariates and genetic variants may include QTL models, GXE interaction models, or heterogeneity models. In addition, the models may be confounded by population structure in covariate distributions. Some test statistics for examining these models depend heavily on assuming a normal distribution of the covariate. Departures from normality can inflate the type I error and reduce the power. Here, we propose several flavors of a new nonparametric method called the quantitative rank association test (QRAT). In the absence of population stratification, a rank-based case-only test applied to marker and covariate data of a single sibling selected from each family (1sib_QRAT) may be applied. To protect against population stratification, a modified test (2sibs_QRAT) is proposed. Both tests are derived from the linkage disequilibrium (LD) coefficient between QTL and marker alleles and genotype-specific covariate distributions. We propose test statistics that are based on alleles or genotypes, similar to the PDT and geno-PDT statistics for a binary affection status. Their respective power depends on the true underlying genetic model, which is typically unknown. A staged design may be used in order to evaluate on part of the sample which test statistic is likely more powerful for the dataset at hand. Here, we present extensive simulation studies with the SIMLA software to evaluate the performance of our proposed tests. Type I error and power were compared to the Monks-Kaplan method implemented in the QTDT package and a newly developed likelihood ratio test for two siblings (2sib_LRT) that relies on the assumption of normality. Our results show that 1sib_QRAT is very powerful in the absence of any form of population stratification or covariate heterogeneity. Under some models, the 2sibs_QRAT is more powerful than QTDT and more robust to deviations from normality.

2099/W

Analysis of the interdependency of Pearson correlation (r^2) and p-values from association tests and development of an evaluation tool. *J.L. Curry[1,2], Y.J. Li[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Biomathemetics Program, Dept of Statistics, North Carolina State Univ, Raleigh, NC.

Pearson correlation (r^2) is the most frequently used measurement for linkage disequilibrium (LD) between two markers. An r^2 ranges from 0 to 1 where 0 signifies no linkage disequilibrium and 1 indicates complete linkage disequilibrium. An LD block or LD bin is arbitrarily defined as r^2 >1/3. By definition, markers within an LD block evolve together and play the same role if they are involved in the development of the disease. Due to this reason, genotyping a tagging SNP (1 represented SNP per block) has been a favorable approach in association studies. However in real data analysis there tends to be a discrepancy between the LD indicated by r^2 and the association signified by p-values. The relationship between r^2 and the p-value from association tests has not been widely investigated. We revisited the theoretical relationship of association test statistics and r^2 and we performed an intensive simulation to examine the impact of r^2 to the p-values derived from population and family based association tests. We created both family based and population based simulations of multiple sample sizes with a range of marker to marker correlations from 0 to 1 and with various disease locus to marker correlations from 0 to 1. We used PDT and chi-squared tests to find significant p-values and thus ascertain proper cut-offs of r^2 depending on the relation of marker to disease locus. We developed a program that will provide assembled visualization of the p-values as they relate to Pearson's correlation and will determine accurate cut-off's of r^2 for verifying association results for both population and family based association tests.

2100/W

Genotyping error detection in unrelated samples. *N. Liu[1], D. Zhang[2], H. Zhao[3,4].* 1) Dept of Biostatistics, Univ of Alabama at Birmingham, Birmingham, AL; 2) Dept of Statistics, Purdue Univ, West Lafayette, IN; 3) Dept of Epidemiology and Public Health, Yale Univ, New Haven, CT; 4) Dept of Genetics, Yale Univ, New Haven, CT.

Even with the advancement of modern technology, data with genotyping errors are still common in genetic studies. Besides the possibility of causing genotyping errors from equipment, such as any damage or loss of performance of some probes of the multiplexed platforms used for genotyping, there are other situations where genotyping errors can also be induced, such as variation in DNA quality/quantity or molecular effects. Many studies have shown that genotyping errors can cause severe problems in genetic studies. To date, however, almost all analytic methods assume that the inputs of the genotype data are without errors. The identification of genotyping errors still remains neglected. The majority of existing genotyping error detection methods is for pedigree data, such as checking for Mendelian consistency and/or Hardy-Weinberg equilibrium (HWE). For unrelated population data, very few methods have been developed for this purpose, besides HWE checking. They mainly rely on external "validation" study, or replicates to get the estimates of error rates, with very few exceptions. We evaluate several models for genotyping error detection in unrelated samples with SNP data. We show that the parameters of these models are not identifiable. However, we also show that with some restrictions on the parameter spaces, the parameters of some of the models are identifiable. Simulation study shows that one of the models performs well, with appropriate coverage probabilities for the estimates and decent power. We also apply that model on HapMap data and another real data to show its usability in practice. The results show that even for high quality HapMap data, there are still SNPs which would have been overlooked by deviations from HWE but are suspicious to genotyping errors. Our work may help researchers to estimate genotyping error rates of their data, and use the estimates of errors in their analysis to increase power and decrease bias, without the extra work to genotype family members or replicates.

2101/W

Sample size calculations in matched case-control studies. *X. Liu, R. Chakraborty, M. Rao.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

The basic premise is a 1:1 matched case-control study, where each case is matched with a control on a number of variables. The goal is to test the equality of k odds ratios stemming from k+1 distinct matching scenarios. In the literature, conditional likelihood approach is used for calculating sample size for detecting specific departures from the null hypothesis of no association with a given power (See Sinha and Mukherjee 2006). In this paper, we use the full likelihood for calculating sample sizes. Comparisons are made between the two approaches.

2102/W

Some Mathematical, Statistical, and Computational Issues Behind the Hardy-Weinberg Equilibrium in the Tri-allelic Case. *M. Rao, X. Liu, R. Chakraborty.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

The Hardy-Weinberg equilibrium phenomenon is well-understood in the bi-allelic case. Formidable mathematical, statistical, and computational problems arise in the tri-allelic and higher-order-allelic cases. Behind the equilibrium phenomenon in the tri-allelic case lies a 3x3 bivariate symmetric distribution of the alleles with identical marginal distributions. Exploiting the fact that the collection of all such distributions is a compact convex set, we produce six basic bivariate distributions on whose edifice inbreeding, equilibrium, estimation, and power calculations revolve around. Each bivariate distribution represents a specific type of mating.

2103/W

Optimal methods to map quantitative trait loci using extreme trait values. *D. Covarrubias[1,2], S.M. Leal[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Statistics, Rice University, Houston, TX.

For mapping quantitative trait loci, one effective study design is to select a subset of individuals with extreme high and low quantitative trait (QT) values. This study design has been implemented to increase power while reducing the number of individuals genotyped or sequenced, and was recently used to identify genes for HDL cholesterol (Cohen et al. 2004) and cardiovascular disease (Arking et al. 2006). There are several frameworks for extreme QT sampling which can be used: phenotyping study subjects until a set sample size of individuals meeting a QT criterion are obtained, or using an existing sample and analyzing only a subset of individuals based upon a QT threshold. For this study, we examined the latter sampling framework for a variety of genetic variances, allele frequencies and sample sizes. Thresholds which optimize power were determined when the QT was dichotomized. Additionally, type I and II error was evaluated for parametric (ANOVA, linear regression, Cochran-Armitage test for trend, Fisher exact test) and non-parametric (Kruskal-Wallis) statistical tests. For the analysis of QTs, the highest power is obtained when the entire sample is analyzed and the power decreases with increasing extreme QT sampling. Additionally, for ANOVA and linear regression, type I error inflates with increasing extreme QT sampling. When the QT is dichotomized, using only those individuals with the highest and lowest QT values, the optimal power is obtained when ~50% of the total sample is analyzed. For this situation, usually the Fisher exact test is most powerful and uniformly controls type I error. If thresholds > 25% are used for sampling, the Kruskal-Wallis test is more powerful than the Fisher exact test and also controls type I error.

2104/W

Weighted approaches for missing SNP data. *G. D'Angelo[1], E. Feingold[1,2].* 1) Dept Biostatistics, Pub Health, Univ Pittsburgh GSPH, Pittsburgh, PA; 2) Dept Human Genetics, Pub Health, Univ Pittsburgh GSPH, Pittsburgh, PA.

Genotype data can be missing in a genetic association study. When modeling the effects of multiple genetic variants simultaneously, typically, complete case analysis is the method of choice leading to significant reduction in sample size and in power. A number of other methods for handling missing data are applicable, but have rarely been used in this context. To address the problem where the SNPs are missing at random (MAR), we compare several standard methods for handling missing data that can be applied or adapted to this problem. The methods compared are the weighted pseudolikelihood, multiple imputation, and the EM algorithm. We apply these methods to an Alzheimer's disease association study. We show that weighting techniques, and specifically the EM algorithm, have the best properties of all the estimators we studied.

2105/W

Detecting Associations in the Presence of Extreme Allelic Heterogeneity: Application to the Rare Variant Common Disease Hypothesis. *B. Li, S.M. Leal.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Association studies are frequently utilized to map variants which are susceptibility loci for common diseases. Critical assumptions of this approach are that the disease is due to a common functional variant which is in strong linkage disequilibrium with genotyped SNP(s) and there is minimal allelic heterogeneity. If the rare variant common disease hypothesis holds, current association based methods will be underpowered due to allelic heterogeneity, low allele frequencies and poor correlation (r^2) with tagSNPs. For common diseases where the underlying etiology is believed to involve extreme allelic heterogeneity, large scale candidate gene sequencing is currently underway to discover multiple causal rare variants. However, which methods are optimal for analyzing this type of data to detect associations is unknown. In this study, we analytically demonstrate that collapsing genotypes and rare variants across multiple loci is more powerful than multi-marker test (Hotelling's T^2) and single marker test (Fisher exact test). Collapsing methods are also robust against misclassifications unless the non-causal variants are common. In that case, collapsing only rare variants gained significant robustness with little loss of power. Empirical findings from simulation studies were consistent with analytical results and, additionally, it was shown empirically that for collapsing methods type I error is well controlled.

2106/W

Allelic dropout does not affect findings of genetic association. *R.B. Ramoni[1,2], C. Hayes[3], M.M. Werler[4], S. Hernandez-Diaz[5], K.T. Kelsey[6], P.L. Williams[7], M.F. Ramoni[2,8].* 1) Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA; 2) Harvard Partners Center for Genetics and Genomics, Harvard Medical School, Boston, MA; 3) Sackler School of Graduate Biomedical Sciences, Tufts University, Boston, MA; 4) Slone Epidemiology Center, Boston University, Boston, MA; 5) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 6) Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA; 7) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 8) Children's Hospital Informatics Program, Division of Health Sciences and Technology, Harvard Medical School and Massachusetts Institute of Technology, Boston, MA.

Allelic dropout (ADO) is one of the most common genotyping errors. When parental genotypes are not available, ADO is detected by testing for deviation from Hardy-Weinberg equilibrium. If a particular locus is found to significantly deviate from such equilibrium, the entire locus is removed from the analysis. Here we present a mathematical model of the effects of ADO on the identification of genetic associations in case-control studies. We provide an analytical proof that the error always biases the results towards no association and, therefore, that any identified associations will hold in the presence of any degree of ADO. These results suggest the reconsideration of previously discarded data and published negative findings, and they should inform the design of future genetic association studies. The findings are broadly applicable because the standard strategy of assessing the validity of genotypes at a locus by testing for deviation from Hardy-Weinberg equilibrium in the control subjects alone implies the assumption that the phenotype will not affect the ADO. Furthermore, the general formulation of the model allows for extension to account for the unusual circumstances in which this assumption does not hold.

2107/W

A log Bayes factor-based 'Taxonomy' approach for large-scale genetic studies. *K. Song, X. Yuan, X. Lin, A. Angelakopoulou, R.A. Gibson, L. McCarthy, L. Griffiths, D. Waterworth, V. Mooser, C. Bowman.* GlaxoSmithKline R&D, King of Prussia PA and London UK.

New methods are needed to identify putative disease-causing variants while dealing with multiple testing issues, gene-gene interactions and population stratification in large-scale genetic studies. A simple visualization method called Taxonomy (v3, http://taxonomy.delrieu.org), which uses empirically-derived log Bayes factor, was recently developed to find genetic variants and to identify heterogeneity for large datasets. Here, we first used various simulations to evaluate the properties of the method and then examined its operating characteristics on a dataset comprising 763 cases with migraine and 769 controls without migraines, genotyped for 5784 SNPs within 1696 genes (described in Drug Discovery Today 2005, 10:177). Whilst there was some overlap between the genes identified using the minimum p-value in single marker analysis and Taxonomy, this new method indicated that 2 genes (out of 98 genes which were associated with migraine with a p value ≤ 0.05 in the minimum p-value with permutation) were markers of stratification rather than disease status. Taxonomy also detected some interesting association for 5 putative susceptibility genes that were weakly (i.e. $p = 0.14$) associated with migraine in the minimum p-value analysis. Altogether, this study indicates that Taxonomy has the potential to separate disease susceptibility SNPs from SNPs associated with diseases due to stratification, and is a novel multivariate method to reduce false positive rates, without multiple testing adjustments.

2108/W

Incorporating endophenotypes into family-based allelic association studies. *W.C. Wang[1], I.S. Chang[2], C.H. Chang[1], C.A. Hsiung[1].* 1) Division of Biostatistics and Bioinformatics, National Health Research Institutes, Taiwan; 2) Institute of Cancer Research, National Health Research Institutes, Taiwan.

For a genetic study in which there are concordant and discordant sibpairs for a complex disease trait and there are also available the measurements of other endophenotypes for each of the individuals, we describe a test for association that utilizes nonparametrically the additional endophenotypes. The usefulness of this method is evaluated in simulation studies, which show that the gain in power is influenced by not only the endophenotypic value but also the correlation between the diagnosis-based phenotype and the endophenotype. Comparison with multivariate FBAT in terms of power will be presented. An additional benefit of our approach is that it provides a method to evaluate the usefulness of endophenotypes. This study is partly motivated by the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRe) study, which included both concordant sibpairs (both sibs being hypertensives or hypotensives) and discordant sibpairs (one hypertensive and one hypotensive sib) and collected several biochemical assay data on metabolic variables. Data from the SAPPHIRe study are used to illustrate the method.

2109/W

Optimizing the power of association studies by using disease samples from other studies to augment the controls. *J. Zhuang[1], A. Morris[1], K. Zondervan[1], F. Nyberg[2], A. Jawaid[3], B. Barratt[3], L. Cardon[4].* 1) Wellcome Trust Centre for Human Genetics, Oxford Univ., UK; 2) Discovery Medicine and Epidemiology, AstraZeneca, Sweden; 3) Research and Development Genetics, AstraZeneca, UK; 4) Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

In the past year, genome-wide association studies have proven to be successful by revealing a number of new disease loci. In doing so they have highlighted the fact that many loci of modest effect remain undetected due to the need for sample sizes involving 1000s-10000s individuals. Large-scale international initiatives such as the Wellcome Trust Case Control Consortium (WTCCC), the Genetic Association Information Network (GAIN), and the database of genetic and phenotypic information (dbGaP), aim to facilitate discovery of modest-effect genes by making genome-wide data publicly available. These resources are designed to improve the detection of disease genes by allowing otherwise disparate disease datasets to be combined at the level of raw data. However, the power to detect allelic association also relies on the size and attributes of the control sample so these same 'disease' samples can, in principle, also dramatically increase power via judicious use as genetically-matched 'controls' for other traits. Using the case-control design, we have developed three strategies for optimally combining external 'cases' to augment control samples and increase power. We present the biological motivation for the problem and the theoretical potential for the public data to contribute striking gains in power. We then use the WTCCC data and a large number of simulations to evaluate the power of the approach and show the retention of nominal significance levels when no real effects are apparent. We demonstrate the practical utility of these procedures in the WTCCC data, in which we show that previously undetected loci can be revealed (and subsequently replicated) which would have otherwise been missed because they were below thresholds of detection.

2110/W

Predicting Gene Coverage: How many SNPs are enough? *A. Mukherjee[1], K. Roeder[2], B. Devlin[3].* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Carnegie Mellon Univ, Pittsburgh, PA; 3) Univ Pittsburgh School of Medicine, Pittsburgh, PA.

In a candidate gene study the strategy is to measure a set of single nucleotide polymorphisms (SNPs) in the vicinity of each gene of interest and test for association. The effectiveness of these studies depends on how well the tag SNPs represent the genetic variation within the candidate genes. An effective set of tag SNPs will reveal association between the phenotype and one or more SNPs even when a causal polymorphism located in the proximity is not measured. Ideally one would choose the tag SNPs based on a complete catalog of genetic variants in the region; however, such a listing is often not available, and the best available resource is HapMap. Although the coverage of HapMap is good on average, it varies by region. The goal of this inquiry is to build a model using available covariates to determine whether or not the coverage of a particular gene is good. If the gene appears to be poorly tagged, additional work could be performed to obtain more SNPs in the region to evaluate the effectiveness of tag SNPs as proxies for potential causal alleles, we estimated how well they predicted unmeasured SNP genotypes in the proximity. To conduct this experiment we relied on the SeattleSNP database, which includes fully sequenced regions of the genome. The two databases have 173 genes with at least one SNP in common. Mean R^2 for each of these genes was calculated by regressing each non-tag SNP on the tag SNPs to determine the R^2. A classification and regression tree (CART) model was developed in which mean R^2 per gene was predicted by the number of tag SNPs, density of tag SNPs, and total SNP density. Including both common and rare SNPs, the average prediction using our CART model was high (55.8%). Even for genes with only 1-2 tag SNPs the mean prediction was good (55.1%). As expected, for genes with low linkage disequilibrium between SNPs, few tag SNPs and high total SNP density, prediction was lower. With our CART model we can estimate the SNP coverage of other genes for which we do not possess a complete listing of SNPs. As an illustration, we estimated tag SNP coverage of the genes from one of our candidate gene association studies.

2111/W

Efficiency of microsatellite markers in linkage disequilibrium mapping for a disease variant. *J. Ohashi.* Dept Human Gen, Grad Sch Med, Univ Tokyo, Tokyo, Japan.

Linkage disequilibrium (LD) mapping for identifying a disease variant has been applied to candidate gene approach and genome-wide screening in association studies. Two genetic markers, single nucleotide polymorphism (SNP) and microsatellite markers, can be used for LD mapping. Although SNP markers, which are the most abundant genetic marker in the human genome, are suitable for detecting a common disease variant with high population frequency, common SNP markers with minor allele frequency (MAF) of more than 15% are hard to detect a rare disease variant due to the low LD between the two loci (i.e., low r2). For such a rare variant, microsatellite marker with multiple alleles may show high statistical power in association studies because one of alleles may be in strong LD with the variant. However, as the number of alleles at microsatellite marker increases, the statistical power may decrease due to the increase in degree of freedom in the chi-square test for comparing allele frequencies between cases and controls. This largely depends on the relationship or LD structure between the microsatellite marker and the disease variant. In this study, taking the LD structure achieved by population history (e.g., random genetic drift, mutation, and recombination) into consideration, we examined the efficiency of microsatellite markers in LD mapping for disease variant. We have used the SelSim software developed by Spencer and Coop (2004) to obtain the simulated haplotype data under the assumption of a symmetric single step mutation model for a microsatellite marker. Our calculations show that microsatellite markers with high mutation rate (e.g., 10-3 per transmission) have more power to detect LD, regardless of the allele frequency of the disease variant, than do microsatellite markers with low mutation rate (e.g., 10-5 per transmission) under otherwise equivalent conditions. The present results suggest that microsatellite markers with multiple alleles can detect LD with a closely located disease variant with low allele frequency.

2112/W

Leveraging the HapMap correlation structure in association studies. *N. Zaitlen[1], H. Kang[1], E. Eskin[2], E. Halperin[3].* 1) Bioinformatics, Univ California, San Diego, La Jolla, CA; 2) Computer Science, Univ California, Los Angeles, LA, CA; 3) International Computer Science Institute, Berkeley, CA.

Recent genotyping technologies have driven down the costs of association studies and have enabled the measurement of SNP allele frequency differences between case and control populations on a genomewide scale. A key aspect in the efficiency of association studies is the notion of "indirect association," where only a subset of SNPs are collected to serve as proxies for the uncollected SNPs. Recently, a new class of methods for indirect association, multimarker methods, has been proposed. Although the multimarker methods are a considerable advancement, current methods do not fully take advantage of the correlation structure between SNPs and their multimarker proxies. We propose a novel multimarker indirect-association method, WHAP, that is based on a weighted sum of the haplotype frequency differences. In contrast to traditional indirect-association methods, we show analytically that there is a considerable gain in power achieved by our method compared with both single-marker and multimarker tests, as well as traditional haplotype-based tests. In order to extend the power and applicability of WHAP and multi-marker methods in general we develop additional techniques that are well studied in the single marker case. First, we describe a novel method to pick tags with the intent of carrying out a WHAP based analysis. Compared to traditional single SNP tagging methods, we observe a strong gain in power with the same genotype cost. Our method can be applied to select SNPs for a follow up study or to develop more powerful whole genome tag sets. Second, we show how to determine which multi-marker tags to evaluate in a population distant from the HapMap populations. The class of multi-marker tests rely on the use of a reference panel such as the HapMap to estimate the correlation between SNPs and markers. However, in many case control studies the population does not match one of the reference populations. We selectively include or exclude multi-marker tests based on local similarity of correlation structure. This technique reduces false positive rate and improves power.

2113/W

Haplotype-based association test for X-linked markers with quantitative trait. *L. Zhang[1,2], E.R. Martin[3], R.W. Morris[4], Y.J. Li[1].* 1) Center for Human Genetics, Dept of Medicine, Duke University, NC; 2) Bioinformatics Research Center, NC State University, NC; 3) Miami Institute of Human Genomics, University of Miami, FL; 4) Dept of Anesthesiology, Duke University, NC.

Haplotype analyses may provide more information than single locus tests in association studies. Many haplotype association methods, family or population-based, have focused on autosomal loci although X-linked genes may be important in some complex diseases. The X-linked loci differ from autosomal loci in their sex-dependent genotypes and mechanism of female X-inactivation. In this study, we develop a family-based haplotype association method of X-linked markers for quantitative traits. We have extended a single locus association method for quantitative trait locus (QTL) on X chromosome to haplotypes. Our haplotype method exhibits three virtues: 1) we have derived solutions for estimating additive genetic value of X-linked QTL and variances of effects through haplotype markers within the likelihood ratio framework. 2) Ambiguous phases of haplotypes and missing parental data can be inferred by EM algorithm conditional on all offspring genotypes and parental mating-type frequencies in the population, which improves computational efficiency and precision of parameter estimation. 3) Dosage compensation (DC) models provide a simple relationship of X-linked additive effects between sexes. Our method has significant power in complete presence or absence of X-inactivation. Properties of our approach are demonstrated by extensive studies using simulated data. The results show that our haplotype-based approach is robust to various biases, including linkage, polygenic effect, and the population admixture, as we vary the sample size (100--2000 families), haplotype frequencies of markers, and allele frequency of X-linked QTL (0.1--0.5). Application of the new method is illustrated by a candidate-gene study of family data with age-at-onset (AAO) of Parkinson Disease (PD) as a quantitative trait. The X chromosome markers RS1800659 and RS979605, located in introns 5 and 12 in MAOA, showed strong evidence of association with patient's AAO of PD(p=0.0075 in non-DC test and p<0.0001 in DC test).

2114/T

Genome-wide association data highlight a series of independent disease signals in regions implicating cyclin-dependent kinase pathways. *N.J. Timpson[1, 3], E. Zeggini[1], M.N. Weedon[2], C.M. Lingdren[1], T.M. Frayling[2], K.S. Elliott[1], H. Lango[2], J.R.B. Perry[2], N.W. Rayner[1], R.M. Freathy[2], J.C. Barrett[1], C.J. Groves[1], A.D. Morris[1], A.T. Hattersley[2], M.I. McCarthy[1].* 1) University of Oxford,UK; 2) Peninsula Medical School, Exeter,UK; 3) MRC CAiTE Centre,UK.

The Wellcome Trust Case Control Consortium recently completed a GWA scan in 1924 UK T2D cases and 2938 controls. Given these data, single-point (SP) result follow-up in additional UK case/control samples and available WTCCC results in other disorders, we aimed to perform SNP-specific, haplotypic and conditional analyses within the WTCCC data-set to dissect the nature of observed association signals and to compare this across T2D and other diseases. A region of chr9 containing genes implicated in the regulation of CDK (cyclin-dependent kinase) has emerged as a confirmed T2D associate in separate follow-up and GW analyses (combined WTCCC/UK/FUSION/DGI OR 1.20 (1.15-1.25) p=2.2x10-15). Further analysis of the genomic structure of this region and comparison to coronary artery disease (CAD) has revealed evidence for (i) two independent signals punctuated by a recombination hotspot, with that upstream of this revealing a second and independent signal for T2D, (ii) strongest association being from haplotypic and conditional analyses indicating the effects of a variant as yet untyped upstream of this feature and (iii) that evidence for association of this region with CAD reveals a third independent association signal. Taking variation matched across all studies, analyses from WTCCC and an independent GWA study gives strong evidence for association between the region of T2D signal upstream of this hotspot and CAD (combined WTCCC/DeCode WGA OR 1.27 (1.22, 1.35). At this SNP there is no evidence for a T2D effect, but this appears to be an independent disease signal (WTCCC CAD signal OR 1.25 (1.19, 1.31) versus 0.97 (0.89 1.05) for T2D). As well as implicating this region in the aetiology of CAD and T2D, analyses suggest that the CAD, upstream T2D signal and downstream T2D signal found are independent. This highlights the importance and complexity of disease risk architecture for this previously unassessed region of the genome.

2115/T

Dynamic Interaction between Gene and Environment. *X. Zhou[1], H. Xiong[3], F. Alert[1], M. Xiong[2].* 1) Dept Internal Medicine, Univ Texas, Houston, Houston, TX; 2) Human Genetics, Univ Texs, School of Public Health, Houston, TX; 3) Department of Computer Science, Texas A&M Univ, Collage Station, TX.

The traditional interaction between the gene and environment is usually defined and measured in terms of joint action of the genotype and environment in causing variations of phenotypes or diseases which are in the steady states. However, the traditional concept of interaction between the gene and environment is insufficient for getting deep understanding gene-environment interaction. We not only need to study static gene-environment interaction, but also dynamic gene-environment interaction. As a proof of principle, we propose to study dynamic interaction between the gene and environment. In this report, we develop dynamic model of the biological systems perturbed by environment, which describe how the gene and environment jointly affect the dynamical changes of the phenotypes and dynamic properties of the biological systems.. We investigate the input and output stability of the biological systems perturbed by the environment. We propose the statistics based on information theory to measure and test dynamic interactions between the gene and environment. The proposed methods for investigation of dynamic interaction between the gene and environment has been applied to gene expression time course data of the systems sclerosis. In this real dataset, we study Two types of interactions between the gene expression and environment. One type of interaction is the joint contribution of the genotype of either targeted gene or other genes and environment to the change rates of the expression of the targeted genes. Another type of interaction is the contribution of the environment alone to the rate changes of the expressions of the targeted gene. Our preliminary results reveal the pattern of dynamic interaction between the gene and environment and detect the interactions between the gene and environment which are difficult to detect by the traditional methods (including longitudinal data analysis) for detection of the gene-environment interaction.

2116/T

A Support Vector Machine Approach for Detecting Gene-Gene Interaction. *S.H. Chen[1], J. Sun[2], L. Dimitrov[2], A.R. Turner[2], T.S. Adams[2], S.L. Zheng[2], H. Grönberg[3], J. Xu[2], F.C. Hsu[4].* 1) Department of Industrial Management, National Yunlin University of Science and Technology, Yunlin, Taiwan; 2) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 4) Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

Although genetic factors play an important role in most human diseases, multiple genes or genes and environmental factors may influence individual risk. In order to understand the underlying biological mechanisms of complex diseases, it is important to understand the complex relationships that control the process. In this paper, we consider different perspectives, from each optimization, complexity analysis, and algorithmic design, which allows us to describe a reasonable and applicable computational framework for detecting gene-gene interactions. Accordingly, support vector machine and combinatorial optimization techniques (local search and genetic algorithm) were tailored to fit within this framework. Although the proposed approach is computationally expensive, our results indicate this is a promising tool for the identification and characterization of high order gene-gene and gene-environment interactions. We have demonstrated several advantages of this method, including the strong power for classification, less concern for over-fitting, and the ability to handle unbalanced data and achieve more stable models. We would like to make the support vector machine and combinatorial optimization techniques more accessible to genetic epidemiologists, and to promote the use and extension of these powerful approaches.

2117/T

Ancestry block correction: A new method to correct for stratification in genome-wide association studies in admixed populations. *J. Estrada-Gil[1], I. Silva-Zolezzi[1], A. Hidalgo-Miranda[1], J. Fernadnez-Lopez[1], E. Hernandez-Lemuz[1], R. Goya-Ogarrio[1], I. Pe'er[2], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine, Mexico; 2) School of Engineering & Applied Science, Columbia University, NY.

Genome-wide association studies (GWAS) have emerged as a powerful approach to identify genetic variants related to common diseases such as age-related macular degeneration and diabetes. Association studies in admixed populations may show spurious results due to population stratification. An approach to correct this effect is to estimate and include individual admixture proportions in the analysis. However, for individuals of admixed ancestry this correction may be of limited benefit since their chromosomes consist of mosaics of blocks derived from ancestral populations. We propose a new method that includes calculations of ancestry block proportions. To compare performance between using individual admixture and ancestry block corrections, we analyzed over 100,000 SNPs in 300 individuals from six different regions of Mexico. Admixture proportions were inferred using 3 sets of Ancestry Informative Markers (AIMs) (2824 with $\delta \geq 0.3$, 1479 with $\delta \geq 0.5$ and 127 with $\delta \geq 0.7$), and ancestry blocks with all markers. Using these data, we simulated three scenarios for GWAS: 1) A model without phenotype; 2) A monogenic model with full penetrance exemplified by the lactase persistence phenotype and, 3) Phenotype with incomplete penetrance. In the first simulation, 100 spurious associations were corrected using either method. In the second scenario, 2 spurious associations (p=3.6E-8 and p=1.2E-7) were corrected by means of ancestry blocks (p=1.3E-2 and p=5.7E-6) but not by individual admixture (p=1.4E-6 and p=1.E-7). For the third model, 64 spurious associations were detected (mean p=4.5E-8). Association p-values for these 64 signals were less significant using ancestry block correction (mean p=0.241) than using individual admixture correction (mean p=0.034). Our results show that in admixed populations, ancestry block analysis may provide better correction for population stratification with a potential benefit for GWAS.

2118/T

A Grid-based web service for the analysis of genome-wide association data. *A. Herrmann[1,2], A. Franke[1], S. Buch[1,2], M. Nothnagel[3], T. Steinke[4], S. Schreiber[1], M. Krawczak[3], J. Hampe[2].* 1) Institute for Clinical Molecular Biology, Christian-Albrechts University, Kiel, Germany; 2) Department of General Internal Medicine, University Hospital Schleswig-Holstein Campus Kiel, Kiel, Germany; 3) Institute of Medical Statistics and Biometry, Christian-Albrechts University, Kiel, Germany; 4) Zuse Institute Berlin, Berlin, Germany.

Genome-wide association analysis has been shown to be a successful approach to the identification of susceptibility factors for complex human disorders. The timely handling and analysis of genome-wide association data poses logistic and computational challenges. A typical experiment involving thousands of individuals will usually generate in excess of a billion genotypes. The Grid implementation may allow a faster administration and analysis of large amounts of disease association data. We have therefore ported and extended the Genomizer stand-alone software (www.ikmb.uni-kiel.de/genomizer) as a Grid application within the MediGRID project framework, which is part of the German e-Science initiative D-Grid. User friendly access is realised by GridSphere Java Portlet technology. To accses the distributed and shared Grid resources use OGSA-DAI, SRB and GLOBUS technologies. Certificate management secures critical data which contains patient information. Currently implemented analysis cover the workflow of an association experiment, including data management, single-point and haplotype analysis, ''lead'' definition, and data visualization.

2119/T

Prediction of osteoporosis candidate genes by computational disease gene identification strategy. *Q. Huang[1], G. Li[1], W. Cheung[1], Y. Song[2], A. Kung[1].* 1) Dept Medicine, Univ Hong Kong, Hong Kong, Hong Kong; 2) Genome Research Center, Univ Hong Kong, Hong Kong, Hong Kong.

Osteoporosis is a complex disease with strong genetic component. To date, more than twenty genome wide linkage scans across multiple populations have been launched to hunt for osteoporosis susceptibility genes. Some significant or suggestive chromosomal regions of linkage to bone mineral density have been identified and replicated in genome-wide linkage screens. However, identification of key candidate genes within these confirmed regions is challenging. Now some bioinformatics tools are available for disease gene identification. These tools use information extracted from public online databases, such as sequence data, medical literature, gene ontology and function annotation, and information on biology, function and gene expression. In this study we used five freely available bioinformatics tools (Prioritizer, Geneseeker, PROSPECTR and SUSPECTS (PandS), Disease Gene Prediction (DGP) and Endeavour) to analyze the thirteen well replicated osteoporosis susceptibility loci (1p36, 1q21-25, 2p22-24, 3p14-25, 4q25-34, 6p21, 7p14-21, 11q14-25, 12q23-24, 13q14-34, 20p12, 2q24-32 and 5q12-21) and identify a subset of most likely candidate osteoporosis susceptibility genes that are largely involved in TGF-β signaling, GM-CSF signaling, axonal guidance signaling, PPAR signaling, and Wnt/β-catenin signaling pathway. The list of most likely candidate genes and the associated pathway identified might assist researchers in prioritizing candidate disease genes for further empirical analysis and understanding of the pathogenesis of osteoporosis.

2120/T

Computational identification of candidate loci for recessively inherited mutation using high-throughput SNP arrays. *M. Laakso[1,3], S. Tuupanen[2,3], A. Karhu[2,3], R. Lehtonen[2,3], L.A. Aaltonen[2,3], S. Hautaniemi[1,3].* 1) Computational Systems Biology Laboratory, Institute of Biomedicine; 2) Department of Medical Genetics; 3) Genome-Scale Biology Research Program, Biomedicum Helsinki, University of Helsinki.

Single nucleic polymorphisms (SNPs) are one of the most abundant genetic variations in the human genome. Recently, several platforms for high-throughput SNP analysis have become available, capable of measuring thousands of SNPs across the genome. Tools for analyzing and visualizing these large genetic datasets in biologically relevant manner are rare. This hinders effective use of the SNP-array data in research on complex diseases, such as cancer. Our major objective is to develop methods for identifying DNA regions that likely harbor recessive mutations. We describe a computational framework to analyze, integrate and visualize SNP-array data. First, the methods to identify biologically interesting regions are implemented as a module (CohortComparator) that can be used for the rapid and integrated analysis of SNP-microarray data. Second, we have constructed a framework (RegionAnnotator) for annotating the genes emerging from CohortComparator analysis. The algorithms are designed to have high sensitivity and the identified regions are ranked using a scoring algorithm. Our method does not assume a close relatedness between the samples. We have also developed annotation tools that automatically query gene IDs, microarray probe IDs, gene ontology information etc. Annotations of the DNA regions are used to integrate genotype information into other sources of data such as gene expressions and literature searches. The methodology has been tested both with simulations and real data. In our case study we analyze 50k and 100k SNP-data from 41 patient samples, from which two samples harbored a MYH mutation, and 51 reference samples. Our results show that the methodology presented here is effective and capable of identifying and ranking loci with recessive mutations, if such exist in the data. In summary, our new software provide means to speed up the process to transform high-throughput SNP data set to biomedical knowledge.

2121/T

Multifactor Dimensionality Reduction 1.0. *J.H. Moore, B.C. White, N. Barney*. Deptartment of Genetics, Dartmouth Medical School, Lebanon, NH.

Multifactor Dimensionality Reduction (MDR) was developed as a computational alternative to parametric statistical methods for detecting, characterizing and interpreting epistasis in genetic studies of common human diseases. MDR uses a constructive induction approach to change the representation space of the data to make interactions easier to detect using classification methods such as naive Bayes or logistic regression. Our goal was to make MDR available to the human genetics community for both applied and theoretical studies through a software package that is open-source, freely-available, user-friendly, and platform-independent. We released the first beta version of MDR in February of 2005 and made it freely available for download via the popular Sourceforge.net website. We describe here a mature version 1.0 of the MDR software that is the result of more than three years of development and testing. The MDR software has been downloaded more than 10,000 times placing it in the top 40 from more than 1,000 bioinformatics software packages maintained on Sourceforge.net. PubMed lists more than 100 published papers with MDR in the title or abstract making it one of the more commonly applied methods for detecting gene-gene interactions. An advantage of MDR is that it can detect epistasis in the absence of main effects. The MDR software provides powerful analytical methods that embrace, rather than ignore, the complexity of the genotype-phenotype mapping relationship that underlies a variety of commun human diseases.

2122/T

REVERSE PHENOTYPING USING MULTIVARIATE DISTANCE-BASED ANALYSIS. *T.G. Schulze[1], O. Libiger[2], A.E. Baum[3], L. Kassem[3], A. Georgi[1], J. Strohmaier[1], F. Schirmbeck[1], A. Karpushova[4], R. Abou Jamra[5], J. Schumacher[5], S. Hoefels[6], M.M. Noethen[4], S. Cichon[4], M. Rietschel[1], F.J. McMahon[3], N.J. Schork[2], NIMH Genetics Initiative Bipolar Disorder Consortium.* 1) DivGen Epidemiology Psychiatry, Centr Inst Mental Health, Mannheim, Germany; 2) Scripps Res Inst, San Diego, CA; 3) GBMAP, NIMH, Bethesda, MD; 4) Genomics, Life & Brain Cntr, Univ of Bonn, Germany; 5) Inst of Hum Genet, Univ of Bonn, Germany; 6) Dept of Psychiatry, Univ of Bonn, Germany.

We recently introduced the concept of reverse phenotyping in complex genetic traits in order to identify genotype-phenotype correlations contributing most to linkage or association findings: genetic marker data is used to drive, or form the basis of, new phenotype definitions. With the advent of whole genome association, there is a need to perform reverse phenotyping for a multitude of phenotypic and genotypic data in order to understand the biological and clinical significance of associated genetic variations. We outline an approach combining the idea of reverse phenotyping with recently developed multivariate analysis methods that consider variation in measures of genomic and phenotypic distance (or similarity) among a set of individuals. We study the utility of different measures of genomic similarity, e.g. IBS allele sharing weighting by allele frequency ancestry. The methodology is illustrated using data from our recently published whole genome association study on bipolar disorder (Baum et al. 2007; initial US study set: 461 cases and 563 controls; German replication set: 772 cases and 876 controls) identifying and replicating 88 SNPs in 80 genes. Our method exhibits great power while maintaining appropriate type I error rates. Varying degrees of missing genotype or phenotype data can be accommodated. Compared to a reverse phenotyping approach based on consecutive single marker or haplotype analyses, our more holistic multivariate approaches provide insights traditional univariate methods cannot. The joint of effect of variations within a gene or across different genes can be flexibly modelled and related to single or cluster of phenotypes.

2123/T

Information Bottleneck Method for Biomedical Paper Clustering. *H. Siu[1], L. Jin[1], M. Xiong[1,2].* 1) Genetics, Fudan University, Shanghai, China; 2) Human Genetics Center, University of Texas, School of Public Health.

Biomedical literature on complex diseases dramatically grow day by day in the internet. Without automatic clustering the articles we will be lost in the huge biomedical literature. We apply Information Bottleneck Method in our research work, and find a good way to shorter time in identifying articles containing complex diseases, genes or markers. Our datasets are download from PubMed databse, we pick out five classes, every classes include 500,1000,2000 three levels articles, each of the accuracy is over 75%, when we pick out 10 classes of articles for the document clustering, accuracy is about 55%. We propose to use information bottleneck to cluster literatures. Our results show that it is possible to develop a method to automatic cluster biomedical articles and that a more powerful software can be design to classify these large data set by disease, genes and pathways, linking disease with pathways.

2124/T

Accurate prediction of deleterious protein polymorphisms. *A. Torkamani[1], N.J. Schork[2].* 1) Graduate Program in Biomedical Sciences; Department of Medicine; and Center for Human Genetics and Genomics, University of California at San Diego, La Jolla, CA 92093; 2) Scripps Genomic Medicine and Department of Molecular and Experimental Medicine, The Scripps Research Institute; Departments of Psychiatry and Biostatistics, Center for Human Genetics and Genomics and Stein Institute for Research on Aging, University of Ca.

Contemporary, high-throughput sequencing efforts have identified a rich source of naturally occurring single nucleotide polymorphisms (SNPs), a subset of which occur in the coding region of genes and result in a change in the encoded amino acid sequence (nonsynonymous coding SNPs or 'nsSNPs'). It is hypothesized that a subset of these nsSNPs may underlie common human disease. Testing all these polymorphisms for disease association would be time consuming and expensive. Thus, computational methods have been developed to both prioritize candidate nsSNPs and make sense of their likely molecular physiologic impact. We have developed a sequence-based method to prioritize nsSNPs and have applied it to the human protein kinase gene family. The results of our analyses provide high quality predictions and outperform available whole genome prediction methods (74% vs. 83%; prediction accuracy). Our analyses and methods consider both DNA sequence conservation - which most traditional methods are based on - as well unique structural features of relevant proteins, such as group membership, domain residence, and protein flexibility. We also provide a ranked list of common kinase nsSNPs that have a higher probability of impacting human disease based on our analyses.

2125/T

High-resolution copy number variation detection: application of an integrated hidden Markov Model on Illumina whole-genome SNP genotyping data. *K. Wang[1], M. Li[2], D. Hadley[1,3], R. Liu[1], J. Glessner[4], S. Grant[4], J. Kim[3], H. Hakonarson[4], M. Bucan[1].* 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Department of Biostatistics, University of Pennsylvania, Philadelphia, PA; 3) Department of Biology, University of Pennsylvania, Philadelphia, PA; 4) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA.

Copy number variation (CNV) refers to segments of DNA sequences that are present at variable copy numbers in the human genome, in comparison to a reference genome assembly. Previous studies typically use array-CGH based methods for CNV detection, with resolution limited to tens or hundreds of kilobases. Here we present a hidden Markov Model (HMM) approach that uses Illumina Infinium HumanHap550 high-density SNP genotyping data for CNV detection. In the HMM model, the emission probabilities of the total signal intensity and allelic intensity ratio for each SNP are modeled through a mixture distribution, indexed by the population frequency of alleles in a large reference population. The HMM transition probabilities are dependent on distances between neighboring SNPs. The HMM parameters are estimated using the Baum-Welch algorithm. Since most CNVs are Mendelian inherited, the pedigree structure can be optionally used a posterior to validate CNV calls. We applied our method on ~1000 samples from disease cohorts and controls, and identified ~22,000 CNVs with median size of 15Kb, representing 10-100 fold increase of resolution over array-CGH based studies. About 35% of the detected CNVs are less than 10Kb, underscoring the importance of studying small CNVs for a comprehensive understanding of human genetic variation. For detected CNVs, we also describe a general strategy of combining PCR and resequencing to identify the exact breakpoint, leading to 10^5 fold increase of resolution in CNV detection and mapping. Our results demonstrate the feasibility of comprehensive genome-wide CNV fine-mapping via high-density SNP genotyping. Given the unprecedented resolution, our method unveils a new avenue towards genetic and functional studies on small and common CNVs.

2126/T

Dynamic Systems Approach to Complex Diseases. *M. Xiong[1], JD. Reveille[2].* 1) Dept Biostatistics, Univ Texas Health Science, Houston, TX; 2) Div of Rheumatology, Univer Texas Medical School, Houston, TX.

In the past century, most biologists have used locus-by-locus approach to uncover the causes of the diseases. Even if they study gene-gene interaction and gene-environment interaction, they only investigate pair-wise interactions from cross sectional studies. In other words, they only investigate interactions in the steady state of biological systems, ignoring dynamic interaction between the genes and between the gene and environment in the time varying biological systems. However, most phenotypic variations, including those involved in complex diseases and differences in drug response, are generated by integrated actions of multiple genetic and environmental factors, through dynamic, epigenetic, and regulatory mechanisms. It is systems dynamics that determine the health status of humans. To discover the true mechanisms of the complex diseases and get into deep understanding of the development of the diseases, we propose a new paradigm based on dynamic systems theory for studying mechanisms of complex diseases. A biological system that consists of phenotypes, genotypes and environments organized into complex networks are taken as a dynamic system. The complex diseases are assumed to arise from dysfunction of dynamic systems. State-space equations will be used to model biological systems. The complex phenotypes will be taken as observed variables. The environments, drugs and some genotypic information will be taken as input variables. The state variables that determine the states of biological systems are hidden. The partial parameters in the state-space models can be functions of SNPs. The extend Kalman filter will be used to estimate the parameters in the model. Statistical methods will be developed to test difference in stability between the normal individuals and unhealthy individuals. Modern control theory will be used to design interventions to improve human health. As a proof of principle, the proposed dynamic approach will be applied to autoimmune diseases. Our preliminary results show that dynamic systems approach to complex disease will open a new way to study mechanisms of the complex diseases.

2127/T

Genetic distribution of three polymorphisms of genes related with Osteoporoses in Mestizos and Amerindian populations from Mexico. *I. Nuño-Arana[1], F.J. Muñoz-Valle[2], L. Sandoval-Ramirez[3], B. Lazalde-Medina[4], H. Rangel-Villalobos[1].* 1) Ciencias Medicas , Universidad de Guadalajara, Ocotlan, Jal., Ocotlan, Mexico; 2) Centro de Investigación en enfermedades reumáticas y músculo-esqueléticas, CUCS-UdeG; 3) División de Genética, CIBO-IMSS; 4) Laboratorio de Genética, Universidad Benito Juarez del estado de Durango.

By means of PCR-RFLPs, we analyzed three different polymorphisms of genes involved in susceptibility to osteoporoses in 765 unrelated individuals from Mexican populations, including Mestizos and five Amerindian groups (Nahuas, Purépechas, Huicholes, Tarahumaras and Mayas). We analyzed the polymorphisms Sp-1 of COL1A1 gene, Bsm I of Vitamin D receptor (VDR), and A163G of the osteoprotegerin (OPG) gene. The purpose was to establish the genotype and allele distribution in this non-previously studied populations. The "s" allele in Sp-1 polymorphism has been implicated in bone mass decreased, due to defect of collagen fibres that it conforms. The Bsm I is a VDR polymorphism, with calcium-dependent response, controversially implicated in osteoporoses. Finally, OPG is a recently discovered polymorphism involved in osteoclastogenesis. In Bsm I the average frequencies for these six Mexican populations in the allele B was 64%. This result was unexpected, considering that allele b has been pointed as the predominant in previous worldwide population studies, particularity in Asian populations. For COL1A1 polymorphism, the allele S had the highest frequency (87%) in Mexican populations, which corresponds to the reported in scientific literature but with more predominance. For OPG polymorphism, the genetic frequencies were according to the reported for most of worldwide populations. The genotype distribution for the majority of loci of Mexican populations was in Hardy-Weinberg equilibrium, excepting to Mestizos for BsmI and Purépechas for COL1A1. These results represent the first data from Mexican populations, specifically Amerindian groups, which could eventually contribute for a better understanding of the risk and susceptibility to osteoporoses in this country.

2128/T

Interactively and jointly contribution of CHRNA4, CHRNB2, BDNF and NTRK2 to tobacco dependence. *M.D. Li[1], X.-Y. Lou[1], G. Chen[1], J.Z. Ma[1], R.C. Elston[2].* 1) Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, VA; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Extensive epidemiological data indicate that vulnerabilities to nicotine dependence (ND) are influenced by genes, environmental factors, and their interaction. Our recent studies support a genetic association of the nicotinic receptor alpha 4 subunit (CHRNA4), brain-derived neurotrophic factor (BDNF), and neurotrophic tyrosine kinase receptor 2 (NTRK2) with ND. Although the interacting effects of BDNF with NTRK2 and CHRNA4 with CHRNB2 have been established experimentally using in vitro and animal models, no human genetic study is reported demonstrating that BDNF interacts with NTRK2 or CHRNA4 with CHRNB2 affecting smoking behavior. To determine if the four genes are affecting ND, we genotyped 6 SNPs for CHRNA4 and BDNF, 9 SNPs for NTRK2, and 4 SNPs for CHRNB2 in a case-control sample containing 275 unrelated smokers with a FTND score of 4.0 or more and 348 unrelated nonsmokers. By using a newly developed algorithm by this group, called generalized multifactor dimensionality reduction (GMDR) method, we found highly significant gene interaction effects on ND for the gene pairs of CHRNA4 and CHRNB2, CHRNA4 and NTRK2, CHRNB2 and NTRK2, and BDNF and NTRK2. Furthermore, we found a significant interaction of CHRNA4 and BDNF on ND. No significant interaction was detected for the genes CHRNB2 and BDNF. Together, this study provides evidence on the presence of interaction among the four genes in affecting ND. Although CHRNB2 alone was not associated with ND in several previously reported association studies on ND, we found it affects ND through interaction with CHRNA4 and NTRK2.

2129/T

Prevalence and effects of gene-gene and gene-nutrient interactions on serum folate and serum total homocysteine concentrations in the United States: findings from the Third National Health and Nutrition Examination Survey DNA Bank. *Q.H. Yang[1], L.D. Botto[2], M. Gallagher[1], J.M. Friedman[3], C.L. Sanders[1], D. Koontz[1], S. Nikolova[1], J.D. Erickson[1], K. Steinberg[1].* 1) Centers for Disease Control and Prevention (CDC), Atlanta, GA; 2) University of Utah, Salt Lake City, Utah, USA; 3) University of British Columbia, Vancouver, Canada.

Abnormalities in the metabolism of folate and homocysteine are associated with conditions that contribute significantly to morbidity and mortality in the United States. Polymorphisms of genes that code for folate-metabolizing enzymes and differences in folate intake are known to affect blood concentrations of folate and homocysteine, but the effects and interactions of these factors have not been studied on a population-wide basis. DNA specimens from 7,159 people who participated in the National Health and Nutrition Examination Survey (NHANES III) during 1991-1994 were genotyped for polymorphisms of genes coding for folate pathway enzymes MTHFR (677C→T and 1298A→C), MTRR (66A→G), and CBS (844Ins68). The influence of these genetic variants on serum folate and serum total homocysteine concentrations was determined with consideration of sex, age and dietary folate intake in three racial groups. In all of the race/ethnicity groups examined, the serum folate and homocysteine concentrations were significantly related to the MTHFR 677C→T genotype but not to the other polymorphisms. People with the MTHFR 677 TT genotype had on average a 20.8% (95% CI 13.8%-27.2%) lower serum folate and 23.8% (95% CI 18.1%-29.7%) higher homocysteine concentration than people with the CC genotype. A moderate daily folic acid intake (mean 150 μg/d, 95% CI 138-162) significantly reduced the difference in mean homocysteine concentration between people with MTHFR 677 CC and TT genotypes. The MTHFR 677C→T polymorphism was associated with significant differences in serum folate and serum total homocysteine concentrations in the United States population prior to the introduction of folic acid fortification. The effect of MTHFR 677C→T on serum total homocysteine concentration appears to be reduced by moderate daily folic acid intake.

2130/T

Imputing Copy Number Variants from Family-Based Signal Intensity Data. *W. Stewart[1], M. Burmeister[2,3], M. McInnis[3], S. Zöllner[1,3].* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Human Genetics, Univ Michigan, Ann Arbor, MI; 3) Dept Psychiatry, Univ Michigan, Ann Arbor, MI.

Recent studies have shown that complex traits such as autism, resistance to HIV infection, and malaria are partially influenced by copy number variants (CNVs). For CNVs in large (>10 kilobases) regions of the genome, signal intensity data from genotyping reactions are commonly used for reliable imputation. However, for CNVs in smaller regions, reliable imputation is much more difficult. We propose a maximum likelihood method to impute the CNVs in smaller regions from family-based signal intensity data. In constrast to common approaches that ignore relationship information, our method is expected to impute the CNVs in smaller regions more accurately since we only consider CNV configurations that are compatible with Mendelian segregation. Specifically, we model single nucleotide polymorphism (SNP) intensity data as a mixture of Gaussian distributions, and we use the Elston-Stewart algorithm to sample CNV configurations conditional on the observed data. We apply our method to a known CNV on 8q24 in a study of 737 bipolar families ranging in size from 3 to 26 members. From the analysis of SNPs subsampled across this region the results show that (1) our method imputes copy number more accurately than an existing approach that ignores relationship information; and (2) that we have the potential to improve the resolution and characterization of CNV boundaries. Currently, we are extending our method to test for association between imputed copy number and disease.

2131/T

Assessing Ancestry in an Admixed Population: STRUCTURE vs EIGENSTRAT. *J.E. Below[1], A. Pluzhnikov[2], M.G. Hayes[2], J. Novembre[1], N.J. Cox[1,2].* 1) Human Genetics, The University of Chicago, Chicago, IL., U.S; 2) Medicine, The University of Chicago, Chicago, IL., U.S.

Several analytic methods to detect population structure have been recently developed. STRUCTURE, which uses a model-based clustering method on multilocus genotype data to assign individuals to populations, has been criticized for its intensive computational time on large datasets and its sensitivity to the choice of the number of clusters. EIGENSTRAT, a newer method that identifies population substructure through principal components analysis, is fast and powerful but there are questions surrounding the biological interpretation of results. We report a comparative analysis of the two methods to detect substructure and estimate proportions of ancestry in an admixed Mexican American (MA) population sampled from Starr County, TX. This dataset includes 286 cases representing the youngest age-at-onset individuals diagnosed with type 2 diabetes and 315 individuals (also from Starr County) sampled without regard to diabetes status. Samples were genotyped using Affymetrix GeneChip Human Mapping 100K arrays. 101,150 SNPs passed QC, and were both typed and polymorphic in all four populations (CEU, YRI, ASN, MA). Genomewide STRUCTURE ancestry proportions were determined for the MA samples using the unrelated individuals from the HapMap populations (60 CEU, 60 YRI, and 89 ASN) as learning populations for STRUCTURE which greatly decreased the computational time to a few hours. Our results showed a high linear correlation (R2=0.9537) between the proportion of European ancestry determined by STRUCTURE, and the ancestry values along the first axis of variation, as estimated by EIGENSTRAT (which did not use HapMap samples, and corresponds to an eigenvalue of 7.353, more than three times the values of the subsequent axes). The strong correlation between the results of EIGENSTRAT and STRUCTURE observed in this admixed sample suggests a fundamental similarity in the underlying basis of how these two methods quantify ancestry within individuals, and we will continue to explore the theoretical frameworks of this similarity in more detail using simulations and mathematical arguments.

2132/T

GMDR: A new software for detection of gene-by-gene and gene-by-environment interaction in the population-based study. *G.B. Chen[1, 2], X.Y. Lou[1], L. Yan[2], J. Zhu[2], M.D. Li[1].* 1) Dept Psychiatry & NB Sciences, Univ Virginia, Charlottesville, VA; 2) Institute of Bioinformatics, Zhejing University, PR China.

A user-friendly computer program is developed to implement the generalized multifactor dimensionality reduction (GMDR) method, a newly proposed approach for detecting gene-by-gene and gene-by-environment interactions underlying complex traits. This software consists of two main components: choosing and computing appropriate statistics and conducting GMDR analysis based on the selected statistic. Within the program, it includes built-in logistic regression and linear regression modules to compute the score statistic for categorical and quantitative phenotypes. As an alternative, user can import self-defined other statistic or score into the program. Furthermore, the program allows adjustment for covariates when using the built-in modules to calculate the score statistic. The output of the program can be in text and visual formats and save as different types of files such as JPG, JPEG, PNG, BMP or EPS. This java-based software is platform-free and can run on different operation systems including MS Windows, Linux and Mac OS. It can also run in the console form for advanced users to efficiently perform large-scale data analysis. The software is available at our website: http://www.healthsystem.virginia.edu/internet/addiction-genomics/. The source code is open to the whole scientific community and allows the user to further extend it. The project is supported by NIH grant DA-12844.

2133/T

Detection of copy number variation from high-density SNP arrays: An integrated Bayesian hidden Markov model approach incorporating pedigree information. *Z. Chen[1], M. Taddesse[1], K. Wang[2], M. Li[1].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA.

Copy number variations (CNVs) refer to gains and losses of genomic elements compared to a reference genome assembly. CNVs are common in humans and some CNVs are associated with human phenotypic variation and susceptibility to disease. Recent advances in genotyping technologies have made it possible to make high-resolution CNV calls using whole-genome SNP arrays. Various studies have demonstrated the heritability of CNVs, however, few have incorporated family structures in the analysis. Here we develop an integrated Bayesian approach that aims to incorporate family relationships when inferring CNVs in the context of parents-offspring trios. We assume that the copy number sequence along the chromosome follows a Markov model with transition probabilities dependent on genetic distances between adjacent SNPs. Specifically, we model parental CNVs through a standard hidden Markov model; given parental copy numbers at a SNP, the offspring's copy number is then modeled through Mendelian inheritance and the dependence on the copy number at the previous SNP is modeled through recombination fraction between the two SNPs. Due to cell-line artifacts or segmental mutation events during recombination, many CNVs might be non-Mendelian inherited. To accommodate such CNVs, we allow for de novo events in offspring's CNV calls and use another HMM to account for the dependence with neighboring SNPs in the same de novo CNV region. By incorporating both family data and allowing for de novo events, our method provides flexibility for the analysis of a wide range of settings, and is expected to improve the accuracy of CNV calls as compared to methods that ignore family relationships. We evaluate the performance of the proposed method and illustrate its practical utility by applying it to the analyses of simulated datasets and the CEU trio data from HapMap.

2134/T

Next-generation Sequencing of 1000 Samples To Detect Rare Variants - Opportunities and Constraints. *F.C.L. Hyland[1], H. Peckham[1], J. Malek[1], E. Cuppen[2], J. Sorenson[1], K. McKernan[1], F.M. De La Vega[1].* 1) Applied Biosystems, Foster City, CA and Beverly, MA; 2) Hubretch Lab, Utrecht, The Netherlands.

Next generation, rapid, low-cost sequencing promises to address a broad range of genetic analysis applications, including quantitative sequencing for identification of somatic mutation profiles in cancer or for allele-specific expression. Additionally, validation of whole genome association studies involves sequencing many samples at specific regions of the genome. Ideally individual samples would be barcoded; in the interim, sequencing of pooled cases or controls can detect rare mutations to elucidate the genetic basis of complex disease. The Applied Biosystems SOLiD system (Sequencing by Oligonucleotide Ligation and Detection) can sequence over 1500 MB of paired-end reads at 32 spots/run, so pooling 33 samples per spot could potentially enable the detection of variants from 50 kb in 1000 individuals at 30x coverage/sample in a single run, while simultaneously detecting large InDels. We developed a model to simulate digital sequencing in pooled samples in the presence of error. We estimate the coverage that is necessary to discover rare variants. We discover that the number of samples that can be pooled is critically dependant on the threshold for SNP calling, which in turn is strongly influenced by the measurement error rate. The higher the error rate, the fewer samples can be pooled for detection of rare variants. Beyond a certain point, increasing the coverage cannot reduce the limit of detection of low frequency variants in pooled samples below the error rate, highlighting the advantage of using two-base encoding to eliminate error reads and increase the base accuracy above 99.9%. We validated this model through SOLiD sequencing of 81 PCR amplicons from exons of EMS-mutagenized C. elegans encompassing ~25kb of sequence with over 1500x coverage on 100 pooled samples (1:200 ratio for alleles). The results were compared with di-deoxy sequencing data carried out independently for each amplicon. To date, 1/3 of the top hits have been validated. Our results suggest that low error rate is the most critical factor for detecting rare variants using next generation sequencing.

2135/T

Pleiotropy and principal components of heritability combine to increase power for association analysis. *L. Klei[1], B. Devlin[2], K. Roeder[3].* 1) Computational Genetics, Western Psychiatric Institute & Clinic, Pittsburgh, PA; 2) Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA.

When many correlated traits are measured the potential exists to discover the coordinated control of these traits via genotyped polymorphisms. A common statistical approach to this problem involves assessing the relationship between each phenotype and each single nucleotide polymorphism (SNP) individually (PHN); and taking a Bonferroni correction for the effective number of independent tests conducted. Alternatively, one can apply a dimension reduction technique, such as estimation of principal components, and test for an association with the principal components of the phenotypes (PCP) rather than the individual phenotypes. Building on the work of Lange and colleagues we develop an alternative method based the principal component of heritability (PCH). For each SNP the PCH approach reduces the phenotypes to a single trait that has a higher heritability than any other linear combination of the phenotypes. As a result, the association between a SNP and derived trait is often easier to detect than an association with any of the individual phenotypes or the PCP. When applied to unrelated subjects, PCH has a drawback. For each SNP it is necessary to estimate the vector of loadings that maximize the heritability over all phenotypes. We develop a method of iterated sample-splitting that uses one portion of the data for training and the remainder for testing. This cross-validation approach maintains the Type I error control and yet utilizes the data efficiently, resulting in a powerful test for association. Results will also be presented on an extension of the method to a multi SNP/gene level analysis.

2136/T

A Platform for the Analysis, Translation, and Organization of Whole-Genome Association Data. *M.D. Ritchie, S.D. Turner, W.S. Bush, S.M. Dudek.* Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN.

Whole-genome association (WGA) has been proposed as a solution to the challenge of identifying disease susceptibility genes for common, complex disease. Recent technological advances enable genotyping hundreds of thousands, or even 1-million single-nucleotide polymorphisms (SNPs) on thousands of samples. We are hindered in exploiting these laboratory advances because strategies for analyzing these data have not kept pace with these technological advances, thus slowing the pace of improved understanding of the genetic contribution to common human disease. Currently, no single analytical method can extract all available information from a WGA study. Because the genetic architecture for diseases varies substantially and in unknown ways, no single analytic method can be optimal for all datasets. Therefore, an integrative platform is needed that accommodates multiple analytical methods to maximize our information extraction and thus maximize our chances of dissecting complex genetic architectures. We have developed a framework that allows the integration of multiple analytic approaches. This framework can ultimately take advantage of the many exciting, novel methods that have been and are currently being developed for both family-based and case-control genetic association studies. This is crucial due to the number of novel methods being developed and the current inability to integrate these methods in a cohesive manner. PLATO (the PLatform for the Analysis, Translation, and Organization of large-scale data) integrates several analytical and knowledge-based filters to identify the important SNPs in a WGA study. The PLATO software package combines this system with a user-friendly graphical interface that allows any number of filter configurations. We have developed and implemented ten primary filters for the analysis of WGA data. Our simulation results demonstrate that using multiple filters can substantially reduce false positive results, while maintaining high power in WGA studies. PLATO will make a comprehensive analysis of WGA data feasible and provide an integral piece of the WGA puzzle for the human genetics community.

2137/T

Confronting Complexity in Late-Onset Alzheimer Disease: Application of Two-Stage Analysis Approach Addressing Heterogeneity and Epistasis. *T.A. Thornton-Wells[1], J.H. Moore[3], E.R. Martin[4], M.A. Pericak-Vance[4], J.L. Haines[2].* 1) Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Departments of Genetics and Community and Family Medicine, Dartmouth Medical School, Lebanon, NH; 4) Miami Institute of Human Genomics, Miller School of Medicine, University of Miami, Miami, FL.

Common diseases with a genetic basis are likely to have a very complex etiology. A new comprehensive statistical and computational strategy for identifying the missing link between genotype and phenotype has been proposed, which emphasizes the need to address heterogeneity in the first stage of the analysis and gene-gene interactions in the second stage. We applied this two-stage analysis strategy to late-onset Alzheimer disease (LOAD) from 654 families and an independent set of 451 cases and 699 unrelated controls. Bayesian Classification found significant clusterings ($p<0.002$) for both datasets, which used the same five SNPs in LRRTM3 as the most influential in determining cluster assignment. In subsequent analyses to detect main effects and gene-gene interactions, SNPs in three genesPLAU, ACE and CDC2were found to be associated with LOAD in particular subsets of the data based on their LRRTM3 multilocus genotype ($p<0.05$). All of these genes are viable candidates for LOAD based on their known biological function. Further studies are needed to replicate these statistical findings and to elucidate possible biological interaction mechanisms between these genes and LRRTM3.

2138/T

Gender influences the association between variation in the Acid Phosphatase 1 (*ACP1*) and percent body fat in Mexican Americans. *YH. Shu[1], J. Hartiala[1,2], A.H. Xiang[1], M. Kawakubo[1], E. Trigo[3], H. Allayee[1,2], J.M. Lawrence[4], T.A. Buchanan[3], N. Bottini[1,2], R.M. Watanabe[1].* 1) Dept of Preventive Medicine, Division of Biostatistics, Keck Schl of Med of USC, Los Angeles, CA; 2) Institute for Genetic Medicine, Keck Schl of Med of USC, Los Angeles, CA; 3) Dept of Medicine, Division of Diabetes and Endocrinology, Keck Schl of Med of USC, Los Angeles, CA; 4) Research and Evaluation, Kaiser Permanente, Pasadena, CA.

Protein tyrosine phosphatases negatively regulate insulin signaling and are candidate genes for obesity and type 2 diabetes (T2D). *ACP1* is a tyrosine phosphatase expressed in adipose tissue and a drug target in obesity. Knocking down ACP1 in liver and adipose using antisense oligos corrects obesity-induced metabolic anomalies in mice. We tested whether variation in *ACP1* is associated with obesity and/or other T2D-related traits in 143 Mexican American families of a proband with previous gestational diabetes mellitus. Subjects were phenotyped by oral (OGTT) and intravenous glucose tolerance test and DEXA scans to measure percent body fat (PBF). Our sample consists of 682 individuals (48.6% male, 51.4% female) with mean age 38.2±12.5 years and mean PBF 31.2±8.8%. Seven tag SNPs were identified from among 14 genotyped across the *ACP1* region. SNPs were tested for association with obesity and other T2D-related traits by variance components and likelihood ratio tests. rs3828329 was significantly associated with PBF (Bonferroni corrected $p=0.037$), whereby, PBF increased ~4.5% with each copy of the T allele. rs3828329 was also associated with several diabetes-related traits (fasting, 30 minute, and 2-hour insulin values from the OGTT; insulin sensitivity; waist-to-hip ratio; BMI), but became non-significant when adjusted for PBF. The interaction between rs3828329 and gender was also significantly associated with PBF ($p=0.0002$). In males, PBF increased by ~10% with each copy of the T allele, but did not change in females. We conclude that variation in *ACP1* is associated with adiposity and secondarily alters diabetes-related quantitative traits in males, but not in females. This effect could be due to disruption in insulin signaling in adipose tissue.

2139/T

Mediating and moderating types of gene-environment interaction effects in genetic epidemiology. *B.M. Chakraborty[1], M.B. Rao[1,2], R. Chakraborty[1,2].* 1) Dept. Environmental Hlth; 2) Ctr. Genome Information, Univ. Cincinnati, Cincinnati, OH.

Gene-environment interaction (GxE) is inferred from heterogeneity of environmental (E) effects across different genotypes or by showing that the genotypes (G) have different effects under varying environment. GxE effects may be significant in the absence of main effects of G and/or E. Distinctions of different forms of GxE effects are made by using concepts of mediators and moderators, analyzed by structural equation models and path diagrams. The hypothesis that the joint effects of genes, exercise habit, and diet on obesity are explained by their mediating type of interaction effects has a mechanistic support. More commonly, GxE interactions may be of moderating type, examples of which are provided here. Such situations arise when effects of each individual gene on the phenotype are modest, and/or there is no association of genotype and environmental exposures. In contrast with the case where some obesity-related genes involved in central nervous systems and energy expenditure and adipocyte differentiation may regulate food intake and energy expenditure, the moderator type of GxE effects may be involved in pediatric asthma, Parkinson's disease (PD), and impairment of neuromotor function. For childhood asthma, genotypes at certain loci (e.g., CD14) may interact with diesel exposure, without any association of genes with diesel exposure. Likewise, for PD, CYP2D6 poor metabolizers are not at increased risk of PD in absence of pesticide exposures, but pesticide's effect on PD is increased by about twofold in poor metabolizers. Without any correlation of pesticide exposure with CYP2D6 genotypes, this becomes a moderating type of pesticide x CYP2D genotype interaction effect on PD. Similarly, childhood lead exposure is associated with impairment of neuromotor function, the strength of which varies by genotypes of at 4 polymorphic sites (DRD2-A, DRD2-B, VDR, and NAT2-Taq1). Association of genotypes with lead exposure is modest (only the VDR locus mildly shows this). We provide a theoretical framework for distinguishing such GxE interaction effects with relevant power computations.

2140/T

Multi-information and Interaction Information for Testing Total Interaction and High-Order Interaction. *G. Peng[1], L. Jin[1], M. Xiong[1,2].* 1) Genetics, Fudan University, Shanghai, China; 2) Human Genetics, University of Texas, School of Public Health.

In the past, most researches mainly focus on studying pair-wise interaction (gene-gene interaction or gene-environment interaction). However, there is increasing evidence to demonstrate that high-order interactions (gene-gene-gene, gene-gene-environment, gene-environment-environment interactions) play an important role in the development of the diseases. The purpose of this report is to develop definition of total interaction and high-order interactions, and statistics for testing total interactions and high-order interaction. Interaction among several genetic and environmental factors is a fundamental concept we often encounter in genetic epidemiology, but rarely specify with precision. In this report, we define high-order interaction as inseparable genetic and environmental effects of the multiple variables. From information point of view, the interaction can be understood as sharing common information causing disease among several genetic and environmental factors. The total interaction is defined as included all interactions from pair-wise interactions to high-order interactions. We use multi-information to measure total interaction and interaction information to measure high-order interaction. The statistics based on information measure are developed to test for total interaction and high-order interaction. Estimation of distribution algorithms is developed to incorporate the test for interaction among more than three genetic and environmental factors into genome-wide association studies. The type 1 error rates and power of the statistics are calculated by large-scale simulations. We show that the power of the newly developed statistics is much higher than that of the logic regression analysis. The developed statistics are applied to the published genome-wide association studies and our current genome-wide association studies of atherosclerosis. Our preliminary results show that the developed statistics have high power to detect high-order interactions.

2141/T

An Approach to Incorporate Linkage Disequilibrium Structure in Genomic Association Analysis. *F. Zhang.* Research Triangle Institute, Resarch Triangle Park, NC.

Genomic association studies often need to analyze a large number of single nucleotide polymorphisms (SNPs) in a chromosomal region or the whole genome to assess linkage disequilibrium (LD) with disease state. However, current analytic methods have some limitations, such as lack of consensus methods of adjusting for multiple testing, computational limitations, and unknown phase issues for haplotype analysis. In this study, we propose combining principal component and regression to analyze population genomic data for associations. This method not only allows testing multiple SNPs simultaneously (thereby addressing the first limitation), but also has flexibility to control for covariates. An illustrative example is presented using a set of 27 SNPs in a gene (MBL) from a population genomic study. Using genotypic data, five principal components were extracted that explain 97 percent; of the total variation at the 27 SNPs. We found one genomic block, defined by the second principal components, that were associated with outcome of interest (p=0.019). The second principal component is weighted heavily to the mid and distal segments of the gene and spans 3 LD blocks identified by Haploview. Compared with traditional single marker-phenotype association analysis, the second principal component captured all of SNPs that were found to be significantly associated with the outcome, using any of the single marker methods: genotypic association, allelic association or Trend test for association. When multiple testing correction was taken into account, all of single markers became no significant, however, our approach still indicated a significant association of the SNPs in the genomic block with outcome of interest. The power to detect a significant association with single SNPs in the sample, for example with a alpha 0.05 and allele frequencies for 0.28 (cases) and 0.213 (controls), is only 41.26 percent. Power may be increased when principal component score is tested, even if the haplotypes are not known.

2142/T

Population specificity may not be enough: a case-based investigation of racial generalization in gene-disease association research. *J. Yu, S.M. Fullerton, J. Crouch, K. Fryer-Edwards, W. Burke.* Center for Genomics and Healthcare Equality, University of Washington, Seattle, WA.

The use of racial and/or ethnic categories in genetic research has received increasing attention, with editors and other commentators recommending greater care in the choice of racial/ethnic labels and attention to the specificity of population description. Specificity in sample description helps guard against inappropriate generalization of population-specific findings to larger, socially-defined, racial groups. It also helps researchers extend preliminary observations to new settings for the robust replication of gene-disease association. Despite the clear importance of sample specificity with respect to these social and scientific purposes, no studies have examined the use of population sampling and description in a defined literature focused on an established gene-disease association. We therefore examined the nature of population description, and its role in result interpretation, in 80 articles which investigated the association of the PPAR-gamma Pro12Ala polymorphism with diabetes and related phenotypes (published between 1997 and 2005). Our analysis suggests that population description varies widely among the collected articles, with differences related more to investigators' country of origin and the source of the study sample than to the journal's impact or year of publication. While very few of the reports hypothesized race-based differences in gene-disease association (5%), and only a small proportion of articles explicitly invoked racial genetic differences in their interpretation of association results (15%), findings from a population in a defined location were commonly generalized to a particular racial group (45%). These results suggest that care in population description does not mitigate the practice of racial generalization. To make progress in this area, greater attention will need to be paid to characterizing the conditions under which generalization by nation-of-origin or race is both empirically and socially justified.

2143/T

Importance of population substructure characterization in linkage studies of admixed populations. *C. Thompson, C. Gray-McGuire.* Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH.

Genetic admixture and the resulting population substructure are known to increase the type I error rate in association studies. However, little work has been done to assess the impact of admixture and population substructure on linkage analysis. Genetic heterogeneity, where underlying genetic factors for disease differ between populations, has been demonstrated in many complex diseases. Currently, in an attempt to create more genetically homogeneous subpopulations, investigators often stratify their linkage analyses by race. However, self-reported race may not accurately reflect ethnicity and its use is controversial. Many algorithms exist for clustering individuals into more genetically homogeneous subpopulations. To understand the effects of admixture and population substructure on linkage, we performed simulations and evaluated the type I error and power of a model-free sibling pair linkage method in a variety of scenarios representing characteristics of admixed populations. The results of the simulations indicate that stratification on inferred subpopulation is an effective way of reducing heterogeneity. Results further indicate that stratification yields the most gain when assortive mating occurs, and when the sample sizes of the subpopulations are large in relation to the number of subpopulations in the sample. As expected, stratification does not work well in cases where the genetic susceptibility locus is the same for all subpopulations or where the lack of distinct subpopulations makes stratification more difficult.

2144/T

Dissecting the genetics of Crohn's disease using the Wellcome Trust Case Control Consortium data. *C.A. Anderson[1], J.C. Barrett[1], M. Tremelling[2], N.J. Prescott[3], S.A. Fisher[3], D.P. Jewell[4], J. Satsangi[5], J.C. Mansfield[6], C.G. Mathew[3], M. Parkes[2], L.R. Cardon[1].* 1) Wellcome Trust Centre for Human Genetics, Oxford, UK; 2) Addenbrooks Hospital, Cambridge, UK; 3) Guy's Hospital, London, UK; 4) Radcliffe Infirmary, Oxford, UK; 5) Western General Hospital, Edinburgh, UK; 6) Royal Victoria Infirmary, Newcastle, UK.

As part of a groundbreaking study, the Wellcome Trust Case Control Consortium (WTCCC) recently published a genome-wide association scan for Crohn's disease (CD). Using 1748 cases and 2938 controls genotyped with the Affymetrix 500K chip, they reported four novel CD risk loci and confirmed associations to five previously known CD risk loci. The four novel CD loci were replicated in a follow-up study of 1182 CD cases and 2024 controls. Given the large number of cases, the high density of markers and the extensive subphenotype information available for each CD case, the WTCCC data represents a rich resource for further investigation of the loci underlying CD. We conducted sub-phenotype analyses of the WTCCC data, fitted interaction models to assess the possibility of epistasis and explored the extent to which further undetected loci are likely to exist for CD. For epistasis assessments, we carried out pair-wise interaction analyses of all known risk loci using the WTCCC main scan data. After accounting for multiple tests only one apparent interaction ($P = 1.9 \times 10^{-3}$) was observed between rs12037853 (1q24) and rs4958847 (*IRGM*). However, this finding failed to replicate in our replication samples ($P = 0.55$), suggesting that the CD genes known to-date act in a statistically independent manner. This finding is in keeping with those from previous gene-gene interaction studies of CD and other complex diseases. Moreover, that further loci exist is without question because we were unable to distinguish between CD cases and controls in the main WTCCC data after calculating the average identity-by-state at the known risk variants. This observation is consistent with the small proportion of phenotypic variance explained by the known risk loci, and highlights the potential for further identification of new CD variants.

2145/T

Conditioning on risk and protective alleles for Crohn disease identifies novel gene interactions. *R. Little[1], P. Van Eerdewegh[1], J. Segal[1], J. Raelson[1], P. Croteau[1], Q. Nguyen[1], S. Debrus[1], J. Hooper[1], H. Clark[2], T. Keith[1].* 1) Genizon BioSciences, St Laurent, PQ, Canada; 2) Genentech, Inc. South San Francisco, CA, USA.

While recent genome wide association studies have successfully identified a number of replicated susceptibility genes for complex diseases, much remains to be done to tease apart the underlying gene-gene interactions contributing to risk. For example, the creation of more homogeneous subsets of patients by conditioning on risk and protective haplotypes in known disease genes has the promise of uncovering genetic heterogeneity as well as epistatic interactions. We performed a GWAS for Crohn's disease (CD) in the Quebec Founder Population and identified the well established CD genes, CARD15 and IL23R, among the top signals. These regions were used for conditional analysis by identifying sets of risk and protective alleles, genotypes, haplotypes and/or haplo-genotypes. Each case was classified as a carrier or non-carrier of the risk and/or protective factors and controls were kept matched to the cases. Association analysis across the entire genome was then performed with these sample subsets, followed by an assessment of genome-wide significance using permutation analyses. Conditioning on cases lacking a specific risk haplotype in IL23R identified the CARD15 region, suggesting that IL23R and CARD15 are independent CD risk factors. Conditioning on cases with a specific risk haplotype in IL23R identified a novel associated locus, indicating an epistatic interaction. Conditioning on cases with a specific protective haplotype in CARD15 enhanced the significance of the IBD5 region, suggesting that IBD5 and CARD15 are independent risk factors. These types of subsetting studies will be useful in dissection of the genetic contribution of highly associated disease genes, but will also yield additional susceptibility loci due to enrichment for a more homogeneous population sample.

2146/T

Higher order interaction integration: how do you minimize multiple comparisons? *C.C. Aragaki, K.E. Klos, E. Boerwinkle.* Epidemiology and Biostatistics, UT School of Public Health: Human Genetics Center, Houston, TX.

In order to elucidate the pathways involved in complex disease, new analytic strategies need to be developed to integrate the human genome and environmental factors. In particular, the sheer number of potential combinations and integration of the multiple biologic pathways are two problems which need to be solved by new methods. One analytic strategy is to combine current differing analytic techniques to minimize each problem. In the following approach, we use Moore's multifactor dimensionality reduction (MDR) within a biological pathway on an intermediate endpoint to determine combinations that impact disease and then integrate multiple pathways in a Hierarchical Bayes approach. METHODS: Using candidate nonsynonymous SNPs in the lipid metabolism and renin-angiotensin pathways and cardiovascular risk factors measured in the Atherosclerosis Risk in Communities cohort study, we determined SNP-environmental factor pathway combinations that impact risk for hypertension and hyperlipidemia. We then regressed these results on cardiovascular events in a hierarchical Bayes Cox regression. RESULTS: We found that genes and environmental factors explained risk variation better than either alone.

2147/T

Information theoretic metrics for visualizing gene environment interactions. *P. Chanda[1], L.E.M. Sucheston[2,3], A. Zhang[1], D. Brazeau[4], J. Freudenheim[5], C. Ambrosone[3], M. Ramanathan[4].* 1) Dept of Computer Science, SUNY-Buffalo, Buffalo, NY; 2) Dept of Biostatistics, SUNY-Buffalo, Buffalo, NY; 3) Dept of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY; 4) Dept of Pharmaceutical Sciences, SUNY-Buffalo, Buffalo, NY; 5) Dept of Social and Preventive Medicine, SUNY-Buffalo, Buffalo, NY.

Good interactive, multi-dimensional visualization tools can provide additional perspectives that assist the user in understanding large multidimensional, Gene Environment interaction (GEI) data at an intuitive level, facilitate subsequent hypothesis generation and enhance knowledge discovery. We extend our previous vizualization approach, VizStruct, based on the information theoretic Kullback-Liebler divergence (KLD), to visualizing GEI. We develop and compare two specific information theoretic metrics, the *K*-Way Interaction Information (KWII) and the Total Content Information correlation (TCI), which are related to the KLD, for visualizing GEI in a diverse range of simulated data sets as well as a Crohn's disease dataset. The KWII and TCI spectra, which are graphical summaries of the KWII and TCI, were found to detect each known GEI in the simulated data sets for each subset of environmental and genotype variables. The patterns in the KWII and TCI spectra were informative of factors such as affected-unaffected misassignment, locus heterogeneity, allele frequencies and linkage disequilibrium. The KWII and TCI spectra were also found to successfully identify the key disease-associated SNPs in the Crohn's disease dataset. Eight of nine significant SNPs identified in previous publications were found. The sensitivity and specificity of these information metrics was further assessed by analyzing KWII/TCI identified SNPs using Pedigree Disequilibrium Transmission (PDT) test in the software package Unphased. Out of the 20 two SNP combinations (treated as haplotypes) and 20 single SNPs, 16 and 15, respectively, were found significant with the PDT. The single SNP and haplotype results indicate good concordance with existing pedigree analysis methods, making both the KWII and TCI promising metrics for visualizing GEI.

2148/T

Exposure to Secondhand Smoke and TGFβ1 SNPs Interact to Decrease Lung Function in Cystic Fibrosis. *J.M. Collaco, L.L. Vanscoy, S. Blackman, A. Bowers, K. Naughton, J. Ellen, G.R. Cutting.* Dept of Pediatrics and Institute of Genetic Medicine, Johns Hopkins, Baltimore, MD.

A major challenge for human genetics is to identify gene-environment interactions that adversely affect health. Using patients enrolled in the U.S. Cystic Fibrosis (CF) Twin and Sibling Study, we determined if exposure to secondhand smoke (SHS) in the home affected lung function and whether SHS exposure modulated the effect of variants in TGFβ1, a modifier of CF lung function. Lung disease severity was defined using forced expiratory volume in 1 second (FEV1), a quantitative measure correlated with survival. To facilitate comparison of patients, lung function measures were converted to disease-specific percentiles. The best CF-specific %ile for FEV1 within the last year was used as a cross-sectional measure. Lifetime average CF-specific %ile for FEV1 was used as a longitudinal measure. Exposure to secondhand smoke was defined as any history of cigarette smoking in the home based on parental report. Cross-sectional lung function measures differed (p=0.001) between patients exposed (mean =0.63; 95%CI: 0.59-0.67; n=211) compared to patients not exposed to SHS (mean=0.70; 95%CI: 0.68-0.73; n=500). Longitudinal lung function measures also differed (p=0.017) between patients exposed (mean=0.551; 95%CI: 0.51-0.59; n=161) compared to patients not exposed to SHS (mean=0.60; 95%CI: 0.58-0.62; n=502). All patients were typed for rs1800469 (-509) and rs1982073 (codon 10), the two TGFβ1 SNPs associated with severe CF lung disease. Patients homozygous for the -509 T allele who were SHS-exposed had lower (p=0.004) longitudinal lung function measures (0.47 ± 0.27; n=22) than non-exposed patients (0.67 ± 0.24; n=44). Similarly, patients homozygous for the codon 10 C allele who were SHS-exposed had lower (p=0.002) lung function than non-exposed patients. SHS exposure did not affect lung function in patients with other -509 or codon 10 genotypes and lung function in non-exposed patients was similar regardless of -509 or codon 10 genotype. These data demonstrate that exposure to second hand smoke dramatically alters the modifier effect of the TGFβ1 genotype upon CF lung disease severity.

2149/T

Information Measure-Based Statistics and Relative Risk and Odds Ratio-Based Statistics for Detection of Gene-Gene and Gene-Environment Interactions. *L. Luo, M. Xiong.* Human Genetics Center, Univ of Texas, 1200 Herman Pressler, Houston, TX 77030.

Over the last three decades, epidemiologists have debated intensely about how to define and measure gene-gene and gene-environment interaction in epidemiologic studies. Whether or not gene-gene or gene-environment interaction is present depends on how effects on risk are measured. Two traditional models (additive model and multiplicative model) have been used to measure effects on risk. Recently, some authors propose to use the concept of linkage disequilibrium (LD) to measure gene-gene interaction and gene-environment interaction. In this report, we propose to use mutual information to measure the gene-gene and gene-environment interaction. Then, the principle questions concerning how to define and test interactions in complex diseases are raised. In this report, we address two fundamental issues in study of interactions. First issue is that we investigate the range of interaction which each definition of interaction can cover. Second issue is the power of each statistic for detection of interaction. To unify our study, we develop four new statistics based on two traditional models for cohort study, and case-control study. We compare the power of six test statistics by large-scale simulation study. To further evaluate their power for detection of interactions, the six statistics have been applied to four published datasets. This study provides extremely valuable information for how to define and test interactions in complex diseases and open a new way for association studies of complex diseases.

2150/T

Mutual Information for Testing Gene-Environment Interaction. *X. Wu[1], L. Jin[1], M. Xiong[1,2].* 1) Dept Genetics, Fudan Univ, Shanghai, China; 2) Human Genetics, University of Texas, School of Public Health.

Abstract Despite current enthusiasm for investigation of gene-gene interactions and gene-environment interactions, the essential issue of how to define and detect gene-environment interaction remains unresolved. In this report, we define gene-environment interaction as a stochastic dependence in the context of the effects of the genetic and environmental risk factors on the cause of phenotypic variation among individuals. We use mutual information that is widely used in communication and complex system analysis to measure gene-environment interaction, and reveal its relationship with the classical concept of interaction odds ratios. We show that the information definition of interaction covers more broad cases of interactions than the logistic regression models. We investigate how gene-environment interaction generate the large difference in information measure of gene-environment interaction between the general population and disease population, which motives us to develop mutual information-based statistics for testing gene-environment interaction. We validate the null distribution and type 1 error rates of the mutual information-based statistics for testing gene-environment interaction using extensive simulation studies. By extensive simulations, we found that the new test statistics were much more powerful than the traditional logistic regression. Finally, in order to further evaluate the performance of our new method, we applied the mutual information-based statistics to three real examples. Our results showed that P-values of the mutual information-based statistics were much smaller than that obtained by other approaches including logistic regression models.

2151/T

Resolving the Power of Multifactor Dimensionality Reduction in the Presence of Many Noise Variables or Genetic Heterogeneity. *S.M. Dudek, T.L. Edwards, M.D. Ritchie.* Ctr for Human Genetics Res, Vanderbilt University Medical Center, Nashville, TN.

In human genetic studies of common, complex disease, an important consideration is the detection of joint effects at several variables. The search space to find such multi-locus associations is very large relative to the number of single locus effects. Conventional parametric approaches such as logistic regression, which were not designed to screen these spaces, suffer from low power due to multiple comparisons and subsequent corrections. The Multifactor Dimensionality Reduction (MDR) algorithm searches these large spaces with an exhaustive approach and has been shown to have good power to detect interactive effects. Prior to this study, the performance of MDR for the detection of gene-gene interaction effects given large numbers of noise variables or varying degrees of genetic heterogeneity was unknown. A variety of 2-locus and 3 locus purely epistatic genetic models with a range of effect sizes were simulated. We explored increasing numbers of SNPs (100, 500, 1,000, 5,000, and 10,000) in datasets consisting of 500 cases and 500 controls. The results show that MDR has power to detect these interactive effects in datasets that exceed the largest candidate gene studies when heritability and effect sizes are moderate to large. Three levels of heterogeneity and four sample sizes were also simulated. The results indicate that selection of a good study population, where heritability and effect size estimates are reasonable, is more relevant to the performance of MDR than sample size. This property of MDR is analogous to a parametric statistic where the power to detect an association is larger when the effect size is doubled rather than the sample size doubled. These results also demonstrate that MDR is robust to locus heterogeneity, regardless of the degree, when the definition of power is liberal. Thus, our results provide additional evidence that MDR is a powerful approach for the detection of gene-gene interactions in the study of common, complex disease.

2152/T

Reducing Selection Bias: Efficiency and Robustness of Parametric & Non-parametric Effect Estimation. *L. Faye[1,2], L. Sun[1,3], S.B. Bull[1,2].* 1) Public Health Sciences, University of Toronto, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; 3) Hospital for Sick Children, Toronto, Canada.

Genome wide association (GWA) studies cast a wide net for genetic associations. Samples showing positive results tend to arise from the tail of the true distribution due to the low power common in GWA studies. When an effect is detected, genetic association parameters are typically estimated in the same sample. Conditioning on observing a large test statistic introduces upward bias into the effect estimate, which is exacerbated by strict testing criteria. We compare two methods proposed to correct selection bias. A statistical resampling approach, based on the bootstrap (Sun and Bull 2005, Genet Epidemiol), is a flexible approach that does not require specification of the data distribution under selection. It can be easily extended to the multiple-marker GWA situation and no assumptions about marker correlation structure are required. In contrast, a maximum-likelihood-based approach (Zollner and Pritchard 2007, AJHG), incorporates information about the distribution of the data and the power to detect association, and considers a single marker in isolation.

We used simulations of selection at a single marker assuming a normally distributed parameter estimate to quantify the bias and relative efficiency of the bootstrap estimator compared to the MLE when the model is correctly specified. We further explore the robustness of the MLE to an incorrectly specified model. Under scenarios with low power and stringent testing, both estimators have moderate bias, but on average the MLE over-corrects while the bootstrap estimates under-correct. When power is between 50% and 80%, we find the bootstrap shrinkage estimator to have smaller mean bias and smaller mean squared error than the MLE. At high power levels, both estimates are unbiased on average, but have larger variance than the original estimate. Evaluation of the performance of each estimator under situations of correct and incorrect model specification is key in selecting a good practical approach.

2153/T

On the analysis of copy-number variations (CNVs) in genome-wide association studies: A translation of the family-based association test (FBAT) approach. *I. Ionita-Laza[1], G.H. Perry[2,3], B.A. Raby[4], B. Klanderman[4], C. Lee[2,5], N.M. Laird[1], S.T. Weiss[4], C. Lange[1,4].* 1) Department of Biostatistics, Harvard University; 2) Department of Pathology, Brigham and Women's Hospital; 3) School of Human Evolution and Social Change, Arizona State University, Tempe, AZ; 4) Harvard Medical School, Channing Laboratory; 5) Harvard Medical School.

It has long been thought that human genetic variation is comprised primarily of single nucleotide polymorphisms (SNPs). However, recent studies have discovered an abundance of kilobase- to megabase-sized DNA segments that vary in copy number among human genomes. These copy number variants (CNVs) can influence levels of gene transcription and translation and therefore may be involved in human phenotypic variation including complex disease susceptibility. Given that copy number data will soon be available as part of many genome-wide association studies, we propose a direct approach to CNV-association testing in family-based association studies that bypasses the issue of uncertainty over CNV calls and genotyping. Instead of establishing associations between CNV calls and the phenotype, we advocate to directly use the raw intensity values that reflect copy number. By replacing the genotypes with the intensities, we are able to translate the family-based association testing (FBAT) approach with its numerous extensions to the analysis of CNVs. We show that, by appropriate conditioning on the intensities within families, the robustness against population admixture and stratification of the family-based approach is maintained and that testing strategies that are based on the idea of conditioning within-family can be applied in a straightforward manner. The power and robustness of the approach are evaluated in simulation studies using two different platforms: array-based comparative genomic hybridization data available for the HAPMAP samples, and Illumina 550K SNP data available for a disease dataset. An application to one of the first genome-wide association studies for CNVs highlights the potential of this approach. This research was partially supported by the following grants: U01 HL065899 and P01 HL083069.

2154/T

Evaporative Cooling feature selection identifies mixed interaction and main effects for SNP association studies. *B.A. McKinney[1], D.M. Reif[2].* 1) Department of Genetics, University of Alabama School of Medicine, Birmingham, AL; 2) National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC.

Many statistical methods show optimal performance for identifying genetic variants with a high marginal effect in the population, and some model-based methods have been used to capture limited interaction effects. Recently developed machine learning methods are able to identify pure interaction effects but have less power to detect additive effects. Evaporative cooling (EC) feature selection is a machine learning method developed to identify gene-gene/gene-environment factors that influence susceptibility to disease or drug/vaccine response without neglecting the importance of variants with high marginal effect. EC is based on a thermodynamic heuristic in which SNPs are treated as a gas of atoms interacting at a certain temperature. The attribute score, analogous to a free energy, combines mutual information and Relief-F, coupled by a tuning parameter analogous to temperature. The most energetic SNPs (least relevant to the phenotype) are recursively removed (evaporated) from the gas, leaving behind a collection of attributes with the lowest information free energy. We compared EC with Random Forest (RF), which also takes into account the context of other attributes when scoring the relevance of an individual feature. Analyses were performed on several simulated interaction models involving 1500 SNPs with 500 cases and 500 controls with 100 replicates for each model. We found that EC has higher power than RF to detect interacting genetic variants. The limited ability of RF to identify interacting SNPs is due to its use of a node-splitting criterion that assumes independence between attributes during decision tree construction. The predictions of EC and RF are comparable to univariate feature selection (logistic-regression) when the relevant SNPs do not interact. We also applied these methods to real genetic data (genotypes at 1442 SNPs across 500 genes) collected to identify biomarkers associated with adverse events following smallpox vaccination of n=108 human subjects. (Supported by AI-64625.).

2155/T

Selecting SNPs using Random Forests. *Y. Meng, L.A. Cupples, L.A. Farrer, K.L. Lunetta.* Boston University, Boston, MA.

Lunetta et al. have shown that when unknown interactions among SNPs exist in a data set consisting of thousands of SNPs, random forest (RF) analysis can be significantly more efficient than standard univariate screening methods in ranking the true disease-associated SNPs from among large numbers of unassociated SNPs. In order to be practical for the analysis of real data, we need to address the question: what methods for selecting subsets of SNPs from a RF for further analysis are most powerful? Here, we evaluate two methods to select SNPs via RFs for further analysis - an iterative procedure (iterative RF), and a significance test of the IM measures (RF IM perm.p) obtained from a RF. We use two simulation datasets, each mimicking the Affymetrix 10k chip: (1) with multiple strong marginal genetic effects and no interaction (GAW 15); (2) with weak marginal genetic effects and strong interaction. The RF methods for choosing a subset of SNPs are compared with three additional methods: single SNP allelic analysis, exhaustive pair-wise allelic interactions analysis, and set association analysis.

For GAW data with strong main effects, but no interaction, RF IM perm.p and set association have the highest power. The power of iterative RFs and single SNP qvalue are lower. For our simulated model with weak main effects but strong interaction, iterative RFs and single SNP nominal p-value methods are similarly powerful; followed by RF IM perm.p, set association and exhaustive pair-wise allelic interactions analysis. We observe that either one of two RF SNP selection methods seem to perform under each of the two models, although no method is the best under both scenarios. Additional simulation models will be presented that clarify the scenarios under which each method performs best. For all procedures other than the iterative RF, the analyst must specify the number of variables or cutoff value for the variables to be selected. The iterative RF procedure provides an automated method for selecting SNPs that does not require pre-specification of selection criteria or the number of SNPs to select. Our simulations suggest that the procedure optimizes the selected number based on the signal:noise ratio in the dataset.

2156/T

Computational efficiency of Logistic regression trees algorithm as a tool for initial screening in Genome-Wide Association Studies. *V.B. Milanov, R.Z. Nickolov.* Department of Mathematics and Computer Science, Fayetteville State University, Fayetteville, NC.

Nowadays genetic epidemiology faces the challenge of dealing with immense number of genetic markers - Genome-Wide Association Studies. Finding a small number of interesting markers for further investigation can greatly facilitate genetic studies. Recently, we have shown that Logistic Regression Tree Algorithms provides an efficient tool for reduction of an initial large pool of markers to a small set of interesting markers with high probability. In this paper we compare the computational capabilities of Logistic Tree with Unbiased Selection (LOTUS) and Random Forest methods to detect an interesting genetic factors involved in disease etiology. Using the simulated data provided for Genetic Analysis Workshop 15 for rheumatoid arthritis (RA), we show how these algorithms perform under different scenarios. Our results indicate that LOTUS is computationally efficient tool for initial screening of large number of candidate markers.

2157/T

A Comparison of Principle Component Analysis and Factor Analysis Strategies for Uncovering Pleiotropic Factors. *X. Wang[1], C.M. Kammerer[1], S.J. Anderson[2], J. Lu[3], E. Feingold[1].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA, 15261, USA; 2) Dept Biostatistics, Univ Pittsburgh, Pittsburgh, PA, 15261, USA; 3) Epidemiology Data Center, Graduate School of Public Health, Univ Pittsburgh, Pittsburgh, PA, 15261, USA.

Principal component analysis (PCA) and factor analysis (FA) are often used to uncover common factors (both genetic and environmental) that contribute to complex disease phenotypes, but little formal evaluation of the performance of these two methods has appeared in the literature. We conducted a comparison analysis using simulated data from nuclear families. We first simulated 7 underlying (unobserved) genetic and environmentally determined traits. Then we derived two sets of 50 complex (observed) traits using algebraic combinations of the underlying components. We next performed PCA and FA on these complex traits. We studied three aspects of the performance of the methods: 1) the ability to detect the underlying genetic/environmental components; 2) whether the methods worked better when applied to raw traits or to residuals (that is, after regressing out potentially significant environmental covariates); and 3) whether heritabilities of composite PCA and FA phenotypes were higher than those of the original complex traits and/or underlying components. Our results indicate that both multivariate analysis methods behave similarly in most cases, although FA is better able to detect predominant signals from an underlying trait. Using residuals in the PCA or FA analyses greatly increases the probability that PCs or factors detect common genetic components instead of common environmental factors, except if there is statistical interaction between genetic and environmental factors. Finally, although there is no predictable relationship between heritabilities obtained from composite phenotypes versus original complex traits, our results indicate that composite trait heritability generally reflects the genetic characteristics of the detectable underlying components.

2158/T

A Supervised Principal Component Approach for Modeling Gene-Gene Interaction. *T. Wang, R.C. Elston.* Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH.

Multiple genetic and non-genetic factors are usually involved in the etiology of complex human diseases. The effect of a genetic variant often depends in an epistatic manner on the presence of other genetic variants, rather than simply acting additively, and this complicates efforts in mapping these genetic variants. Although modeling interactions in the analysis may be of limited value for establishing biological mechanisms, it can potentially improve power to identify disease variants that have relatively modest marginal effects. However, modeling all possible interactions involves a severe penalty owing to the greatly increased number of tests or degrees of freedom in an association analysis, which has been called the "curse of dimensionality". A more desirable strategy to maximize power should have the ability to allow for interactions while at the same time avoiding a considerable penalty arising because of a large number of interactions being possible. Recently, various approaches have been adopted to reduce dimensionality in detecting interactions. Here, we consider a semi principal component approach that compresses information from multiple correlated SNPs in a local genomic region in order to model gene-gene interactions. Our approach combines a supervised approach for SNP selection with an unsupervised approach for data compression, which provides a parsimonious way to detect gene-gene interaction. We perform a simulation study that demonstrates the validity and superior power of this method over those of several other approaches.

2159/T

Detection of multi-locus genetic interaction in aspirin-intolerant asthma with multifactor-dimensionality reduction analysis. *S.H. Kim[1], H.H. Jeong[2], H.Y. Lee[1], M.K. Kim[2], B.Y. Cho[1], J.S. Lee[3], K.B. Wee[2], H.S. Park[1].* 1) Department of Allergy and Rheumatology, Ajou University School of Medicine, Suwon, Suwon, Korea; 2) Department of Infromation & Communication, Ajou University; 3) Department of mathmatics, Ajou University.

Background and objective: Aspirin-intolerant asthma (AIA) is a common phenotype of aspirin hypersensitivity and affects about 10~20% of asthmatic patients. Recently, the single gene polymorphism associated with the AIA susceptibility has been investigated, but identification of multi-locus single nucleotide polymorphism (SNP) set in association with the susceptibility has not been investigated Subjects and methods: In this study, we selected 23 SNPs in 13 candidate genes for 94 asthmatics with aspirin hypersensitivity (AIA) and 152 asthmatics without aspirin hypersensitivity (aspirin-tolerant asthma, ATA) and genotyped each SNP by a primer extension method. Multi-locus genetic interactions were examined with multifactor-dimensionality reduction (MDR) to test all multi-locus SNP combinations for the efficient prediction of AIA. Result: Through a MDR analysis, we identified four-locus gene-gene interaction models that predict AIA disease risk among asthmatic patients with 65.16 % balanced accuracy and a cross-validation consistency of 70 %. Conclusion: These results suggest that significant epistatic effect of four-locus genetic interaction may exist in the susceptibility for AIA in asthmatic patients which may be a useful in vitro method to diagnose the AIA with acceptable sensitivity.

2160/T

genomeSIMLA: a data simulation package to explore the human genome. *T.L. Edwards, W.S. Bush, S.D. Turner, E.S. Torstenson, S.M. Dudek, M.D. Ritchie.* Ctr Human Genetic Res, Vanderbilt Univ, Nashville, TN.

In the quest for disease susceptibility loci, many novel statistical and computational methods are in development. Data simulation is necessary to evaluate the performance of these methods before their utility can be demonstrated in real data applications. However, it is difficult to emulate the properties of genetic data in human populations which are the result of complex demographic history. Explicitly modeling all linkage disequilibrium (LD) parameters observed in real data with many variables is computationally infeasible; additionally, synthetic models often lack the complexity of real data. Rather than modeling human population history or LD characteristics, we use a forward-time population simulator that uses random mating, genetic drift, recombination and population growth to allow a population to naturally obtain LD features. We have developed a software package, genomeSIMLA, that uses these properties to simulate data on a genome-wide scale for both case-control and family-based study designs with linkage disequilibrium patterns that resemble those observed in human populations. Positions of real human markers can be used to estimate the expected frequency of recombinant gametes under the Haldane or Kosambi models which can be applied to simulations to emulate patterns of LD observed in human populations. After a pool of chromosomes has developed suitable LD, datasets can be drawn by randomly sampling chromosomes with replacement. Disease-susceptibility effects of multiple genetic variables with any mode of inheritance as well as interactions between them may be modeled using a prospective logistic regression model. In contrast, purely epistatic interaction models can also be simulated. genomeSIMLA provides a robust data simulation package for creating whole-genome data to evaluate novel analysis approaches in a realistic context on a scale relevant to modern genetic epidemiology. A graphical user interface is provided for ease of use. Additionally, a website is available where pools of chromosomes using human markers with similar LD to the HapMap data may be downloaded to draw datasets from or increment further generations for new LD.

2161/T

Reduction of measurement noise in a pooling-based GWA study using multimarker analysis of SNPs in linkage disequilibrium. *N. Homer[1], W. Tembe[2], S. Szelinger[2], M. Josephson[2], J. Pearson[2], D. Stephan[2], S. Nelson[1], D. Craig[2].* 1) University of California - Los Angeles, 5554 Gonda, 695 Young Drive South, David Geffen School of Medicine at the University of California - Los Angeles, Los Angeles, CA 90095-7088; 2) The Translational Genomics Research Institute, 445 N. Fifth Street Phoenix, AZ 85004.

Recently emerged Genome-wide association (GWA) studies utilizing hundreds of thousands of single nucleotide polymorphisms (SNPs) have the potential to revolutionize our ability to identify the common genetic influences of complex traits and diseases. Additionally, numerous pooling-based studies have found associations for complex diseases such as Diabetes, Supra Nuclear Palsy, and Memory Loss. Pooling-based GWA studies are cost effective and have the power to detect significant associations. Nevertheless, the inherent difficulties of pooling-based studies are to correctly predict allelic frequency and more importantly to reduce measurement noise. In this vein, multi-marker statistics can leverage SNP linkage disequilibrium to increase resolution. Using known linkage disequilibrium from the HapMap project, we are able to improve our test of significance for each SNP and thus increase our power to detect associations. We evaluate numerous methods to combine correlated tests of significance and develop our own tests based on the underlying error model. Using pooled samples from a subset of the HapMap population we are able to assess these methods against the traditional single marker analysis and known individual genotype data and conclude an increase in power when using the multi-marker methods.

2162/T

Approaches to testing regional significance in whole genome association scans. *P.H. Kuo[1], E.J.C.G. Van den Oord[2], B.S. Maher[1].* 1) Department of Psychiatry, VIPBG, VCU, Richmond, VA; 2) Center for Biomarker Research and Personalized Medicine, VCU, Richmond, VA.

The recent escalation of whole genome association scans (WGAS) has introduced an entire set of novel problems to statistical genetics. A major issue that faces the field is interpretation of results from these scans. The primary difficulty arises from the large number of significant single marker signals these scan will generate. An alternative to assessing interesting regions across the genome is to analyze many markers in concert. Importantly, several investigators, including Marques-Bonet et al (2005), Hoh & Ott (2000) and Guedj et al (2006) have proposed methods for testing multiple markers simultaneously that do not account for, or are naïve to, genetic or physical distance. We propose a methodology that tests for clusters of significant signals across a region of the genome while accounting for the correlation between the markers. Our method, using effect statistics, corrects for the initial signal in the region to assess residual significance beyond that signal. The approach is especially useful in regions (genes) where multiple modest-sized signals are present. Similar to the previously described approaches, significance is assessed via permutation. We examined the performance of the approach on simulated whole genome association scan data.

2163/T

Powerful new methods for genome-wide copy number association studies. *C.L. Lambert[1], A. Baker[1], D.M. Hawkins[2], D.A. Peiffer[3].* 1) Golden Helix, Inc., Bozeman, MT; 2) Sch of Statistics, Univ of Minnesota, Minneapolis, MN; 3) Illumina, Inc., San Diego, CA.

Few genome-wide association studies (GWAS) have been published involving copy number variations (CNVs). This is primarily due to a lack of reliable methods, workflows and infrastructure for conducting GWAS with CNVs. The most commonly used CNV ascertainment methods are based upon Hidden Markov Models. While fast in performance, these methods generally suffer from low sensitivity and high false discovery rates (FDRs), resulting in missing small regions of CNVs, adding noise to the data, and ultimately reducing the power to find CNV associations with complex diseases. Circular Binary Segmentation (CBS) methods have shown superior sensitivity and FDRs but suffer from slow run times, making it nearly impractical to calculate CNV for the hundreds to thousands of patients in current GWAS. We present a new segmenting method based on dynamic programming that searches through all possible CNV change-points in a chromosome to find the most optimal with respect to an error metric, without suffering from the combinatorial explosion associated with such a search. We present benchmarks showing that our implementation runs dramatically faster than CBS, while maintaining equal or better sensitivity and FDR. We also specially tune our methods to extract signals, and perform appropriate normalizations on intensity data from various Illumina whole genome SNP genotyping array platforms, for maximal CNV detection. Once CNV calls have been made for all SNPs, we demonstrate how, by reducing genome-wide scans from ~550k SNPs to a few thousand "tagging" CNV segments, we are able to reduce multiple testing correction by at least an order of magnitude, dramatically increasing the power for GWAS with CNVs. Standard statistical tests can then be used. We demonstrate the complete workflow on several case/control data sets of 1000+ patients using the Illumina Hap550 and Hap300 BeadChips. Our methods make it possible to discover **statistically significant** associations using small CNV changes in genome-wide scans for complex diseases on large case/control and quantitative trait studies.

2164/T

Multi-locus analysis of whole genome association studies, and ridge regression to account for linkage disequilibrium. *N. Malo[1], O. Libiger[1], N.J. Schork[1,2].* 1) Scripps Genomic Medicine, The Scripps Research Institute, La Jolla, CA; 2) Medical School, University of California, San Diego, La Jolla, CA.

The use of whole genome association (WGA) studies for the identification of genes and genetic variations that influence common, complex diseases such as hypertension, cancer, and depression will continue to grow as cost-effective high-throughput genotyping technologies are developed. As a result, appropriately flexible yet robust data analysis strategies for analyzing WGA data will be essential. We emphasize the need to accommodate phenomena such as linkage disequilibrium via simple extensions of traditional regression models. We describe the use of regression analysis models for WGA that are very intuitive and flexible. We propose the use of ridge regression, a special case of Bayesian regression, to account for correlation. We showcase the utility of the method on previously published WGA data, and via a simulation study. We also consider limitations of the proposed approach as well as areas for further research.

2165/T

Method for identifying "ethnic outliers" among samples genotyped for genomewide or large-scale association studies. *R. McGinnis[1], W. McLaren[1], WTCCC[2], P. Deloukas[1], M. Inouye[1].* 1) Wellcome Trust Sanger Institute, Cambridge, UK; 2) Wellcome Trust Case-Control Consortium.

Genomewide association scans can be improved by minimizing genotype and allele frequency differences between cases and controls caused by ethnic admixture rather than disease susceptibility. Yet the need to collect and handle thousands of DNA samples occasions the inclusion within cases or controls of sporadic samples whose ethnicity differs from the main bulk of samples in an intended study design (such as the British Caucasian samples of the Wellcome Trust Case Control Consortium [WTCCC]). In addition to inflating disease association statistics, inclusion of "ethnic outliers" can also produce SNP departures from Hardy-Weinberg equilibrium, thereby complicating assessment of genotyping platform quality. Here we report a very effective method for identifying ethnic outliers that works by examining autosomal SNPs with (a) zero heterozygote genotype counts and few counts for the rarer homozygote or (b) zero counts for the rarer homozygote and very few for the heterozygote. We tabulated (a)-type and (b)-type SNPs separately for each set of WTCCC disease cases among 6 diseases genotyped on the AFFY500K chip and among the 4 other diseases genotyped on a 14000+ mainly non-synonymous SNP panel. By summing counts of the infrequent genotype in (a)-type or (b)-type SNPs separately for each case in a particular disease set, we identify ethnic outliers as those samples with high total counts separated from the main body of the distribution. For example, from one set of 1970 cases genotyped on AFFY500K, three samples each gave >200 counts for the rarer homozygote of (a)-type SNPs with the next highest sample giving only 28 counts. Among the SNPs contributing >200 counts, 47 SNPs were shared by at least two of the three prospective outlier samples and when HAPMAP genotype frequencies were examined these SNPs were found to be monomorphic in CEU and CHB/JPT but highly polymorphic in YRI, thus illustrating the method's effectiveness. We will present similarly definitive results from (b)-type SNPs and from other WTCCC disease sets and will explain our method in detail.

2166/T

Association mapping in admixed populations. *S. Myers[1,2], J. Marchini[2], A. Price[1,3], D. Reich[1,3], N. Patterson[1].* 1) Broad Institute of MIT and Harvard, Cambridge, MA; 2) Department of Statistics, Oxford, UK; 3) Harvard Medical School, Boston, MA.

Genome-wide association studies offer a powerful framework for identifying mutations contributing to human disease. Performing these studies in admixed populations, such as African Americans and Hispanics, should enable researchers to identify many variants affecting disease risk. Differing allele frequencies and linkage disequilibrium structures across human populations imply admixed groups are likely to be especially useful both for the identification of new mutations, and in fine-mapping causal variants. Despite these potential benefits, association mapping has thus far concentrated on examining European populations. One reason for this, and a key issue to address, is the fact that there are a number of methodological challenges specific to performing association studies in admixed populations. For example, it is important to infer information about case and control admixture "chunks", to prevent false positive associations and to fully exploit available information. We have developed and implemented an analytical framework to address such factors. Our approach uses dense genotype data to probabilistically infer admixture segments, and impute untyped SNPs, using previous variation surveys, e.g. the HapMap, as a framework. These inferences are integrated into a Bayesian full-likelihood methodology, providing a natural weighting of both broad-scale "admixture LD" information and fine-scale association information. Applying this method to simulated and real African American datasets demonstrate that typing 500,000 or more markers across the genome provides exquisite information about African American population ancestry (capturing over 95% of available information), and allows highly accurate SNP imputation. Finally, we describe the application of our approach to detect prostate cancer risk variants in 650 African American cases and controls. This revealed SNP rs6983267 as being the most strongly associated with disease status. Further, most of the other top associations are strongly replicated in several additional human populations.

2167/T

Genome-wide association scan for height in 6,671 individuals from Finland and Sardinia. *S. Sanna[1,2], A.U. Jackson[1], G. Usala[2], C.J. Willer[1], M. Dei[2], L.L. Bonnycastle[3], S. Lai[2], Y. Li[1], M. Uda[2], M.R. Erdos[3], H. Shen[4], A. Shuldiner[4], A. Cao[2], R.M. Bergam[5], D. Schlessinger[2,6], F.S. Collins[3], M. Boehnke[1], G.R. Abecasis[1], R. Nagaraja[5], K.L. Mohlke[7].* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) National Human Genome Research Institute, Bethesda, MD; 3) Istituto di Neurogenetica e Neurofarmacologia (INN), CNR, Cagliari, Italy; 4) University of Maryland, School of Medicine, Baltimore, MD; 5) Keck School of Medicine of USC, Los Angeles, CA; 6) Gerentology Reasearch Center, NIA, Baltimore, MD; 7) Dept Genetics, University North Carolina, Chapel Hill, NC.

Height represents a classic example of a highly heritable quantitative trait. In our sample, heritability analysis shows that genes can explain >80% of the variation in height. Nevertheless, with the exception of a few rare Mendelian syndromes, gene-identification has proved difficult despite many parallel mapping efforts. Genetic influences on height are probably due to the contribution of several loci of small effect. We have carried out a meta-analysis of genome-wide association results from two different groups, ProgeNIA and FUSION. The first sample consist of 4,305 individuals from 570 families from Sardinia, the second includes 2,366 mostly unrelated Finnish individuals. Since the two groups worked with two different platforms (Illumina 300K and Affymetrix 500K respectively), SNPs appearing only in one platform were imputed to allow direct comparison of results across studies. To control inflation of type I error due to outliers and departure from normality, quantile normalization was applied to each trait prior the analysis. In both GWA scans, we evaluated the additive effect of each SNP, adjusting the model for familiality and covariates. In our combined results, the top associated SNP (p= 4.0*10-7) maps to a region of LD containing several genes, including one previously implicated in growth. Replication is ongoing, but preliminary results on 2017 Finnish and 858 Amish samples support our initial finding (p=1.7*10-3), with the same direction of effect. Further detailed SNP analysis of the region is necessary to refine the responsible gene.

2168/T

The Whole-genome Association Study Pipeline (WASP): A Comprehensive Tool for Large-Scale Association Studies. *D.P. Sexton, J.L. McCauley, J.T. Giles, W.S. Bush, Y. Bradford, J.L. Haines.* Center Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

Whole genome association studies generate vast amounts of genotypic data produced by rapidly evolving technologies. Studies of complex diseases contain ever increasing sample sizes and phenotypic measures. Genotype quality assurance is nothing novel, these checks were once trivial to perform given the constraints of small datasets with few markers. However, data management can be a very time consuming task if not automated in large complex datasets. We have explored the difficulties of managing these data for ongoing family-based and case-control studies and have subsequently developed the Whole-genome Association Study Pipeline (WASP) software tool. The principle goal of this tool is to aid in storing, evaluating, formatting, and analyzing genotypic and clinical data from the latest large-scale genotyping studies. The WASP application implements a battery of quality control procedures to assess and analyze these data. The currently available procedures are the examination of marker and sample genotyping efficiency, allele frequency calculations, checks of Mendelian error and gender discrepancies (based on available chromosome X or Y genotypes), and tests of Hardy-Weinberg Equilibrium. Additionally, the application can retrieve and format data for other software programs such as the Graphical Representation of Relationships (GRR) program, STRUCTURE and EIGENSTRAT. Beyond the quality control aspect of this application, WASP can perform standard tests of association using the TDT, for family-based datasets and the chi-square test of association for case-control datasets. Additional analyses currently include the Cochran-Mantel-Haenszel test, and the Armitage Trend test, with additional analytic extensions in development. In addition to the command line procedures used in WASP, we have created a graphical user interface (GUI) data plotter (WASP Plotter) that allows the user to visually examine the data in a rapid and interactive manner. As datasets reach and exceed billions of datapoints, such tools will become a necessity for virtually all large-scale genotyping studies.

2169/T

Quantifying and correcting for the winner's curse effect in genetic association studies. *R. Xiao, M. Boehnke.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Genetic association mapping is a powerful method to detect genetic variants that predispose to human disease. Investigators are also interested in estimating the genetic effect on disease risk of each identified variant. Estimates of genetic effect based on initial positive findings tend to be upwardly biased, a phenomenon known as the winner's curse. Overestimation of genetic effect size in initial studies may cause follow-up studies to be underpowered and so to fail. In this paper, we quantify the impact of the winner's curse on the uncorrected maximum likelihood estimator (MLE) of the allele frequency difference and odds ratio between cases and controls in association studies. We then propose an ascertainment-corrected maximum likelihood method (see also Zoellner and Pritchard 2007) and an ad hoc bias-correction method to improve the estimate of the allele frequency difference. We extend these calculations to two-stage association studies. We show that the overestimation of the genetic effect by the uncorrected MLE decreases as the power of the study increases for both one- and two-stage studies. Simulation results demonstrate that the ascertainment-corrected maximum likelihood estimator reduces overestimation by different degrees, depending on the sample size, true genetic effect size, and the chosen significance level, while the bias-correction method further improves the estimator performance. We recommend using the ascertainment-corrected maximum likelihood estimator or the bias-corrected estimator unless study power is expected to be high.

2170/T

A population-based WGAS identifies novel genes associated with androgenic alopecia, while confirming association with androgen receptor. *X. Yuan[1], D.W. Waterworth[1], K.S. Song[1], V. Mayor[2], M. Firmann[2], G. Waeber[2], P. Vollenweider[2], V. Mooser[1].* 1) GlaxoSmithKline R&D, King of Prussia PA, RTP, NC and London UK; 2) CHUV University Hospital Lausanne Switzerland.

BACKGROUND: Androgenic alopecia (AGA, or male pattern baldness) is a major unmet medical need and shows some association with premature cardiovascular diseases (CVD). AGA has a genetic component, and the androgen receptor (AR) has been associated with this condition. No genome-wide linkage or association scan has been reported so far for AGA. Here, we performed a nested case-control study within the Affymetrix 500K genotyped Lausanne population-based study to identify novel genes associated with AGA, as assessed using the Hamilton classification, in men aged 35-75. METHODS: A total of 591 cases with AGA (aged 35 to 65 years with Type V-VIIIA) were compared to 561 discordant controls without AGA (aged 45-55 years with Type I/IA, or aged 55-75 years with Type I - IIA). In the primary analysis, single point analysis was performed using logistic regression and the Armitage trend test. RESULTS: A robust association was identified between 2 SNPs within the AR gene and AGA (OR = 1.4, CI = 1.1-1.8, p = 0.01; and OR = 1.6, CI = 1.3-1.9, p < 0.0001). Similarly, we found some degree of association between AGA and other candidate-genes for AGA, such as SRD5A2, CYP19 and keratins. In addition, we identified 411 SNPs within 87 genes with P-values < 10E-4, 49 of which are expressed in hair follicle. Further analyses including biological data mining and statistical analysis are being performed to examine closely the genes of interest, and a replication dataset is being sought for in an attempt to verify these results and thereby identify novel potential drug targets for AGA.

2171/T

A powerful approach via forest to identifying gene and gene-gene interactions revealing a resistant haplotype associated with age-related macular degeneration. *H.Z. Zhang, X. Chen, C.T. Liu, M.Z. Zhang.* Dept Epidemiology/Public Hlth, Yale Univ Sch Medicine, New Haven, CT.

Multiple genes and interactions among genes and among genes and environmental factors are believed to underlie most complex diseases. However, such interactions are difficult to identify. While there have been recent successes in identifying genetic variants for complex diseases, it remains to be difficult to identify gene-gene and gene-environment interactions. To overcome this difficulty, we propose a forest-based approach and a concept of variable importance. Analyses of both real data and simulated data based on published genetic models demonstrate the effectiveness of our approach. For example, our analysis of published data set on age-related macular degeneration (AMD) not only confirmed a known genetic variant (p-value <0.005) for AMD, but also revealed an un-reported haplotype surrounding single nucleotide polymorphism (SNP) rs10272438 on chromosome 7 that was significantly associated with AMD (p-value = 0.045). These significance levels are obtained after the consideration for a large number of SNPs. Thus, the importance of this work is two-fold: a powerful and flexible method to identify high-risk haplotypes and their interactions, and the revelation of a potentially resistant region for AMD.

2172/T

The ordered penetrance test for detecting single-locus association and gene-gene interaction. *M. Song[1], D.L. Nicolae[2].* 1) Department of Statistics, The University of Chicago, Chicago, IL, USA; 2) Department of Medicine and Statistics, The University of Chicago, IL, USA.

In genome-wide studies that search for loci affecting complex traits, two stage strategies, where the analyses in the second stage are done only on markers associated in the first stage have become a common choice for researchers. In this talk, we propose methods for detecting association and interaction in a 2-stage strategy which is shown to be more powerful than the classic approaches. Our method makes use of the fact that many traits are monotone in penetrance and mean and incorporating this knowledge can dramatically increase power. For qualitative traits, we develop likelihood ratio tests for both association and interaction where the asymptotic distributions for both cases are shown to be Chi-bar-squared (i.e. weighted sums of chi-squared distributions). Our simulation studies for various models show that the ordered penetrance tests are more powerful compared to other popular tests especially at genome-wide scale. For quantitative traits, analogous tests based on ordered means are proposed and asymptotic results are obtained. We will also show an important extension of our method to testing untyped variation.

2173/T

A shrinkage regression approach to tackle the HLA region. *C. Vignal[1,2], A. Bansal[2], C. Hoggart[1], D. Balding[1].* 1) Imperial College, London; 2) GlaxoSmithKline, UK.

Many autoimmune diseases have been associated with the HLA region, but the presence of linkage disequilibrium (LD) has meant that finding causal elements has been difficult. Multivariate association analyses can perform better than univariate methods, however, there can be problems when the number of variables exceeds the number of observations or in the presence of correlated predictors.

We adopt a Bayesian-inspired shrinkage regression approach for multilocus analysis of correlated data in which each regression coefficient is assigned a prior distribution that strongly favors zero values. We consider two shrinkage priors, the Laplace or double exponential distribution, and the normal-exponential-gamma distribution. Parameter inference is based on the posterior mode and terms with non-zero posterior modes indicate marker-disease associations.

We applied this approach to a case-control association study on rheumatoid arthritis (RA) using SNPs spanning the HLA region, together with genotypes from the multiallelic HLA-DRB1 locus. The latter is a known RA risk factor that was included in all our models without shrinkage. After controlling for type-I error, we found fewer positive SNP associations than in single-point tests, suggesting that LD might be better-handled. These results were supported by a simulation study. We selected a set of SNPs in various degrees of LD with HLA-DRB1. For each marker, case-control labels were randomised within the HLA-DRB1 allelic classes to simulate causal SNPs, while maintaining LD with HLA-DRB1. Our results showed that the shrinkage approach provides a substantial benefit, both in terms of maintaining statistical power to detect multiple causal variants and in the reduction of false positive associations.

2174/T

Bayesian Mapping of Quantitative Trait Loci for Multiple Complex Traits Using Variance Components. *J. Liu[1], Y.J. Liu[1], X.G. Liu[1,3], H.W. Deng[1,2,3].* 1) Dept Basic Medical Sci, Univ Missouri, Kansas City, MO; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China; 3) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shanxi 710049, P.R. China.

Joint mapping of quantitative trait loci (QTL) for multiple correlated traits plays an important role in unraveling genetic architecture of complex traits. Compared with the single-trait analysis, joint mapping addresses more questions and has advantages on power of QTL detection and precision of parameter estimation. Some statistical methods have been developed to map QTL underlying multiple traits, most of which are based on maximum-likelihood methods. We develop here a multivariate version of the Bayes methodology for joint mapping of QTL using Markov chain Monte Carlo algorithm. We adopt a variance component method to model complex traits in outbred populations. The method is robust, can deal with an arbitrary number of alleles with arbitrary patterns of gene actions, and allows for multiple phenotype data of various types in the joint analysis. Under a Bayes framework, parameters including the number of QTL are estimated based on their marginal posterior samples, which are generated through two samplers, Gibbs sampler and reversible jump MCMC. In addition, we calculate the Bayes Factor related to each identified QTL to test coincident linkage vs. pleiotropy. The performance of our method is evaluated in simulations with full-sib families. The results show that our proposed Bayes joint mapping method performs well for mapping multiple QTL in situations of either bivariate continuous traits or mixed data types. Compared to the analysis for each trait separately, Bayes joint mapping improves statistical power, provides stronger evidence for QTL detection and increases precision in estimation of parameter and QTL position. We also applied the proposed method to a set of real data for further assessing our proposed method.

2175/T

Efficient Control of Population Structure in Model Organism Association Mapping. *H. Kang[1,2], N. Zaitlen[4], C. Wade[6], A. Kirby[6], D. Heckerman[5], M. Daly[6], E. Eskin[2,3].* 1) Computer Sci Engineering, Univ California, San Diego, La Jolla, CA; 2) Department of Computer Science, University of California Los Angeles, Los Angeles, CA; 3) Department of Humna Genetics, University of California Los Angeles, Los Angeles, CA; 4) Bioinformatics Program, University of Calfornia San Diego, La Jolla, CA; 5) Microsoft Research, Redmond WA; 6) Broad Institute of MIT and Harvard, Cambridge, MA.

Genome-wide association mapping in model organisms such as inbred mouse strains is a promising approach for the identification of risk factors related to human diseases. However, genetic association studies in inbred model organisms are confronted by the problem of complex population structure among strains. This induces inflated false positive rates, which can not be corrected using standard approaches applied in human association studies such as Genomic Control or Structured Association. Recent studies demonstrated that mixed models successfully correct for the genetic relatedness in association mapping. However, the currently available mixed model methods suffer from computational inefficiency and unknown convergence properties. We propose a new method, Efficient Mixed Model Association (EMMA), which corrects for confounding due to population structure in model organism association mapping. Our method takes advantage of the specific nature of the optimization problem in applying mixed models for association mapping, which allows us to substantially increase computational speed and reliability of the results with improved convergence properties and global optimization. We applied our EMMA method to in silico whole genome association mapping of inbred mouse strains involving hundreds of thousands of SNPs. We also performed an extensive simulation studies to estimate the power of EMMA under various effect of SNP, population structure, and multiple measurements. In spite of limited power of model organism association mapping due to the limited number of inbred strains, we are able to identify significantly associated SNPs, which fall into known QTLs or genes identified through previous studies without an inflation of false positives.

2176/T

A powerful and flexible multi-locus association test for quantitative traits. *L.C. Kwee[1], D. Liu[2], D. Ghosh[3], X. Lin[4], M.P. Epstein[5].* 1) Dept Biostatistics, Emory Univ, Atlanta, GA; 2) Center for Statistical Sciences, Brown Univ, Providence RI; 3) Dept Biostatistics, Univ Mich, Ann Arbor, MI; 4) Dept Biostatistics, Harvard Univ, Cambridge, MA; 5) Dept Hum Genetics, Emory Univ, Atlanta, GA.

For association mapping of quantitative traits, debate exists regarding the most efficient approach for analyzing tag SNP genotype data within a candidate gene of interest. A popular approach tests each tag SNP individually, but such tests could lose power due to incomplete linkage disequilibrium (LD) between the genotyped tag SNP and the trait-influencing variant. Alternatively, one can jointly test all tag SNPs simultaneously within the gene (using genotypes or haplotypes), but such tests have large degrees of freedom that can also compromise power. Here, we consider a semiparametric model that uses LD information from multiple tag SNPs simultaneously in analysis but still produces test statistics with small degrees of freedom. We fit this model using least-squares kernel machines, which we show is identical to analysis using a linear-mixed model (which we can fit using standard software packages like SAS and R). Using simulated tag SNP data from the International HapMap Project, we demonstrate our approach has superior performance relative to existing approaches for association mapping of quantitative traits for common causal variation. Our approach is also flexible, as it allows easy modeling of covariates and, if interest exists, high-dimensional interactions among genetic and environmental predictors.

2177/T

Gene-Gene Interaction between FGF20 and MAOB in Parkinson Disease. *E. Martin[1,2], X. Gao[1,2], W. Scott[1,2], G. Wang[2], G. Mayhew[1,2], J. Vance[2].* 1) Center for Genetic Epidemiology and Statistical Genetics, Univ Miami, Miami, FL; 2) Miami Institute for Human Genomics, Univ Miami, Miami, FL.

The fibroblast growth factor 20 (FGF20) and monoamine oxidase B (MAOB) genes are reported to be associated with PD risk, and both are involved in the dopamine bio-pathway. We investigated the joint effect between polymorphisms in FGF20 and MAOB genes, to see if there was evidence of statistical interaction with risk of PD. All subjects analyzed were white, and families with known parkin mutations were removed. A total of 736 families were used in the final analysis. Statistical analysis was performed by Conditional Logistic Regression (CLR) using sibships as strata. Because MAOB is located on chromosome X and the prevalence of PD differs by sex, we stratified the data set on sex and analyzed males and females separately. Significant two-locus gene-gene interactions were found in white females using CLR between the polymorphism rs1721100 of FGF20 and the polymorphism rs1799836 of MAOB, and between the polymorphism rs1721082 of FGF20 and rs1799836. The risk alleles for each single SNP identified from CLR, rs1721100 C, rs1721082 T and rs1799836 A, are consistent with previous reports. Using indicator variables for the SNP genotypes, rs1721100 G/C with rs1799836 A/A showed significant interaction (P = 0.021), compared with the reference group rs1721100 G/G with rs1799836 G/G. rs1721082 T/A with rs1799836 A/A also showed significant interaction (P = 0.019), compared with rs1721082 A/A with rs1799836 G/G. Using an allele-dose model for the risk alleles, rs1721100 and rs1799836 showed significant interaction (P = 0.019), rs1721082 and rs1799836 also showed significant interaction (P = 0.030) with PD risk. Variants in FGF20 and MAOB have non-independent effects on PD risk in females of our family-based data set. This suggests a statistical interaction between alleles in these genes.

2178/T

Simulation and visualization tools for gene discovery studies of complex human diseases. *S. Schmidt[1], R.-H. Chung[1], X. Qin[1], X. Lou[1], M. Schmidt[2], E.R. Martin[2], E.R. Hauser[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Inst Human Genomics, Univ of Miami, FL.

We have integrated two software packages that facilitate simulation studies for complex human diseases: an enhanced version of our previously distributed SIMLA package (Schmidt et al. 2005) that is now available with a Graphical User Interface (GUI) for generating the control file, and our recently developed visualization tool SIMLAPLOT (Qin et al. 2007). Specifically, we have implemented the following features: simulation of unrelated case-control datasets; simulation of up to three modifier loci, each of which can generate a categorical phenotypic feature, such as disease severity; simulation of a biallelic quantitative trait locus (QTL) that may influence the distribution of a continuous disease risk factor and/or generate a disease-unrelated trait for QTL analysis; simulation of X-linked disease loci and sex-specific relative risks; simulation of up to four blocks of markers in linkage disequilibrium (LD), which may be in LD with one or two distinct susceptibility variants; LD calculation tool for generating founder haplotype frequencies given user-specified values of D' or r^2; and a sibling recurrence risk (λ_s) calculation tool for several complex disease models. SIMLAPLOT graphically illustrates different models by which continuous environmental or clinical covariates may influence the risk of complex diseases, in concert with genetic susceptibility. Examples for such models include gene-environment interaction, the QTL mechanisms described above, and genetic main effects with covariate-based heterogeneity. SIMLAPLOT may be used to better understand the role of various model parameters in a SIMLA control file by graphically displaying the relationship between disease locus (or QTL) genotypes and covariate values. When applied to real datasets, plots produced by SIMLAPLOT may assist in the interpretation of statistical analysis results and the exploration of plausible disease models.

2179/T

A multi-locus χ^2 test for case-control genetic association studies. *G. Zhang[1], R. Chakraborty[1], M.B. Rao[1], L. Jin[1,2,3].* 1) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 2) School of Life Sciences, Fudan University, Shanghai, China; 3) CAS-MPG Partner Institute of Computational Biology, Shanghai, China.

It is commonly believed that haplotype based association test is more powerful than single-locus test in studying genetic association between a set of biallelic SNP markers (i.e. tagging SNPs) and a complex trait. However, the marker haplotype need to be inferred statistically from unphased genotype data by assuming Hardy-Weinberg equilibrium (HWE); and the additional information from the use of haplotypes comes at the cost of increased degrees of freedom. Recently, some researchers have indicated test procedures based on unphased multi-locus genotypes might be more powerful than haplotype-based methods. The most commonly used multiple-locus genotype association test is the Hotelling's T^2 test and related procedures. In this study, we develop a simple multiple-locus χ^2 test for simultaneously testing allele frequency differences at multiple loci between cases and controls. Under the null hypothesis of no association, the allele frequency difference of each marker locus asymptotically follows a normal distribution, and the correlation of allele frequency differences between pair of loci is given by the pairwise linkage disequilibrium between the two loci. Therefore, we model the allele frequency differences of multiple loci by multivariate normal distribution and assess the statistical significance of allele frequency differences of multiple loci between cases and controls by χ^2 test. We evaluate the power of the suggested χ^2 test by simulated and real data sets. The results indicate the multiple-locus χ^2 test is more powerful and robust than haplotype-based association test or Hotelling's T^2 test.

2180/T

Machine Learning Methods for Detection of Epistasis under Low Penetrance. *K.K. Nicodemus[1], Y.Y. Shugart[2].* 1) GCAP, CBDB/NIMH/NIH, Bethesda, MD; 2) Johns Hopkins SPH, Baltimore, MD.

Machine learning (ML) algorithms may be useful in the detection of epistasis in large-scale studies. Although the misclassification rate (MR) is one way to evaluate ML methods, in conditions of low penetrance expected in genetic studies, reduction in MR may be modest. Another way to measure performance is using measures of variable importance (VI). We simulated 250 replicates, including 3 genes not associated with case status and 2 genes that participated in a 2-SNP interaction (N SNPs = 199; N cases = N controls = 500). Prevalence = 0.10 and the odds ratio for interaction was 2.5 (baseline penetrance = 0.09, penetrance for double risk homozygotes = 0.24). One causal SNP was in a gene with strong LD; the second causal SNP was in a low LD gene. Null replicates were created by permuting case status. Methods evaluated were random forests (RF), Monte Carlo (MCLR) or logic regression (LR) and generalized boosted regression (GBR). For all 3 ML methods we calculated 1) detection rates (DRs) (% replicates where causal SNPs were ranked in the top 5% important) and permutation based p-values 2) MR for cases and controls using an independent test and 3) training dataset. Algorithms used a classification/logic tree as base learners. We also performed bivariate logistic regression. Under conditions of modest penetrance, all ML methods showed prediction (test set) MR near 0.5; no different from random guessing. Using the training set, both LR and GBR obtained a 10% reduction in MR; RF did not show any reduction. MCLR VI measures detected the correct causal SNPs in 32 and 51% of the replicates (p-values ranged from 0.14-1.8e-4); the DRs using GBR were 26 and 69% (p-values ranged from 0.059-1.0e-4). RF detected the causal SNP in low LD in 100% of the replicates (p-values ranged from 0.048-1.0e-5) but did not detect the causal SNP in high LD. Logistic regression DRs were lower: 24 and 29%. In reduced penetrance conditions, no reduction in MR for predicting case status was observed; reduction in MR using the training set was modest. MCLR and GBR outperformed logistic regression in detecting causal SNPs using VI. Ongoing work is considering complex, multi-SNP pathways.

2181/T

A generalized combinatorial approach for detecting gene by gene and gene by environment interactions. *X.-Y. Lou[1], G.-B. Chen[1], L. Yan[2], J.Z. Ma[3], J. Zhu[2], R.C. Elston[4], M.D. Li[1].* 1) Dept Psychiatry & Neurobehavioral Sciences, Univ Virginia, Charlottesville, VA; 2) Institute of Bioinformatics, Zhejiang University, Hangzhou, P. R. China; 3) Dept Public Health Sciences, Univ Virginia, Charlottesville, VA; 4) Department of Epidemiology and Dept Biostatistics, Case Western Reserve University, Cleveland, OH.

The widespread multifactor interactions pose a significant challenge to identifying genetic determinants involved in complex diseases. The traditional methods are typically underpowered because of the problem referred to as the "curse of dimensionality". Currently available combinatorial approaches, such as the multifactor dimensionality reduction method (MDR), the combinatorial partitioning method (CPM), and the restricted partition method (RPM), are promising tools and have a straightforward correspondence to the concept of the phenotype landscape that unifies biological, statistical genetic and evolutionary theories. However, they do have limitations, such as not allowing for covariates, which restrict their practical use. In this study, we develop a generalized MDR (GMDR) by using a class of more efficient and comprehensive statistics (e.g., the score statistic) that permits adjustment for discrete and quantitative covariates and is applicable to both dichotomous and continuous phenotypes in various population-based study designs. Benefiting from eliminating the background noise due to risk-conferring covariates, the new method has the increased prediction ability and statistical power compared with the existing combinatorial approaches in the literature. Computer simulations support our theoretical expectation and indicate that the GMDR method has superior performance in its ability to identify epistatic loci. In summary, GMDR can serve the purpose of identifying contributors to population variation better than do the other existing methods. The project is supported by NIH grant DA-12844.

2182/T

Moving towards a combined framework for association with extensions to meta-analysis. *B.M. Neale[1,2,3], P.C. Sham[4], P.I.W. deBakker[2,3], S. Purcell[2,3], M.J. Daly[2,3,5].* 1) SGDP Centre, King's College London, United Kingdom; 2) Broad Institute, Boston, MA; 3) CHGR, Massachusetts General Hospital, Boston, MA; 4) Psychiatry and Genome Research Depts, University of Hong Kong; 5) School of Medicine, Harvard, Boston, MA.

We extend the TDT to incorporate information from singletons and suggest a correction for the analysis of SNPs imputed from the linkage disequilibrium (LD) structure from HapMap. Both of these developments are useful for meta-analysis and improving power of association studies. Using probands in a case/control design positively correlates with the TDT evidence under the null. Identifying a source of association signal independent of the transmission information will improve the power. We propose modelling parents as *half-cases* as half the genetic material is shared with a case. From simulation, parental genotypes do not correlate with the TDT under the null and deviate from control frequencies under the alternative. Based on Mitchell (2000), case/control and TDT association information can be combined and tested using a single degree of freedom. This parental association information is subject to other population-based difficulties such as stratification, and so due diligence is necessary. We apply this to genome-wide association studies (GWAS) of ADHD and autism.

Imputing untyped variation in samples based on LD is viable for GWAS. These untyped variants are known imprecisely because of the uncertainty inherent in LD and the sampling variance associated with the HapMap. We explore the two approaches to the generation of the SNP data for analysis: *best guess* and *dosage*. *Best guess* imputes the most likely allele, and *dosage* generates a probabilistic counts of alleles. Our simulation and empirical results show that the *dosage* approach is preferable. However, *dosage* is conservative under the null. To resolve this, we propose a correction to the variance of the association test statistic. By doing so, we can now utilize imputation to combine evidence across non-overlapping SNP sets using Z-score combination. We apply this GWAS of Crohn's disease for meta-analysis.

2183/T

Multiple Imputation to Correct for Measurement Error in Genetic Structured Association Testing. *M.A. Padilla[1], J. Divers[2], L.K. Vaughan[1], D.B. Allison[1], H.K. Tiwari[1].* 1) Section on Statistical Genetics, Dept of Biostatistics, University Alabama at Birmingham, Birmingham, AL; 2) Section on Statistical Genetics and Bioinformatics, Dept of Biostatistics, Wake Forest University, Winston-Salem, NC.

Structured association testing (SAT) is association testing that takes into account population substructure. Within this framework, a SAT model was developed in the form of a linear model using admixture estimates as covariates to control for population substructure. However, like any statistical model, it assumes that all variables are measured without error. Measurement error can cause biased parameter estimates and confound residual variance in linear models. It's been shown that admixture estimates can be contaminated with measurement error causing SAT models to suffer from the same afflictions. Multiple imputation is presented as a viable tool for correcting measurement error problems in linear models with emphasis on correcting measurement error contaminated admixture estimates in the context of a SAT linear model. Several multiple imputation methods are presented and compared, via simulation, in terms of controlling Type I error (false positives). In addition, both non additive and additive genotype coding were also investigated. Results indicate that multiple imputation can be used to correct for measurement error in admixture estimates in SAT linear models. However, the data should be of reasonable quality, in terms of marker informativeness, because the method uses the existing data to borrow more information in which to make the measurement error corrections. If the data are of poor quality then there is little information to borrow in order to make measurement error corrections.

2184/T

Combinatorial alellic risk scores for pulmonary tuberculosis vary in Mexican mestizos according to Amerindian ancestry. *P. Zoochlorella, C. Rangel.* Instituto de Medicina Genómica, Mexico City 01900, MEXICO.

Most individuals within the Mexican population are considered "mestizo", having originated from the admixture of Amerindian groups with Spaniards and, to a lesser extent, Africans. The complex admixture process has resulted in genetic differences between geographical regions. The purpose of this study was to evaluate the existence of regional differences in polymorphisms associated with susceptibility to pulmonary tuberculosis (PTB) in México, both at the level of individual genotypes and higher-order interactions, i.e. combinatorial genotypic categories. Several functional polymorphisms influence susceptibility to PTB, including SNPs in macrophage chemotactic protein-1, interleukin-10, interleukin 12-receptor B1 and the phagosomal solute carrier family 11 member 1. We determined genotypic frequencies in *MCP1* (rs1024611), *IL10* (rs1800896), *IL12RB1* and *SLC11A1* (rs17235409) in 1,150 healthy mestizos from six geographically distant states in Mexico. Our results show that the MCP1-2518 GG genotype exist in a higher frequency in Mexican mestizos compared to European and African populations, suggesting that the G allele could have been contributed by Amerindian populations. The state of Sonora exhibits a significantly lower frequency of the GG genotype compared to the rest of the states and this is consistent with an ancestry analysis by chromosomal region revealing that individuals from this state shows a lower Amerindian contribution in the 100 kb region where *MCP1* is located. We developed **C**ombinatorial **G**enotype **B**ins, CGBs, an R-programmed query algorithm to a relational database which ranks PTB risk scores according to a probability matrix of the reported odds ratio values for individual SNPs. Three of the CGBs, corresponding to high and moderate PTB risk scores, showed significant differences between Mexican mestizos and individuals with higher Amerindian ancestry. Our results support the existence of regional differences in genomic variations associated with susceptibility to PTB in Mexico. CGBs is a useful tool in higher-order genomic analysis, and its potential in PTB risk assessment will be evaluated in case-control studies.

2185/T

Case-only analysis ignoring control genotypes is efficient for detecting gene-gene interactions in case-control studies. *C. Li[1], M. Li[2].* 1) Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Dept of Biostatistics and Epidemiology, Univ of Pennsylvania, Philadelphia, PA.

Complex diseases often result from the interplay of multiple genetic and environmental factors. When we suspect two genes interact to modify disease risk, we may want to test for their interaction. Screening for interaction has also been proposed for genome-wide studies. However, the commonly used approaches often suffer from low power. In case-control studies, the most commonly used approach for gene-gene interaction analysis is logistic regression, in which we compare two joint genotype distributions, one for cases and one for controls, to determine if their genotype distributions are significantly different. When the subjects are sampled from a homogeneous population and the two loci of interest are unlinked or linked but are in linkage equilibrium, the control genotypes may provide little additional information over the prior knowledge of independence between the loci in the population, but contribute additional variation that has to be taken into account, lowering the power to detect interaction. To reduce such variation, we propose case-only analysis, in which the control genotypes are ignored, as an efficient and powerful alternative test of gene-gene interaction even when control genotypes are available. The appropriateness of the case-only analysis relies on the definition of "no-interaction", which is scale dependent. Using analytical arguments and simulations, we show that (1) under the multiplicative definition of no interaction, i.e., additivity on the log-risk scale, the case-only approach is more appropriate and more powerful than logistic regression and the latter has inflated type I error, and (2) under the additive definition of no interaction, i.e., additivity on the penetrance scale, both approaches may have inflated type I error rates, but the case-only approach can control the type I error rate better than logistic regression when the relative risks are near one (e.g. RR=1.5). Our results indicate that case-only analysis is a powerful alternative to logistic regression for detecting gene-gene interactions, even when the control genotypes are available.

2186/T

A powerful test of association of multiple markers with disease using kernel scores. *I. Mukhopadhyay[1], A. Thalamuthu[2], E. Feingold[3], D.E. Weeks[4].* 1) Department of Statistics, Burdwan University, Burdwan, West Bengal, India, 713104; 2) Genome Institute of Singapore, 60 Biopolis Street, #02-01, Genome, Singapore 138672; 3) Department of Human Genetics and Biostatistics, 130 DeSoto Street, A305 crabtree Hall, Pittsburgh, PA 15261, USA; 4) Department of Human Genetics and Biostatistics, 130 DeSoto Street, A303 crabtree Hall, Pittsburgh, PA 15261, USA.

When multiple genes might influence disease risk, it can be useful to globally test for the simultaneous effect of the multiple genes on disease risk. Based on kernels we propose a powerful test for testing association of multiple markers acting simultaneously on disease, using case-control data. We used the idea of analysis of variance (ANOVA) with the scores of symmetric kernel functions on genotypes of each marker (Schaid et al, 2005) as observations. We compare the variation between cases and controls and the variation within each class that would eventually led us to propose a testing procedure for the detection of association. We study each marker separately and combine them to get a global statistic that is finally used to test for disease-marker association. We carried out a simulation to calculate the Type I error and power of our new statistic, varying liability loci from one to five out of a total of ten markers. For a variety of relative risks and allele frequencies, our proposed statistic has much higher power than some other statistics in the literature. We studied several different kernels and it appears that no particular kernel turns out to be the best in all models; however there is very little difference in power among them.

2187/T

Bayesian choice of optimal number of subpopulations from multilocus genotype data. *H. Gao, K. Bryc, C.D. Bustamante.* Dept.Bio.Stat.& Comp. Bio. , Cornell University, Ithaca, NY.

Strong population stratification is a known factor that inflates the false positive rates substantially in association mapping tests. The popular software STRUCTURE performs Bayesian classification of individuals into subpopulations conditional on the input of the number of clusters. We present a Bayesian model selection criterion--Deviance Information Criterion (DIC)--to determine the number of the clusters underlying a given sample, which fits the Bayesian clustering algorithm of STRUCTURE well and can be easily integrated into the Markov Chain Monte Carlo framework. We also performed extensive coalescent simulations under various genetic contexts (e.g. population differentiation with migration or partial self-fertilization, hierarchical population stratification) to evaluate the accuracy and robustness of the DIC approach vs. several other methods, such as the likelihood approximation method implemented in STRUCTURE, the ΔK method and the EIGENSTRAT approach. It turns out the DIC outperforms the rest methods in most genetic scenarios, with its percentage of correct hittings of true number of subpopulations always close to 100%, which implies it can facilitate correcting for the confounding effect caused by population structure in the whole-genome association studies.

2188/T

PCA-correlated SNPs for structure identification in worldwide human populations. *P. Paschou[1], E. Ziv[2], E.G. Burchard[3], M.W. Mahoney[4], P. Drineas[5].* 1) Dept of Molecular Biology & Genetics, Democritus University of Thrace, Greece; 2) DGIM, Institute for Human Genetics, UCSF; 3) Depts of Biopharmaceutical Sciences & Medicine, Pharmaceutical Sciences & Pharmacogenetics, UCSF; 4) Dept of Mathematics, Yale University; 5) Dept of Computer Science, RPI.

Existing methods to ascertain small sets of markers for the identification of human population structure (δ, Fst, Informativeness, etc.) require prior knowledge of individual ancestry. Based on Principal Components Analysis (PCA) and recent results in Theoretical Computer Science, we develop a novel algorithm that, applied on genomewide data, selects small subsets of SNPs (PCA-correlated SNPs) that reproduce the structure found by PCA on the complete dataset, without use of ancestry information. Evaluating our method on a previously described dataset (10,805 SNPs, 11 populations), we demonstrate that, achieving in most cases 99% genotyping savings, PCA-correlated SNPs can be effectively used to assign individuals to particular continents or populations. We validate our methods on the HapMap populations and achieve perfect intercontinental differentiation with 14 PCA-correlated SNPs. The Chinese and Japanese populations can be easily differentiated using less than 100 PCA-correlated SNPs ascertained after evaluating 1.7 million SNPs from HapMap. We show that structure informative SNPs are not portable across geographic regions. However, we manage to identify a general set of 50 PCA-correlated SNPs that effectively assigns individuals to one of nine populations. Compared to the measure of Informativeness, our methods, although unsupervised, achieved similar results. Applying our algorithm on a novel Puerto Rican dataset (192 individuals 7,257 SNPs) we show that PCA-correlated SNPs can successfully predict population structure and admixture proportions. We subsequently validate these SNPs for structure identification in an independent Puerto Rican dataset. The algorithm that we introduce runs in seconds, even on genomewide data, and will facilitate the identification of population substructure, the study of admixed populations as well as stratification assessment in multi-stage whole-genome association studies.

2189/T

Human population stratification and genetic association studies. *X. Sheng, G. Zhang, R. Chakraborty.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Population stratification becomes relevant for case-control association studies when allele frequencies are different in cases and controls due to systematic ancestry differences of subjects classified as cases and controls. This may cause spurious associations, and leads to both false positive and false negative findings. Recently, several statistical approaches have been proposed using genomic markers to control for this confounding effect. In this study, we describe a new method that efficiently corrects for stratification by regressing the pairwise genotypic difference on the pairwise genetic distance (computed from genomic markers) of all case-control pairs. A new test statistic *T* is formulated to measure the genotypic difference between cases and controls, adjusting for stratification contribution. Significance level is determined by the null distribution of *T*, which is generated from the genomic markers by using permutation. The current existing approaches (Genomic Control and Structured Association) are compared to our new method by simulating different disease association studies, under a variety of parameter settings. Allele frequencies from the African and European data of the HapMap project as well as simulated allele frequencies from a uniform distribution were used in such simulation experiments. Results suggest that our procedure has a correct nominal type-1 error rate in the presence of different levels of population stratification. In most scenarios we considered, our method has a larger power and, in some cases, substantially larger power than that of existing methods. In terms of power, the Structured Association method is closest to our new approach, but the latter requires substantially smaller computational time, which implies its ease of application in large-scale or even genome-wide association studies. (Research supported by the NIH grant GM41399 to RC).

2190/T

Bayesian Model Search and Selection for Association Studies. *M.A. Wilson, M.A. Clyde, E.D. Iversen, Jr.* Dept. of Statistical Science, Duke University, Durham, NC.

Modern genotyping techniques allow vast amounts of data to be collected for genetic association studies. With this volume of data comes an increased need for statistical methods that are able to efficiently sort through the enormous number of models given the available genetic and non-genetic data. In addition, it is increasingly the case that there is prior data on the structure and function of genetic pathways and their interaction with environmental factors. Analyses that ignore this information and focus, instead, on marginal associations will have diminished power. We describe a Bayesian model selection technique utilizing Evolutionary Monte Carlo that searches over models including genetic and environmental main effects and their interactions in a computationally efficient manner. The approach formally incorporates prior data on pathways, thus restricting the model search space. As alternatives, we consider SNP-by-SNP and gene-by-gene approaches in which each SNP (or gene) is analyzed separately and where pathway- or study-wide association is determined by a secondary analysis, e.g., of test statistics or associated p-values derived from the primary analyses.

Using Hapgen, we simulate a set of case-control pathway studies of SNP data that reflect true patterns of linkage disequilibrium and minor allele frequencies. We utilize these data sets to compare the power of the competing analytical strategies described above. We describe how the power of each of these analytical strategies depends upon the true model of association, sample size, and strength and extent of association. As part of this study, we investigate the performance of Evolutionary Monte Carlo as we change the parameters of the tempering scheme, the number of iterations run and the assumed penalty function/prior. Finally, we describe the advantages and disadvantages of each approach in terms of model complexity, computational tractability and analytical simplicity. We have found that SNP-by-SNP methods are surpisingly powerful given their simplicity and that Bayesian model selection techniques, while more computationally demanding, provide a promising alternative.

2191/F

Financial Incentives for the Procurement of Oocytes for Research: In Search of Ethical and Political Consistency. *R. Isasi.* Ctr Recherche en Droit, Univ Montreal, Montreal, PQ, Canada.

The recent South Korean scandal involving fraud and gross ethical violations in stem cell research has re-opened the debate on both the appropriateness of allowing healthy women to provide oocytes for research use and on the use of financial incentives. As the South Korean case illustrates, the debate is increasingly reduced to a confrontation between ethics, science and the welfare of women. It is plausible that the expansion of international research efforts, paired with the growing trend towards liberalizing stem cell research policies, will have the inevitable effect of increasing the demand for the human reproductive materials needed to conduct such research. The scarcity of oocytes available for conducting research for the derivation of stem cell lines have caused concerns over: the possible emergence of a "black market", the growing trend towards increasing financial incentives for donors, and, the appropriateness and sufficiency of current regulatory frameworks to safeguard donors. While consensus exists regarding the impermissibility of commercializing the donation of human reproductive materials, divergence exists regarding the amount of compensation that is reasonable to offer and the conditions under which compensation should be granted. Providing financial incentives for the procurement of oocytes for research is a controversial issue that can only be situated within the larger context of the donation of other human material (blood, organs). Likewise, it must be analyzed in the context of the overall acceptability of providing financial rewards to donors or providers of gametes and embryos for assisted reproductive technologies. In this presentation I will (1) explore the use and the implications of providing financial incentives for oocyte donation (with special emphasis on stem cell research) and (2) analyze the models (e.g. free market, pure gift, fixed compensation, minimum wage, and reimbursement of expenses) proposed in the literature and implemented in various jurisdictions. Examples will be drawn from the regulatory frameworks adopted by 17 countries that are members of the International Stem Cell Forum.

2192/F

Alterations in family planning in female reproductive-aged BRCA mutation carriers. *A.M. Bakke[1], M. White[2], S. Ross[3], L.P. Shulman[4], A.P. Trivedi[3].* 1) Center for Genetic Medicine, Graduate Program in Genetic Counseling, Northwestern University, Chicago, IL; 2) Cancer Risk Clinic, University of Chicago, Chicago, IL; 3) Department of Psychiatry, Northwestern University Feinberg School of Medicine, Chicago, IL; 4) Division of Reproductive Genetics, Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL.

Objective: We investigated if and how reproductive-age female BRCA mutation carriers alter reproductive decisions after BRCA result disclosure. **Methods**: 41 out of 126 eligible mutation carriers completed surveys regarding whether or not their and their partners' attitudes regarding family planning changed after receiving their test results. **Results**: 56% of participants desired additional children at the time of testing. Of this subgroup, 52% reported a change in their reproductive planning. 26% reported they desired fewer children after testing, and 17% desired more children than before testing. 26% wanted to start childbearing earlier, and 48% wanted to stop childbearing earlier than planned due to the desire to pursue oophorectomy. 22% of participants indicated other BRCA positive women in their family altered their family planning due to carrier status. Compared to their partners, participants perceived themselves to be more concerned about the chance of passing the BRCA mutation on to their children ($p<0.001$) and their own mortality ($p=0.013$). Of the three participants who designated they were single at the time of the survey, two indicated that their BRCA status affected their ability to have a committed relationship. More than one-third of participants indicated they would like to have family planning issues specifically discussed in a genetic counseling session. **Conclusion**: Young BRCA mutation carriers frequently alter their reproductive plans after learning their carrier status. Participants were more concerned about their own mortality and passing on their BRCA mutation to their children than they believed their partners to be. A subset of women would likely find value in discussing family planning issues in the genetic counseling session.

2193/F

Family history of chronic diseases in Mexico: genomic tool for the risk establishment in public health. *P.F. Oliva-Sanchez[1], E. Velasco -Mondragon[2], R. Lopez-Ridaura[3], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine, Mexico; 2) School of Public Health and Policy, Morgan State University,MD; 3) National Institute of Public Health, Mexico.

The objective of this study was to evaluate the association between chronic diseases, like diabetes type 2 (DT2), hypertension (HTA) and metabolic syndrome (SM), with family history (FH) in its different components (antecedents paternal or maternal or both) on Mexican adult population. An analysis of the National Health Survey of 2000 (ENSA 2000) was made. This Survey was implemented by the National Institute of Public Health and the Mexican Ministry of Health, by means of the application of an interview on a random representative sample of adults over 20 years in Mexico. Non - adjustment and adjustment OR was calculated by logistic regression analysis. We evaluate the association between chronic diseases and family history. Sample size of participant's adult subjects in the survey was of 45,294. The 7, 5% (3,334) of them had DT2, the 31, 6% (14,004) HTA, the 2, 5% (696) SM. Was observed that those individuals who reports antecedents of DT2 in both parents they have 5, 11 ($p < 0, 0001$) grater possibility to suffering DT2 than those individuals without antecedents of this disease. In the adults who had SM was observed 9, 15 ($p < 0, 0001$) odds ratio on those who had the antecedents of DT2 in both parents in comparison with the individuals without antecedent of this disease. Another important finding is the interaction of HF with the body mass index (BMI). When we performed the stratification by groups of BMI, the OR of DT2 associated to FH in both parents on thin individuals, turned out to be higher in comparison to the same association in people with overweight and obesity. These results suggested that FH represents more the genomic component than the environmental component in causation of DT2.We considered that FH is a tool in genomic medicine and public health that serves to detect groups of greater genomic vulnerability.The HF represents a tool to detect individuals and/or families in risk. This strategy will be applied in better health policies, in terms of the diseases control in Mexico.

2194/F

Initial actions to implement health policies related to Genomic Medicine in Mexico. *P. Oliva, E. Barrientos, C. Lara, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

The availability of the human genome sequence has indicated that 0.1% of the human sequence varies between individuals. Combinations of these nucleotide variations influence risk to common health problems as well as response to commonly used drugs. Systematic analysis of these genetic variations will lead to important implications for public health. Genomic medicine will result into a more individualized, predictive and preventive medical practice with significant implications to individual health, life quality, medical practice, health finances and opportunities in the current knowledge-based economy. Mexico has committed to develop genomic medicine in benefit of its population. In 2004, the Mexican Congress created the National Institute of Genomic Medicine (INMEGEN) to develop scientific research in genomic medicine. In the last two years, Mexico has implemented a robust scientific and technological infrastructure. In addition, the Genome Diversity Project of Mexico, has shown different allele frequencies and haplotype patterns compared to other populations, indicating that genomic medicine needs to be developed according to genomic structure of the target population. Initial whole genome scan studies in complex diseases and epidemiologic studies are in their way, and cohort studies will follow. INMEGEN have established strong interactions with the public, academic and private institutions, both domestic and international. These efforts have been strengthen by international interactions established with WHO, PAHO and the OCDE. In addition, the Mexican Congress has a remarkable interest in developing legal bases that stimulate scientific research in genomic medicine in the context of the national public health policies. There are currently nine bills related to genomic medicine in the Mexican Congress, including those related to protecting against ethical challenges. In the next decade, progressive applications of genomic medicine will post important challenges to public health systems, and will require coordinate efforts to fully translate this new knowledge into benefits for the Mexican population.

2195/F

Translation and Regulation of Personalized Medicine is Here and Now. *P.F. Terry[1], S.F. Terry[2].* 1) Genomic Health, Inc., Personalized Medicine Coalition, *Coalition for 21st Century Medicine, Wash., DC; 2) Genetic Alliance, Inc. Wash., DC.

Ensuring timely patient access to high quality, safe and effective genetic/genomic technologies is important to all stakeholders: scientists, providers, regulators, payers, public health officials, and patient organizations. Accelerating scientific advancements and their disruptive impact on healthcare delivery is a major challenge for the genetics community. Establishing appropriate oversight and balanced regulation to encourage the maturation of genetic technologies through the transition to routine clinical applications is critical. All stakeholders must work to ensure that these transition steps are accelerated and responsible. Current technological advancements, exponential knowledge generation, rapid product life cycles, innovative precision tools, and the diversity of novel clinical studies, genetic discoveries, compelling biological associations, and the various innovative delivery models have burdened the traditional regulatory schema. We explore solutions for validation of clinical claims for genetic/genomic tests, development and implementation of least burdensome regulatory approaches to products and service delivery models, restrictions on off-label use, evolving clinical utility claims, coding and reimbursement for genetic/genomic tests, and the incentives of value-based pricing for both components (Rx & Dx) of personalized medicine solutions. We review a variety of efforts and solutions proposed by advocacy organizations engaged in the debate over defining appropriate oversight and regulation of modern genetic/genomic testing services.

2196/F

Community Concerns Regarding Genomic Medicine. *S. Hahn[1], K. Powell[2], S. Letvak[2], D. Spoon[2], C. Christianson[2], D. Wallace[2], S. Blanton[1], P. Lietz[3], M. Pericak-Vance[1], V. Henrich[2].* 1) Miami Institute for Human Genomics, University of Miami, FL; 2) The University of North Carolina at Greensboro, NC; 3) Moses Cone Health System, Greensboro, NC.

The Guilford County Genomic Medicine Initiative is a demonstration project aimed at developing a model to incorporate genomic medicine into community health care. Included in this model are broad-based education programs for target populations: the community, health professionals, and patients. Community focus groups were conducted as part of the educational needs assessment. One question focused on participants' concerns about the use of genetics in medicine, as these may influence their acceptance and use of genomic medicine services. Furthermore, concerns may stem from lack of complete information or misconceptions that may be addressed by focused education. 13 focus groups were conducted with a total of 121 participants. The average group size was 9, ranging from 6 to 16. Overall, the demographics approximated the ethnic and racial diversity in Guilford County. Focus group transcripts were analyzed and coded for themes. Common themes include the cost of genomic medicine to the individual and affordability to all (equity); unanticipated physical harm from the use of technology; mistrust in the government, doctors, and/or scientists; downstream effects such as overpopulation from healthier people; playing God/disturbing the natural order; need for regulations; privacy; and genetic discrimination. Concerns about one or more moral issues such as genetic engineering (e.g. cloning and stem cells), choosing traits, and abortions resulting from genetic information were also raised in almost all focus groups. In some cases, responses were grounded in personal experiences, and in many cases reflected topics in the media. Some respondents mentioned concerns about issues they did not understand or were unsure of, and some had misconceptions about the use of genetics in medicine. These data reveal perceptions that must be acknowledged in order to produce an effective education program and were used to generate questions for a community telephone survey that further examined areas of concern.

2197/F

There are gender differences in attitudes toward the genomic studies applied to medicine and genomic literacy in Japan. *I. Ishiyama[1], A. Nagai[1], K. Muto[2], A. Tamakoshi[3], K. Mimura[4], M. Kokado[5], T. Tanzawa[6], Z. Yamagata[1].* 1) Department of Health Sciences, School of Medicine, University of Yamanashi, Japan; 2) Institute of Medical Science, University of Tokyo, Japan; 3) Department of Clinical Research Management, National Hospital for Geriatric Medicine, Japan; 4) Ochanomizu University Graduate School of Humanities and Sciences, Japan; 5) Kyoto University, Japan; 6) Department of Science Education, Shizuoka University, Japan.

The aim of this study was to assess gender differences in public attitudes toward the promotion of genomic studies applied to medicine, the level of genomic literacy and the relationship between attitudes and literacy , analyzing the data of nationwide opinion survey. The participants of 4,000 people (age 20-69) were selected using the stratified two-step randomization from Japanese public. They were asked the following items by postal questionnaire. Pros and cons of the promotion of genomic studies applied to medicine, level of genomic literacy, demographic and socioeconomic background, and attitudes about science in general. We examined the relationship between approval of the promotion and the level of genomic literacy, using logistic regression models stratified by gender. The response rate was 54.3% (male 51.1%, female 58.6%), and 69.4% of all respondents were in favor of the promotion (male 73.8%, female 65.6%). Males were more likely to approve of the promotion than females. The mean score of genomic literacy was 20.2 (range 0-30). There was a significant difference between the score of males and females (male 20.4, female 20.0). Multivariable analysis showed that approval was related to the high score of literacy. This relationship was stronger in male than in female (for the highest quintile of score versus the lowest, male: adjusted odds ratio (OR) 3.36; 95% confidence interval (CI) 1.88-5.98, female: adjusted OR 1.86; 95% CI 1.17-2.95). It also showed that a relationship between approval and income was stronger in female than in male. We need further discussions and research to analyze the factors that affect the attitudes toward genomic studies in male and female.

2198/F

Experiences of genetic discrimination are common in persons at risk for Huntington disease. *Y. Bombard[1], L. Currie[1], G. Veenstra[1], J. Paulsen[2], J. Bottorff[1], M. Hayden on behalf of the Canadian Respond-HD resear[1].* 1) University of British Columbia, CAN; 2) University of Iowa, USA.

Genetic discrimination (GD) is a potential risk associated with genetic testing (GT). GD is the perceived differential treatment of asymptomatic individuals (AI) on the basis of their actual or presumed genetic differences. The fear of GD has prevented individuals from undergoing GT and participating in genetic research. Such effects are significant as GD directly hinders potentially beneficial engagement with genetic medicine as well as important scientific advances. Although the concern for GD is widespread, there is paucity of evidence indicating whether GD exists in general and in HD in particular. The aims of this study were to examine the nature & extent of GD and to assess whether GT is associated with increased levels of GD. A cross-sectional survey of 293 AI from families at risk for HD was undertaken using a self-report questionnaire. The sample comprised 233 AI (response rate of 80%): 167 AI who underwent GT (83 who have the mutation & 84 who do not) and 66 AI who chose not to be tested. GD was reported by 93 respondents (40%) and occurred most often in life & disability insurance, by friends, when making reproductive decisions and establishing relationships. GD did not differ in prevalence between tested & untested respondents (p=0.236). Family history (FH) rather than GT was reported as the major reason for GD. Predictors of GD included: discovering the FH under the age of 19 (OR:4.5, p=0.001), being aware of the FH for > 10 years (OR:2.0, p=0.004) and knowing people with HD symptoms or who have died (OR: 1.5, p=0.033). Distress was found to be associated with the experience of GD (p=0.011). CONCLUSIONS: GD is common in this sample. FH is the major determinant of GD. Those that discover their FH at a younger age and know of their FH for longer are at greater risk for GD. Overall, participating in GT is not associated with increased levels of GD. GD is a significant mental health and social issue for persons at-risk for HD. To our knowledge, this is the first study to report GD among AI who have participated in GT compared to those who chose not to be tested.

2199/F

Attitudes, beliefs and anticipated reactions towards breast and prostate cancer risk genetic testing. *A. Carnevale[1], S. Romero-Hidalgo[1], N. Urraca[1], D. Parra[2], A. Villa[3], R. Lisker[3].* 1) Coord. Medicina Genomica, ISSSTE, Mexico; 2) Hospital Regional "IZ", ISSSTE, Mexico; 3) Instituto Nacional de Ciencias Médicas y Nutrición "SZ", Mexico.

The genomic-based technology is rapidly permeating biomedical research; however, translating the genomic information to improve human health requires research into the social consequences. OBJETIVE: The aim of this study was to investigate the attitudes, beliefs and anticipated reactions towards cancer risk genetic testing in a group of non-high risk women and men and to analyze the factors that may influence the intention to test. METHODS: In-person interviews of 859 (397 men and 462 women) outpatients attending to the four tertiary care hospitals of the ISSSTE in Mexico City were conducted. Two different questionnaires, one for women and one for men, explored different aspects about genetic testing of a high risk gene for breast or prostate cancer, respectively. Descriptive statistics, contingency tables and logistic regression were used in the data analysis. RESULTS: About 68% of respondents believe that the test could save their lives and that the results might provide valuable information to their family members. Women were significantly more motivated to get genetic testing, more aware about the benefits of the test, and also more concerned about getting cancer, than men. Adjusted for hospital, the factors: beliefs of benefits, concern about getting cancer, and consequences derived from a positive result were statistically significant with intention to test. People anticipated feeling more guilt, more regret and less relief if they choose not to be tested. More women than men anticipated feeling guilt, sadness and regretful if tested positive. On the other hand, 95% of participants agreed to follow doctor's advice in case of a positive result. CONCLUSIONS: The results suggest that the success of genetic testing will depend jointly on people's knowledge about the benefits of the test and on people's distress about possible consequences derived from a positive results. It is also important that medical doctors be prepared to provide objective advice to those at high risk of getting cancer.

2200/F

Moore, Greenberg, and and Catalona: Should patients retain any property rights in donated tissue samples? *S.M. Carter[1,2], S.J. Gross[1].* 1) Reproductive Genetics, Montefiore Medical Ctr, Bronx, NY; 2) Rutgers University-School of Law, Newark, NJ.

In **Moore v. Regents of Univ. of California, 793 P.2d 479 (Cal. 1990)**, the plaintiff underwent treatment for hairy cell leukemia. The defendants obtained numerous tissue samples from him over 7 years under the guise of patient care because of their great commercial and scientific value. After concealing their research activities, they established a cell line from Moore's T-lymphocytes that they patented for commercial development. Moore alleged breach of fiduciary duty, lack of informed consent, intentional infliction of emotional distress, and conversion. A majority of the California Supreme Court concluded that Moore did not have a cause of action for conversion but did have a claim for breach of fiduciary duty and lack of informed consent. In **Greenberg v. Miami's Children's Hosp., 264 F. Supp 2d 1064 (S.D. Fla. 2003)**, the plaintiffs with a family history of Canavan disease provided blood and tissue samples to develop genetic testing. After the research team isolated and patented the gene, they restricted any activity related to the Canavan disease gene. The parties finally agreed to allow the Hospital to collect royalties for clinical testing but to permit license free use for research. In **Wash. Univ. v. Catalona, 437 F. Supp. 2d 985 (E.D. Mo. 2006)**, research participants (RPs) donated tissue samples for cancer research and signed an informed consent relinquishing ownership rights to any medical products derived from research with their sample. They could also withdraw from the research at any time but did not retain the right to have their samples transferred to another institution. The PI moved to another university and requested the RPs to release their samples to him continue his research. The court ruled that the University was the sole owner of the research samples.

We discuss why researchers cannot take patients' altruism for granted, must seek partnerships that will facilitate research, and whether patients should retain any rights in donated tissue.

2201/F

Integrating Genomics into Public Health Practice: Views of Stakeholders in Tobacco Control. *M. Dingel[1], A. Hicks[2], M. Robinson[2], B. Koenig[2].* 1) North Dakota State University, Fargo, ND; 2) Mayo Clinic, Rochester, MN.

For well over a decade genomic research, or more properly, the promise of genomic research, has dominated the scientific landscape. Scientists and others have begun to predict how genomic research might contribute generalizable knowledge that would improve human health at the population level, a major goal of public health. However, public health and genetics hold different priorities, research techniques, worldviews, and requirements for evidence. The genetics of nicotine addiction serves as an illustrative case study for the issues in combining these two fields because of the scope of the problem - the World Health Organization estimates that a billion people will die of tobacco-related disease in this century. It is also an area in which both traditional public health measures, like increased taxes and indoor smoking bans, and pharmaceuticals, like nicotine replacement therapy, have had some success. In order to gauge how key constituents in the public policy debate on tobacco control perceived the tensions and promises of integrating genetics into public health programs, our team conducted 86 interviews between Jan 2004 and Aug 2006 with stakeholders in tobacco control (19 clinicians, 20 scientists, 25 prevention workers, 11 pharmaceutical employees, and 11 health payers). These interviews reveal both hopes and concerns of combining these fields. While there is strong support for traditional public health programs among all stakeholders, these individuals also recognize that public health programs struggle to combat the powerful forces of the tobacco industry while dealing with the individual needs of those most at risk. Stakeholders recognize that alternatives to current tobacco control initiatives are needed, but problems of acquiring adequate funding for both traditional and new genetic approaches remain. These concerns mirror contentious debates in Science and JAMA about the efficacy and cost-effectiveness of genetic approaches to tobacco control. For genomic approaches to integrate into public health practice there must be honest evaluation of the strengths and weaknesses of each approach and a reckoning of what counts as evidence of efficacy.

2202/F

Opinions of Japanese Life Scientists on Ethical, Legal and Social Implications of Behavioral Genetics. *J. Higashijima[1], K. Kato[1,2], K. Takahashi[1].* 1) School of Biostudies, Kyoto university, Japan; 2) Institute for Research in Humanities, Kyoto University, Japan.

With recent progress in the life sciences, especially in genome sciences, behavioural genetics related research has come into a new phase, being able to elucidate human nature from genetic information. Historically, behavioural genetics has not only been important in the academic world but also dramatically influenced society, with respect to it's close relationship to eugenic social policies. This is why it is particularly significant to tackle research on the Ethical, Legal, and Social Issues (ELSI) of behavioural genetics along with and/or in anticipation of its progress. Then what are the ELSI resulting from behavioural genetics now, and in the future? The problem is that existing controversial issues in behavioural genetics are hindering the formation of general consensus for its reliability and/or interpretation of scientific conclusions of bahavioural genetics. Though there have been some discussions about the ELSI of behavioural genetics in some journals, most of them do not reflect a variety of opinions amongst the basic researchers in the life sciences. In this study, we interviewed 64 Japanese front-line life scientists in basic research to clarify both the heterogeneity and homogeneity of their opinions about putatively hot topics in behavioural genetics related research, especially about higher-brain function related issues within the global context of emphasis on the relationship between science and society. Most respondents agreed with the existence of the potential implications in behavioural genetics related research. They also agreed with the necessity of more global discussions or judgments in/from society about related topics. It seems necessary to consider practical border line to distinct genetic therapy and genetic enhancement in cognitive ability. Moreover, it is strongly suggested to form better relationship between scientists and mass-media to improve the quality of scientific information to the society, and to enrich the social discussion about the ELSI in behavioural genetics.

2203/F

Genetic discrimination: a survey of cancer genetics professionals' knowledge, attitudes, and practices. *C. Huizenga[1], K. Lowstuter[2], K.C. Banks[3], V. Vandergon[1], C.S. Malone[1], V.I. Lagos[2], J.N. Weitzel[2].* 1) California State University, Northridge, Northridge, CA; 2) Clinical Cancer Genetics Department, City of Hope National Medical Center, Duarte, CA; 3) St. Joseph Hospital, Orange, CA.

Genetic discrimination is an issue of concern among health care providers as well as patients. Lack of knowledge about anti-genetic discrimination laws as well as attitudes about genetic discrimination risk among health care providers may serve as a barrier to obtaining cancer genetics services. This study aimed to assess knowledge, attitudes, and practices regarding genetic discrimination among cancer genetics professionals (CGPs), determine if attitudes of CGPs regarding genetic discrimination have changed over time by comparing our findings to those of previous studies investigating this topic, and to compare CGPs' knowledge and attitudes regarding anti-genetic discrimination laws to that of primary care providers (PCPs). The PCP data were obtained from an unpublished study conducted at City of Hope National Medical Center. A 39 question, anonymous, internet-based survey was conducted of the National Society of Genetic Counselors Familial Cancer Special Interest Group. One hundred and fifty three responses were obtained (34% response rate). The mean total knowledge score of CGPs regarding anti-genetic discrimination laws was significantly greater than that of PCPs ($p<0.001$). A higher percentage of CGPs in this study than in a previous study said that if they were to undergo genetic testing, they would bill their insurance for the cost of genetic testing. The majority of CGPs (94%) perceived the current risk of genetic discrimination to be low, very low, or theoretical and 64% expressed confidence in the current federal anti-genetic discrimination legislation. These results show that CGPs have a higher level of knowledge regarding anti-genetic discrimination laws than PCPs, that concern about genetic discrimination has decreased among CGPs since earlier studies, and that the majority of participants have confidence in current legislation and perceive the risk of genetic discrimination to be low to theoretical.

2204/F

Ethical, Legal and Social Aspects of the Mexican Genomic Variability Project. *C. Lara, A. Hidalgo, I. Silva-Zolezzi, E. Balam, L. Del Bosque, S. March, E. Barrientos, G. Jimenez-Sanchez.* National Institue of Genomic Medicine, Mexico.

Genomic medicine is a priority for the Mexican Government to contribute improving health care for the Mexican population. The Mexican population has a unique origin, more than 80% of the population is considered Mestizo resulting from the admixture of any of 65 indigenous groups with the Spaniards and, in a lesser extent, Africans and Asians. We are conducting a Genome Diversity Project in the Mexican population. The project aims to genotype over 1 million SNPs per individual and produce a comprehensive description of LD patterns, haplotype diversity and sharing, as well as a comparative analysis with other populations. The project was approved by the appropriate Scientific, Ethic and Biosafety Review Boards. We collected anonymous samples from ten states of Mexico representing the country's geography. We implemented a community consultation and consent process strategy that included state government officials, indigenous community leaders, university authorities and members of the local student and scientific community. This strategy operated 2-3 weeks before sample collection and included a brochure using simple language, 4-6 open access informative sessions, an informative poster, general and specific information via TV, radio and printed press. We have conducted 59 informative sessions and collected Informed Consent Forms for 2,800 samples. As a part of this strategy our Institute has established ten collaborative agreements resulting in research collaborations and training local students at the National Institute of Genomic Medicine of Mexico. Thus far, over 200,000,000 SNPs have been genotyped. We ensured availability of enough public information, community engagement process, individual consent, protection individual information, biobank management and confidential treatment of resulting data. This project has led to the largest genomic database for Mestizos and Mexican Amerindian DNA repository worldwide. We expect that results from this project will contribute to generate public knowledge about genomics and sets the basis for best practices in genomic research.

2205/F

Implications of the Property Approach in a Biobanking Context. *J.E. LeGrandeur, T. Caulfield, N. Ries.* University of Alberta, Edmonton, Alberta, Canada.

The status and ownership of tissue samples in biobanks continues to be a controversial issue subject to much debate. For example, to what degree do research participants have continuing control over tissue samples that were previously collected? Do researchers need to re-consent for each new research project? Can participants withdraw their consent and request destruction of the sample? In examining these issues, many biobank policy documents focus on traditional consent norms, for example a right to withdraw consent at any time, so long as the samples remain identifiable. Some commentators have suggested that property principles ought to be given more consideration. Indeed, years after the landmark decision in *Moore v. Regents of the University of California*, which debates the ownership of extracted genetic material, the *Washington University v. Catalona* case has, again, raised a number of serious questions regarding the issue of whether patients have a property interest in their tissue.

In this presentation, we will explore: 1) the role of property principles in existing biobank policy documents (i.e., to what degree is property law considered?); 2) existing literature recommending a heightened role of property law in this context; and 3) the possible ramifications of emphasizing property law as compared to other legal/ethical principles (e.g., autonomy based consent law and confidentiality/privacy principles). This analysis will include such considerations as whether tissue donation to biobanks can/should be considered a "gift", and the impact of a property perspective on commodification and policy concerns.

2206/F

Variation in Preferences for Future Use of DNA Among 2226 Genetic Research Participants. *S.M. Lewis[1], K. Spates[1], P. Raska[2], G.L. Wiesner[1,3].* 1) Dept. Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Dept. Bioethics, Case Western Reserve Univ, Cleveland, OH; 3) Center for Human Genetics, Case Western Reserve Univ, Cleveland, OH.

Researchers in human genetics must balance the advancement of gene discovery with ethical considerations. To promote respect for autonomy and privacy of research participants in genetics studies, guidelines for informed consent recommend that participants indicate whether their DNA may be used in future research. However, research progress could be impeded if participants limit access to this valuable resource. In order to assess the proportion of participants who do not wish to have their DNA used in future studies, we examined the preferences of 2226 participants enrolled in the Colon Neoplasia Sibling Study, a family genetic study that aims to identify colon neoplasia susceptibility genes. In this study, participants choose one of three options for use of their DNA in future research: 1) DNA may be used for future research studies without further contact if identifying information is removed (UNRESTRICTED); 2) DNA may be used for future research if participant is re-contacted and consents (RECONTACT); 3) DNA may not be used for future research studies (NO FUTURE USE). Results showed that overall many participants are willing to allow their DNA to be used for research purposes in the future, as 49% chose UNRESTRICTED use, 46% chose RECONTACT, and only 4% chose NO FUTURE USE. Examination of these choices by self-identified racial categories showed a significant difference ($p< 0.001$) between the choices of the 1894 Caucasian and the 317 African American participants. 53% vs. 29% chose UNRESTRICTED use, 45% vs. 57% chose RECONTACT and 3% vs. 14% chose NO FUTURE USE for Caucasians and African Americans, respectively. The odds for African Americans to select NO FUTURE USE or RECONTACT were 2.6 times higher than Caucasians (95% CI [2.0-3.4]). Understanding the root causes underlying the differences in participants' preferences for future use of their DNA may help researchers more productively address issues of privacy, autonomy, and ascertainment when designing and conducting their genetic studies.

2207/F

Primary Care Clinician Perceptions of Genetic Discrimination and Limited Knowledge of Protective Laws and Cancer Genetics Creates Barriers To Care. *K. Lowstuter[1], S. Sand[1], C. Lee[2], B. Schwerin[3], G. Uman[4], K. Banks[5], C. Gonzalez[6], M. Juarez[6], J. Weitzel[1].* 1) City of Hope, Duarte, CA; 2) Cal Med Assoc Foundation, Sacramento, CA; 3) Cancer Legal Resource Ctr, Los Angeles, CA; 4) Vital Research Inc, Los Angeles, CA; 5) St Joseph Hospital, Orange, CA; 6) Cal Latino Med Assoc, Monterey Park, CA.

Primary care clinicians (PCC), such as nurse practitioners and physicians, are often gatekeepers to specialty services. We surveyed PCCs to explore the extent to which potential knowledge gaps and/or opinions of cancer genetics, genetic discrimination and protective laws influences cancer genetics referrals and consequently access to risk-appropriate cancer screening and prevention. Pre-qualification postcards and invitations were sent to a random stratified sample of California Medical Assoc. members and to all members of California Latino Medical Assoc. and California Assoc. of Nurse Practitioners. The survey contained 47 items on demographics, opinions, knowledge, and practices regarding cancer genetic testing. The majority of responders were physicians (62%, 734/1181). Although 96% of responders viewed genetic testing as beneficial to their patients, 75% stated genetic testing is likely to be declined by patients due to fear of genetic discrimination. The majority did not know that federal law (HIPAA) prohibits health insurance discrimination in the group market on the basis of genetic test results (61%) or that California State law prevents genetic information from being used as a criterion for health insurance coverage decisions (67%). When given five hypothetical family cancer histories only 30% correctly identified four or more scenarios as appropriate or inappropriate for genetics referral. Of the 55% who had not referred patients for genetic cancer risk assessment, 11% indicated concern over health insurance discrimination as a reason. Results indicate knowledge gaps and misperceptions as possible barriers to referral for genetic cancer risk assessment. Education of PCCs regarding cancer genetics and current, as well as, future genetic discrimination laws may help to promote access to appropriate care.

2208/F

Parental perceived value of a diagnosis for Mental Retardation (MR): A qualitative comparison of families with and without a diagnosis for their child's MR. *N.L. Makela[1], C.A. Marra[2], P. Birch[1], D.A. Regier[2], J.M. Friedman[1].* 1) Human Genetics, University of British Columbia, Vancouver, B.C., Canada; 2) Centre for Health Evaluation and Outcome Sciences, Providence Health, UBC, Vancouver B.C., Canada.

Background: Although the adoption of Array Genomic Hybridization (AGH) for diagnosis of submicroscopic genomic copy number alterations that cause mental retardation (MR) is likely to affect practice, its value to families of children with MR is largely unknown. We used qualitative methods to investigate the value that families of a child with MR place on obtaining a precise genetic diagnosis. Method: Using telephone interviews, 161 parents of children between the ages of 5 and 10 years with MR were interviewed to determine the value they place on receiving a precise genetic diagnosis. 24.8%(N=40) had a child with chromosomal abnormality identified cytogenetically, 4.3%(N=7) had a child that was diagnosed clinically but could not be confirmed by laboratory testing, and 70.8% (N=114) had a child with idiopathic MR. 65 of the idiopathic MR cases had a condition that explained some of the child's symptoms but did not provide an etiological diagnosis of the MR. Twenty of the families (10 with a genetic diagnosis and 10 without a diagnosis) also participated in taped interviews to provide a qualitative comparison. Results: No differences on the value placed on obtaining a precise genetic diagnosis could be found between the two groups. Most parents in both groups strongly valued a genetic diagnosis, but a few did not. Reasons included the avoidance of stereotypes and prejudice. Many felt that the timing of diagnostic testing was important, and higher values were placed on early testing provided that the families were emotionally ready. Parents' perceptions of what a precise diagnosis can offer were not always realistic, and many perceived it as critical in obtaining support and services for their children. Few cited family planning as a strong factor. Conclusions: Parents of children with MR strongly valued obtaining a precise genetic diagnosis. Most cited access to support services as the reason.

2209/F

Geneticists' views of societal and ethical implications of research: results from a national survey. *J. McCormick, A. Boyce, M. Cho.* Ctr Biomedical Ethics, Stanford Univ, Palo Alto, CA.

While past studies on research ethics have shown that scientists have major concerns about scientific misbehavior, it is also important to characterize a broader spectrum of geneticists' concerns -- what considerations do they give to the broader societal and ethical implications of life science research? How do scientists view socially "controversial" areas of research? Has public discussion of ethical, legal, social, and policy (ELSP) issues in genetics influenced geneticists to think more about the ethical and societal issues of their research? We have conducted a national survey and interviews to determine how and what life scientists think about the relationships between life science and society, and the ELSP implications of their research. We mailed 2000 surveys to seven different institutions across the United States and conducted follow-up interviews. Our sample population included researchers from range of academic positions and a number of departments, including genetics. Our preliminary findings suggest possible differences between genetics researchers and the larger life science population. When asked about ethical and societal concerns a researcher might have in the course of her research, geneticists were more likely to deal with issues around the use of race and ethnicity as a research variable, to encounter unexpected findings in research subjects, and to perceive they work in a field the public finds controversial than the broader life science population. In general, researchers have a broad span of societal and ethical concerns, ranging from specific issues in animal and human subjects research, to socially controversial topics in genetics and stem cell research, to broader issues concerning the environment and natural resources and the nation's healthcare system. Finally, nearly all of the scientists were concerned about the increasing politicization of science, yet have mixed views on how scientists, wearing their "scientist hat," becoming involved in partisan politics damages the credibility of the scientific community.

2210/F

Sharing research results from complex disease genetics studies: A community based participatory research approach. *K.K. McGlone, E.M. Drew, G.V. Mohatt, R.L. Pasker, B.B. Boyer.* Center for Alaska Native Health Research, Institute of Arctic Biology, University of Alaska Fairbanks.

The Center for Alaska Native Health Research (CANHR) conducts studies in Yup'ik Eskimo communities in order to understand the interactions between genetic, nutritional and psychosocial risk factors for obesity and diabetes. CANHR employs a community-based participatory research (CBPR) approach, in which participating community leaders are viewed as co-researchers and are involved in all steps of the process from generating a common research question to the interpretation and dissemination of overall results. Collaboration on procedures for sharing results is imperative to the partnership underlying CBPR, as it builds capacity within the community to understand and utilize study results. CANHR investigators have collaborated with regional healthcare providers, tribal leaders, and university-, local- and national-IRBs to identify culturally appropriate mechanisms for sharing general research progress. Using a CBPR approach to disseminate results of multifactorial disease genetics studies is yet unprecedented. NBAC guidelines and the NHLBI working group (Bookman *et al*, 2006) recommend that genetics results should only be disclosed to participants under limited circumstances. This guidance conflicts with the goal of the community as co-researcher that is fundamental to CBPR. We plan to apply our CBPR process to genetics research, to avoid ethical dilemmas and to sustain the relationship of trust among partners, while respecting NBAC recommendations. To do so, we will hold several meetings, including a traditional scientific presentation among CANHR investigators, followed by focus groups with tribal leaders, and then with selected participants to identify and resolve ethical issues and to develop a culturally and ethically appropriate presentation to convey emerging results to all participating communities. We conclude that both researchers and participants should benefit from population-based genetics research, and that it is essential to move forward as co-researchers in the CBPR enterprise.

2211/F

Screening for sickle cell disease on dried blood: the application of a new ELISA-test on African newborns. *L. Mutesa[1], F. Boemer[1], L. Ngendahayo[2], S. Rurisa[3], E.K. Rusingiza[4], N. Cwinya-Ay[4], D. Mazina[5], P.C. Kariyo[6], R. Schoos[1], V. Bours[1].* 1) Center for Human Genetics, Univ Liege, Belgium, Liege, Belgium; 2) Department of Pathology, National University of Rwanda, Butare, Rwanda; 3) Department of Obstetrics and Gynecology, National University of Rwanda, Kigali, Rwanda; 4) Department of Pediatrics, National University of Rwanda, Butare, Rwanda; 5) Department of Public Health, University of Liege, Liege, Belgium; 6) Department of Pediatrics, University of Burundi, Bujumbura, Burundi.

Objectives: To evaluate the feasibility of a systematic neonatal screening for sickle cell disease in the region of Great Lakes in Central Africa using a new approach with limited costs. Setting: Sickle cell disease is a major public health problem in Africa. Methods: Between July 2004 and July 2006, 1825 newborn dried blood samples were collected onto filter papers in four maternity units from Burundi, Rwanda, and the East of the Democratic Republic of Congo. We tested the presence of hemoglobin C and S in the eluted blood by an enzyme-linked immunosorbent assay (ELISA) test using a monoclonal antibody. All ELISA positive samples (Multiple of median above 1.5) were confirmed by a simple molecular test (PCR-restriction). The statistica software version 7.1 was used to create graphics and to fix level of MoM cut-off, whereas the chi-square of Pearson was used to compare the genotype incidences between countries. Results: Among the 1825 newborn samples screened by ELISA-test, 97 (5.32 %) were positive. Sixty (3.28 %) of these samples were heterozygous for Hb S, 4 (0,22 %) for Hb C, whereas 2 (0,11 %) newborns were Hb SS homozygotes. Conclusions: The lower cost and the high specificity of ELISA-test are appropriate for developing countries, and such a systematic screening for sickle cell anemia is therefore feasible.

2212/F

Community Centered Family Health History. *J. O'Leary[1], N. Bonhomme[1], J. Williams[2], M. Williams[2], P. Kyler[3], S. Terry[1].* 1) Genetic Alliance, Washington, DC; 2) Clinical Genetics Institute, Intermountain Healthcare, Salt Lake City, UT; 3) Genetic Services Branch, MCHB, HRSA/DHHS, Bethesda, MD.

Family health history is an accessible tool which captures heredity, diet, and environment; allows a health care provider to diagnose conditions and understand risk; increases health and genetics knowledge for the individual and the family; and promotes conversations about health in the family and community. It is impossible to create a one-size-fits-all family health history tool, given the diversity of individuals, families, and communities. In the community context, a flexible approach is required, best articulated by the organizations and individuals that are directly involved with them. Genetic Alliance is partnering with a diverse group of communities to create customized family health history tools. We hypothesize that accessible tools produced by the community, for the community, will promote conversations about health within the family and translate knowledge of family health history into healthy choices. Each community involved in the project adapts the "Does It Run In the Family?" family health history toolkit, disseminates it to community members, and evaluates its usefulness through baseline and follow-up surveys. Evaluation of the project serves the dual purpose of measuring the utility of family health history in promoting healthy choices and determining necessary modifications of the toolkit for the creation of an online customizable version. The current "Does It Run In the Family?" template was created using feedback from all partners and the National Advisory Committee. Partner organizations and their Community Advisory Boards customized that template to create their community-specific toolkits. The online version will streamline this process by allowing users to choose photos, personal health stories, and quotations from an online file library. In addition, Genetic Alliance will be releasing an RFP for national and community organizations to beta test the customizable online toolkit. The honorariums available will include costs for developing a tailored family health history tool and printing.

2213/F

Genetic discrimination by phenotype: the law versus the insurers in health. *A. Ordonez, F. Suarez, E. Diaz.* Inst de Genetica Humana, Pontificia Univ Javeriana, Bogota, DC, Colombia.

The epidemiologic transition in Colombia is the main reason by which in the country, the second cause of mortality in the newborns, is the birth defects. Nevertheless, birth defects are not covered by the insurances of health. Objectives: to determine the set of laws that guarantees the access to the health services to the individuals affected by birth defects and to determine causes by which the attention in clinical genetics is not a priority of the health system. Methods: systematic revision of the legal regulations of the Colombian system of health. Revision of the norms of the insuring companies in health, and of the health maintenance organizations (HMOs), in relation to the attention of birth defects. Results: the Colombian law through the act of the childhood and adolescences, Colombian congress Law 1098 2006, stipulates that the affected with birth defects must receive appropriate medical attention, diagnostic, treatment and counseling. In contrast, the basic plan of health insurance and the HMOs in Colombia only cover one consultation to the geneticist, and no diagnostic test or genetic test asked for, by the geneticist, are covered. The insurers warn to the parents of the patient, about this situation and explain that this restriction is based on the pre-existence of the pathology, which means that the diseases exist before the individual were assured, a situation that prevents him/her the access to the health insurance and attention to the congenital or genetic diseases. The Colombian law of security in health, Colombian congress law 100-1993, prohibits the implementation of this concept (pre-existence), but the insurers apply it currently. Conclusion: the birth defects constitute, in the affected individuals, a discrimination mark into the health system which moves them away of an suitable attention, a discrimination that we have denominated genetic discrimination by phenotype, in which the people with congenital malformations, are warned by the insurers about the supposed limitations that the health plans have in relation to their pathology, a warning based on a concept that the Colombian law prohibits.

2214/F

Can patents on genetic tests inhibit the development of genomic diagnostics? An analysis of case studies. *B.L. Pierce[1], S. O'Connor[2], J.L. Stanford[3], M.A. Austin[1,3].* 1) Institute for Public Health Genetics, University of Washington, Seattle, WA; 2) School of Law, University of Washington, Seattle, WA; 3) Department of Epidemiology, University of Washington, Seattle, WA.

Patents on genetic tests are controversial for many reasons, including their potential inhibition of the development of genome-based diagnostics. Such inhibition might occur if multiple parties are assigned patents on genetic tests that relate to a specific clinical problem. Any of these parties can legally block the commercialization of a product that tests all relevant variants. The aim of this project was to search for evidence for such a scenario using case studies. Selected cases were required to be conditions with at least three genetic risk factors, corresponding to at least three patents. For each case, relevant genetic risk factors were identified by reviewing the scientific literature. Relevant patents were identified using gene- and disease-specific searches of the U.S. patent and trademark office's patent database. For each patent, the issue date, assignees, and claims were catalogued. Long QT syndrome (LQTS) and maturity onset diabetes of the young (MODY) have moderate levels of known locus heterogeneity (6-8 genes). Five LQTS genes are the subject of patents, all assigned to the same party, with a co-assignee on patents for two of the five. Five MODY genes are the subject of patents (3 total), all assigned to the same party. Cystic fibrosis is characterized by allelic heterogeneity within the CFTR gene. All patents on testing CFTR variants, with one exception, are held by a single party (3 patents) with a coassignee on two. The remaining CFTR patent was licensed to a company owning the rights to test all other variants. In conclusion, we observed two cases of unified and one case of fragmented patent rights. The fragmented rights were unified via licensing, suggesting that it is unlikely that patents have seriously inhibited the development of tests related to these case studies. However, as more genetic risk factors are discovered, future genetic testing products may require securing patents from multiple parties, potentially inhibiting the development of valuable genomic tests.

2215/F

Roles of Advocacy Groups in Genetic Research of Complex Traits: The Example of Autism. *H.K. Tabor, M. Lappé, M.K. Cho.* Ctr Biomedical Ethics, Stanford Univ, Palo Alto, CA.

Historically, patient and family advocacy groups for genetic diseases have played key roles in research. Research on Huntington's disease, cystic fibrosis and hemophilia has been directly influenced by the work of advocacy groups in raising funds for research, organizing subjects for participation, and making recommendations about screening and genetic counseling. One of the roles of advocacy groups for rare Mendelian traits has been the creation of collaborative relationships between families and researchers in genetic research studies. These interactions, and the ethical and social issues involved, have been documented in work by social scientists and ethicists, as well as by advocates themselves. However, little research has been published on the roles of advocacy groups in genetic research on complex diseases, and whether the ethical and social issues involved in these roles differ from those in the context of Mendelian traits.

We examined the roles of advocacy groups in genetic research for one complex disease, autism. We conducted semi-structured interviews with leading genetics researchers in autism and the founders and leaders of the largest autism advocacy groups. Interviews were analyzed using qualitative textual analysis. We will present data on how interactions between advocacy groups and researchers influence the framing of causality of autism, particularly the relative influence of genetic causation. We will describe ethical and social issues identified by interviewees in the effort to identify causal genes through the creation of large-scale databanks of DNA and phenotypic data for complex traits and through large-scale collaborations across multiple research groups. We will also present findings on the influence of advocacy groups on the short and long term goals of genetic research, consent of family members for participation in genetic research for complex traits, and researcher-participant trust. We will also discuss researcher and advocacy group perspectives on the advantages and disadvantages of their interactions, the future of genetic research of autism and on the future roles of advocacy groups.

2216/F

What interests and values should guide biobanking? Lessons from two experiments in deliberative public consultation. *H. Walmsley[1], R. Abadie[2], D. Hartell[1], M. Burgess[1], B. Koenig[2].* 1) University of British Columbia, Vancouver, BC, Canada; 2) Mayo Clinic College of Medicine, Rochester, MN.

What interests and values should guide biobanking? Existing governance frameworks were developed for small-scale research projects and are based upon personal autonomy and individual informed consent. Large-scale and networked collections of biological specimens and data pose new problems. These range from the expense and unwieldy nature of the consent process for researchers, to complaints about commercialization and unauthorized use of samples by indigenous groups, to fears of data linkage by privacy advocates, and debates about the relative value of 'biobanks' versus 'cohorts' to public health. Transparent public engagement with biobanking is long overdue. We provide lessons learned from two deliberative public consultations: one conducted in British Columbia (BC), Canada, the second in Olmsted County, Minnesota (MN). A proposal for a BC-wide BioLibrary and Mayo Clinic plans for an institutional biobank provided the opportunity for citizens to shape planning for an actual, as opposed to a hypothetical, biobank. This joint project draws from theories of deliberative democracy and pioneering examples, such as the Citizen's Assembly in BC. Our aim: to facilitate a genuinely inclusive public debate. An innovative community engagement structure was developed and implemented in BC and MN. The engagement exercise included two full weekends (4 days) of face-to-face deliberation in large and small groups. Professional moderators facilitated the discussion. Diverse expert and stakeholder presentations, background readings circulated ahead of the event, and physical models of the proposed biobank features provided the stimulus for informed yet open-ended deliberation by 25 demographically-stratified citizens at each site. Recording of all sessions and online discussions and a members-only website facilitated research on the event. The challenge to the deliberants: what interests and values should guide biobanking? The challenge to the research team studying the events: what methods allow for "authentic" public consultation about complex scientific topics by citizens in a democracy?

2217/F

Public-Private Partnerships and Genetic Research: Data-Sharing Issues. *D.N. Wholley.* Research Administration, Foundation for the NIH, Bethesda, MD.

Public-private partnerships are becoming increasingly important in creating large-scale collaborations and community resource projects for scientific research. These projects allow researchers to tackle opportunities and solve problems in the "pre-competitive" sphere which a single entity cannot tackle effectively alone, either because of scale, lack of available funding, or because the very nature of the problem requires cooperation amongst academic, government, and industry partners. The resulting requirements to share data and infrastructure, however, carry with it significant responsibilities: protecting the confidentiality and respecting the consent of study participants, ensuring that use of data is not restricted by premature or predatory claims on intellectual property, balancing the needs of principal investigators to publish results versus the imperative to provide broad public access to data, and managing conflicts of interest, antitrust, and confidentiality in dealings with commercial partners. My talk therefore will focus less on specific technical standards for interoperability than on the business and policy infrastructures that enable and sustain them. I will cite several examples from current public-private partnerships managed by the Foundation for the National Institutes of Health (FNIH) such as the Genetic Association Information Network (GAIN) and The Biomarkers Consortium.

2218/F

Public opinion for Direct-to-consumer (DTC) genetic testing in Japan. *T. Ohata, A. Tsuchiya, M. Watanabe, F. Takada.* Dept of Medical Genetics, Graduate school of Medical Science, Kitasato Univ, Sagamihara, Japan.

In recent years, DTC genetic testing has gradually become popular. In this situation, consumers are buying tests by their own choices, collecting information from the internet or magazines. In clinical scene, medical professionals require to provide instruction and counseling for genetic testing, following some guidelines. On the other hand, the organization of testing companies, "Council for Protection of Individual Genetic Information", made their own guideline voluntarily. But there is no comprehensive regulation system for these testing in Japan.

On February 2007, we performed the inquiry survey based on 3,000-people scale, to hear their opinion on DTC genetic testing. Their ages were ranged from 20 to 69 years old. Twenty seven point five percent of the people answered "I have used it", or "I know it", and 46.6% answered "Genetic testing, provided at any place other than a hospital is convenient". Although the visibility of DTC genetic testing was not so high, the expectation for doing it at any place other than a hospital was high.

About the regulation in this questionnaires, we provided 5 choices which were "Our government should regulate it by law", "Academic societies should make guidelines which virtually regulate it", "Some organization of companies should control it voluntarily", "Each company should make a decision voluntarily", and "No particular regulation is necessary", and the 66.8% of them expected the regulation by the government.

These results will serve as a basis for initial trials when the regulation for DTC genetic testing will be considered near future.

2219/F

Social attitudes toward genetic testing and social image of "gene" in Japan. *A. Tsuchiya, T. Ohata, M. Watanabe, F. Takada.* Medical Science, Kitasato University, Sagamihara, Kanagawa, Japan.

A purpose of this presentation is to analyze social attitudes toward genetic testing, especially about polygenic disease, in Japan. This analysis includes correlation between image of gene, an extent of genetic determinism, and the other basic properties like age, gender and the other ones. I used 3000 Samples which were collected by random sampling through out Japan. The ratio of basic properties - age, gender, marriage and region - is distributed according with the one of national census in 2005.

The output below is multiple regression analysis that dependent variable is the degree of needs to genetic testing about life-style related disease and independent variables are an extent of genetic determinism(12-48),image of gene(bad/good 1-4)and the other basic properties(gender,marriage).

	Coef	Std	Err	p>|t|
women (dummy)	.187	.039	4.79	0.000
married (dummy)	-.038	.040	-0.96	0.335
genetic determination	.020	.003	6.83	0.000
image of gene	.134	.019	6.92	0.000
cons	1.485	.188	7.91	0.000

One output of this analysis is that the needs of genetic testing about life-style related disease is affected especially by an extent of genetic determinism and image of gene, controlling the other variables(gender, marriage)..

2220/F

Age of diagnosis vs. outcome of infants with Severe Combined Immunodeficiency. *J. Davis[1], K. Chan[1], J. Puck[2].* 1) NHGRI,NIH Bethesda, MD; 2) Dept.of Pediatrics, UCSF, San Francisco, CA.

Severe combined immunodeficiency(SCID)is a rare disorder characterized by lack of T cells and antibody responses. Though genetic etiologies are diverse, all affected infants have very few T cells. SCID is asymptomatic at birth. Unless family history is positive, SCID infants are diagnosed only after serious infections arouse suspicion; those not recognized in time for intervention die of infections. Bone marrow transplantation (BMT) for SCID is life-saving if performed early; Buckley et.al (2004) reported 95% vs 70% survival, respectively, for BMT before vs after 3.5 m of age. We have developed a SCID newborn screening test based on quantitative PCR of T-cell receptor excision circles (TRECs), which are abundant in normal neonatal blood, but absent in SCID blood regardless of genotype(Chan and Puck, 2005). To examine whether universal newborn screening for SCID would be beneficial, we designed a structured interview for parents that reviewed each month of their affected infant's first 2 years. Parents of 39 SCID patients born from Jan 2000 through Dec 2004 consented to sharing their infant's history either after enrolling in our mutation study or by responding to our notice on the website www.scid.net. Based on their vivid recollections, we recorded numbers of clinic visits for infections, hospital stays, treatments, age at SCID diagnosis, age at BMT, and outcome. A recognized family history, present in only 7 cases (18%), led to early diagnosis (mean age of 2 m). These infants all received antibiotics and immunoglobulin, had BMTs at a mean age of 3 m, and were surviving at least 2.5 y post-BMT. In contrast, the 32 SCID infants (82%) with no known family history had a mean age of diagnosis of 9 m, with one diagnosed only at autopsy. Eight sporadic SCID infants died of infections before treatment could be instituted, and 10 others died after receiving BMT or PEG-ADA enzyme treatment, yielding an overall survival of only 44% for this group. We suggest that testing all newborns for TRECs could rescue SCID infants by giving sporadic cases access to early care currently available only to those with prior affected relatives.

2221/F

Perennial challenges in genetic screening policy-making. *A. Andermann[1,2], I. Blancquaert[1,3], S. Beauchamp[1].* 1) Agence d'évaluation des technologies et des modes d'intervention en sante (AETMIS), Montreal, Canada; 2) McGill University, Montreal, Canada; 3) Université de Montréal, Montreal, Canada.

INTRODUCTION: Genetic screening policy-making is highly complex and deals with many sensitive issues on multiple levels. A systematic approach is therefore needed to promote greater transparency and accountability. OBJECTIVES: As part of a longer process in the development of a decision guide for genetic screening policy-making that included a series of literature reviews and consultations, local and international experts were consulted to determine whether a draft of the guide was considered useful and how well it addressed challenges in genetic screening policy-making. METHODS: Self-completion questionnaires and a copy of the draft decision guide were sent to 66 local and international experts and high-ranking officials in the fields of population-based screening, public health and genetics as well as 51 stakeholders who had been involved in previous consultations. Reminders were sent to non-respondents. Data was analyzed using thematic coding and validated using an inter-judges technique. RESULTS: With an overall response rate of 29% (n=34/117), it was considered that the decision guide is an improvement over existing lists of criteria. In particular, the decision guide attempts to address and make explicit many of the perennial challenges in genetic screening policy-making, including: 1) the lack of evidence with regard to rare diseases, 2) balancing individual-level and population-level concerns, 3) ensuring the protection of individuals and communities, and 4) ultimately reaching consensus as to whether the benefits outweigh the risks of screening. CONCLUSIONS: Although it may not be possible to entirely resolve all perennial issues associated with genetic screening policy-making, the decision guide encourages documentation of evidence, trade-offs, and reasoning underpinning recommendations, thus promoting greater transparency, and allowing decisions to be revisited over time.

2222/F

An interdisciplinary approach to genetic screening policy-making. *I. Blancquaert[1,2], A. Andermann[1,3], S. Beauchamp[1].* 1) Agence d'évaluation des technologies et des modes d'intervention en sante (AETMIS), Montreal, Canada; 2) Université de Montréal, Montreal, Canada; 3) McGill University, Montreal, Canada.

INTRODUCTION: Decision-making with regard to population-based genetic screening programs is highly complex. Judging the utility of screening, which entails an evaluation of whether the benefits outweigh the risks, requires that a large range of factors are taken into consideration. OBJECTIVES: In developing a decision guide to support policy-makers faced with difficult decisions regarding the introduction or expansion of population-based genetic screening, a consultative process was used which sought to integrate the views of stakeholders as well as experts from a number of relevant disciplines. METHODS: Two rounds of consultations with local stakeholders and with local and international experts in the fields of population-based screening, public health and genetics led to the transformation of an initial list of 20 criteria, synthesized from over 54 published lists of criteria, into a more elaborate decision guide. RESULTS: The decision guide was structured according to the logic of the decision-making process that emerged from the consultations, which involves three nested decision nodes. Some considerations (e.g. scientific, ethical, legal, social, organizational, economic, political, etc.) are more relevant at specific stages in the decision-making process, and therefore, are more prominent in certain nodes of the decision guide. Although multiple types of expertise need to be called upon during this process, different disciplines have different perspectives, methodologies, and vocabularies which stem from different paradigms. Thus, an interdisciplinary approach also raises many challenges. CONCLUSIONS: Genetic screening policy-making requires the input of many different types of evidence and expertise. This led to the production of an interdisciplinary decision guide which has the advantage of making explicit the broad range of issues involved, articulating the different considerations according to the logic of the decision-making process, highlighting trade-offs and promoting a dialogue between different stakeholder groups.

2223/F

Data Sharing - A good idea in principle? *J.S. Kaye.* Ethox Centre, University of Oxford, Radcliffe Infirmary, Woodstock Road, Oxford.

The scientific benefits of sharing sequence data are well established. This has lead to considerable investment by funding bodies, to establish new genomic resources that can be used by all, but also to develop the means to link and share existing collections. The initial data- sharing principles were articulated in the Bermuda Principles 1996, and more recently the Fort Lauderdale Tripartite-Agreement 2003. Both of these documents relate to sequence information. Since then, data sharing policies have been put in place by major funding bodies, such as the NIH, the European Commission, and the MRC (UK) as a requirement of funding, which apply to all types of data generated through the research process. The rationale behind such initiatives is to utilize publicly-funded research data to its fullest extent, by opening up such collections to other researchers, thereby reducing unnecessary duplication of data-sets, enabling new lines of enquiry and speeding up the process of knowledge production. The basic principle is that all data should be shared, unless controllers of datasets can establish good reasons why this should not be so. These requirements have implications for the present practice such as how to protect the privacy rights of the research participants; standardise procedures; ensure trust between researchers; fair acknowledgement of use of the resource; and the apportionment of intellectual property rights. Using Europe as a case study, the purpose of this paper is to outline some of the legal obstacles and issues that arise out of the push by funders to share samples and data, in order to hi-light areas of the law that need development and require further policy consideration.

2224/F

Lack of clinical use, of molecular, enzymatic and cytogenetics diagnostics test, at medical genetics services, in Bogotá, Colombia. *F. Suarez, A. Ordonez, E. Diaz.* Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia.

Molecular, enzymatic and cytogenetics diagnostics tests are an essential part of the diagnostic process of the patients attended at the genetics consultation. Objectives: to determine the frequency of use of molecular, enzymatic and cytogenetics diagnostics test in the consultation of medical genetics of three hospitals of the city of Bogota. Methods: revision of 600 clinical histories, of the three hospitals. After the clinical charts were reviewed, a survey to the parents of the patients, was made, asking about their perception about the genetic tests. Results: 600 clinical histories were reviewed; the patients were attended between the 01/9012004 to 01/12/05. A definitive diagnostic was reached in 284 cases (47, 3%); of which 109 cases (38, 4%) were patient with Down syndrome. A definitive diagnostic obtained through molecular tests was accomplished in only just 4 cases (1,4%): 1 case of Cystic Fibrosis and 3 cases of Muscular Dystrophy of Duchenne. The diagnostic through FISH was made in a case of Prader Willi (0,4%). The diagnostic of a metabolic pathology through enzymatic test was carried out in two cases of Morquio syndrome (0,7%) and a case of a homocystinuric child (0,4%). The molecular, enzymatic tests and FISH were asked for in the clinical history in 80% of the cases. The most asked laboratory test was Karyotype, in 66% of the patients, but it was made in less than 50% of the cases. The survey to 264 parents of the patients in which it was not possible to reach a definitive diagnostic showed that: the genetic tests, were not covered by their health policies (81,8%); the genetics consultation was not covered in their health policies (88.3%); the insurers do not know where, of what kind of laboratory companies is able to make that type of laboratory test (62%). Conclusions: the diagnostic genetic tests are not broadly used in the consultation of medical genetics at Bogota city; the main causes are the lack of cover on the part of the insurance health policies and the deficiency of specialized laboratories. The negative consequences of this situation in the context of clinical attention of the affected by genetic diseases, are discussed.

2225/F

Assessment of Current Information Resources for Newborn Screening Conditions. *E.S. Reese, B. Chen.* Division of Laboratory Systems, National Center for Preparation, Detection, and Control of Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Background: In 2005 the American College of Medical Genetics (ACMG) recommended a national uniform newborn screening (NBS) panel. Many states have adopted these recommendations, resulting in an increased need by healthcare professionals and the public for information regarding diagnosis, intervention, and management of the disorders. However, the desired information may not always be publicly available. Based on recommendations from the CDC-hosted "Quality, Access, and Sustainability of Biochemical Genetic Testing Working Meeting" in October 2006, we assessed the current information resources for NBS diseases and related genetic testing and explored ways to develop a common information portal for general practitioners, specialists, laboratories, and the general public. Methods: Twenty commonly used websites were assessed for information regarding basic information, genetic testing information, laboratories, testing algorithm and availability, sensitivity/ specificity of genetic tests, interpretation of test results, and disease management for the 84 diseases on the ACMG NBS panel. Findings: The quality and quantity of the information varied among the websites. Five websites contained information provided by external links, two contained restricted access, and two contained no applicable information. The most common information elements were basic information and availability of genetic tests for newborn genetic diseases. Many websites lacked information on test algorithms, sensitivity/specificity, interpretation, and disease management. Conclusions: Availability and accessibility of NBS information is an increasing public health need. Using the information compiled, we have developed a searchable database, available on the CDC website, that can direct users towards general and disease-specific information about NBS conditions.

2226/F

Public Policy Issues Surrounding Genetic Information and Long-Term Care Insurance. *E.M. Ramos[1], K.L. Edwards[2], E. Ramos[3], W.A. Kukull[2], C.A. Watts[2]*. 1) NHGRI, Bethesda, MD; 2) U. of WA, Seattle, WA; 3) ASHG, Bethesda, MD.

Objective: Information gleaned from genetic tests for Late-onset Alzheimer's disease (LOAD) may affect access to long-term care (LTC) insurance. We applied a policy framework to dissect this difficult issue. **Background:** LOAD is a prevalent, complex neurodegenerative disease that leads to severe disability and the need for expensive nursing home or at-home care. LTC insurance is one mechanism to cover these costs. It has been difficult to elucidate the genetic and environmental stimuli that influence LOAD onset. Only APOE has been verified as a genetic risk factor. As new research tools are implemented, such as genome-wide association studies, additional genetic variants may be uncovered. **Methods:** A literature search was conducted to inform the elements of the policy framework including outlining relevant background information pertaining to genetic testing, LTC insurance, and adverse selection; identifying the stakeholder groups and analyzing their interests and concerns; generating a list of potential policy options to regulate the use of genetic information in LTC insurance; and evaluating these options to determine the consequences of implementation. **Results:** We identified insurance applicants and their families, insurance companies offering LTC policies, state and federal governments, employers, and genetic testing companies as the primary stakeholders. Each group has distinct interests that define their public policy positions. Policy options range from prohibiting the use of all genetic information in LTC insurance to imposing no regulations and allowing market forces to drive the future of LTC insurance. **Conclusions:** Policymakers must balance complex issues including equity, accessibility, and affordability when instituting legislation that regulates use of genetic information in the LTC insurance market. Many policy options exist and each needs careful evaluation to ensure that appropriate coverage for LTC insurance is available for consumers. **Disclaimer:** This abstract was prepared while Dr. Ramos was employed at the U of WA. The opinions expressed here are the author's own and do not reflect the views of the Dept. of Health and Human Services.

2227/F

Direct to Consumer Genetic Testing: A Report from Japan. *M. Watanabe, A. Tsuchiya, T. Ohata, T. Sumida, F. Takada.* Clinical Genetics, Graduate School of Medical Sciences, Kitasato Univ, Sagamihara, Kanagawa, Japan.

The goal of the proposed presentation is to discuss the challenges of constructing appropriate regulation for Direct-to-Consumer genetic testing in a society, based on the experience of Japan.

The market of DTC genetic testing service in Japan began around the year 2000, and today there are about 10 providers. At the moment, there is no regulatory system specifically designed for provision of genetic testing in Japan. However, the committee organized voluntarily by industries dealing with human genetics is now constructing a voluntary standard on the provision of genetic testing. Nevertheless, the result of our survey shows that the majority of citizens in Japan prefer governmental regulation for the provision of genetic testing.

Preference in Types of Regulation for DTC Genetic Testing

Governmental Regulation	66.8%
Guidelines by Academic Societies	23.77%
Guidelines by Industry Associations	5.9%
Self-regulation of each company	1.73%
No regulation necessary	1.8%

The proposed presentation aims to present a case of policy making for genetic testing and its difficulty, by providing an analysis of Japanese experience.

(Direct-to-Consumer genetic testing can include both genetic testing advertised directly and sold directly to consumers. However, in this paper, "DTC genetic testing" indicates only such genetic testing sold directly to consumers.)

.

2228/F

PUBLIC EDUCATION FOR LOW-INCOME PREGNANT WOMEN ON PRIMARY PREVENTION OF BIRTH DEFECTS IN RIBEIRãO PRETO-SãO PAULO-BRAZIL. *I. Gomy[1], M.L.O.C. Mesquita[2]*. 1) Medical Genetics, Ribeirão Preto School of Medicine, University of São Paulo, Brazil; 2) Pastoral da Criança, Ribeirão Preto, São Paulo, Brazil.

Approximately eight million children with serious birth defects are born each year - 6% of all births worldwide. At least 3.3 million children under age five die yearly due to congenital anomalies and about three million of those who survive are handicapped. Birth defects are a global problem, but their impact is greater in middle- and low-income countries where there have been as much as 95% of deaths of children with congenital anomalies. In Brazil, infant mortality rate is 22.5/1000 live births (2004). In South and Southeast areas where infectious diseases are controlled, birth defects are the second cause of early infant deaths. Brazil's prevalence rate of congenital anomalies is 57.2/1000 live births whereas global prevalence ranges from 82/1000 (Sudan) to 39.7/1000 (France). These huge disparities are due to several risk factors, such as maternal exposure to teratogens and lack of public maternal health and preventive measures. High-income countries have some experience on preventing birth defects by up to 70% with actions such as food fortification with folic acid on prevention of neural tube defects. There are non-profitable and non-governmental organizations programs in Brazil that effectively plays a role on prevention of infant mortality, such as those of "Pastoral da Criança". **Objective**: to provide low-income pregnant and childbearing age women with educational and healthy actions about primary prevention of birth defects. **Methods**: educative talks and explanatory leaflets were given monthly from January to December 2006 during "Pastoral da Criança"'s programs in Ribeirão Preto, southeastern Brazil. **Results**: awareness and comprehension improved on how simple and feasible those preventive actions are. **Conclusion**: effective public education initiatives are urged to be taken by public health policy of developing countries on primary prevention of birth defects, in order to minimize morbidity and to decrease infant mortality rates.

2229/W

Safety of sapropterin dihydrochloride (sapropterin) in children with phenylketonuria (PKU) on a phenylalanine (Phe)-restricted diet. *D. Grange[1], C. Whitely[2], H. Christ-Schmidt[3], A. Dorenbaum[4], H. Levy[5].* 1) St Louis Child Hosp,St Louis,MO; 2) U Minnesota Med Ctr,Minneapolis,MN; 3) Statistics Collaborative Inc,Washington,DC; 4) BioMarin Pharmaceutical Inc, Novato, CA; 5) Child Hosp, Boston, MA.

Intro:Sapropterin, an oral formulation of tetrahydrobiopterin, can lower blood Phe in PKU. We report safety data from a double-blind, placebo-controlled Ph 3 study in children with PKU controlled on a Phe-restricted diet. Methods:Children (4-12yrs) diagnosed with PKU with hyperphenylalaninemia (≥1 blood Phe measurement ≥360μmol/L), controlled (blood Phe ≤480μmol/L) on a Phe-restricted diet for ≥6months received sapropterin 20mg/kg/day, for 8 days (Part 1). Responders (≥30% reduction in blood Phe and blood Phe ≤300μmol/L [5mg/dL] on Day 8, arbitrarily defined) entered Part 2 and were randomized 3:1 to receive sapropterin 20mg/kg/day or placebo for 10 wks. Phe supplement was prescribed at Wk 3 and adjusted bi-weekly according to blood Phe level. Safety was monitored by adverse events (AEs), physical exam and clinical lab tests. Results:89/90 children had known responder status (Part 1), 50(56%) were responders and 46(51%) were randomized (sapropterin=33,placebo=12,1 did not receive drug). Most AEs were mild and considered unrelated to study drug; 15/90(17%) subjects in Part 1 and 9/33(27%) sapropterin subjects and 3/12(25%) placebo subjects in Part 2 had AEs considered possibly/probably related to treatment. Compared with placebo, the sapropterin group had a higher incidence of mild AEs in the respiratory and gastrointestinal (GI) disorder System Organ Classes (rhinorrhea[21%vs0%] and cough[15%vs0%]; diarrhea[9%vs0%] and vomiting[12%vs0%]) and reported more mild headaches (21%vs8%). No child reported a severe AE or withdrew due to an AE. 2 serious AEs were reported in Part 2 (sapropterin, infection; placebo, appendicitis), both considered moderate in intensity and unrelated to study drug, and both resolved during the study. Concl:Sapropterin 20mg/kg/day, has an acceptable safety profile in children with PKU on a Phe-restricted diet despite higher incidences of mild respiratory, GI and neurological AEs than for placebo.

2230/W

Safety and efficacy of sapropterin dihydrochloride (sapropterin) treatment over 22 weeks in patients with phenylketonuria (PKU). *L. Randolph[1], J. Baker[2], J. Bergoffen[3], P. Harmatz[4], A. Morris[5], E. Crombez[6], M. Seashore[7], H. Christ-Schmidt[8], A. Dorenbaum[9].* 1) Child Hosp,Los Angeles,CA; 2) Kaiser Permanente Med Ctr,Oakland,CA; 3) Kaiser Permanente Genetics Dept,San Jose,CA; 4) Child Hosp,Oakland,CA; 5) Royal Manchester Child Hosp,-Manchester,UK; 6) David Geffen Scl Med UCLA,Los Angeles,CA; 7) Yale,New Haven,CT; 8) Statistics Collaborative Inc.,Washington,DC; 9) BioMarin Pharmaceutical Inc.,Novato,CA.

Intro:Sapropterin, an oral formulation of tetrahydrobiopterin, can decrease blood phenylalanine (Phe) levels in some patients with PKU. We report 22-week efficacy and safety data from an open-label Ph 3 extension study of sapropterin in PKU patients who previously responded to sapropterin. Methods:80 patients (≥8yrs) with PKU, elevated blood Phe (≥600μmol/L) and who had relaxed or abandoned a Phe-restricted diet were enrolled. Design: 6-wk forced-dose titration phase (all patients received 3 consecutive 2-wk courses of sapropterin at 5, 20 and finally 10mg/kg/day), followed by a 4-wk dose-analysis phase (sapropterin maintained at 10mg/kg/day) and 12-wk fixed-dose phase (patients received 5, 10 or 20mg/kg/day based on their blood Phe level at Wk 2 and 6 visits). Results:Mean(SD) age was 20.4(9.6)yrs; 59%37; patients were male; 79 patients completed the study. Mean(SD) blood Phe concentration decreased from 844(398)μmol/L (14.1[6.6]mg/dL) at Wk 0 to 645(393)μmol/L (10.8[6.6]mg/dL) at Wk 10 and 652(383)μmol/L (10.9[6.4]mg/dL) at Wk 22 (end of fixed-dose phase). At Wk 22, 46%(36/79) patients had a ≥30% reduction in blood Phe compared with Wk 0. Adverse events (AEs) were reported by 68/80 patients (85%); all but one (tooth abscess considered to be unrelated to study drug) were mild/moderate in severity, no patient withdrew due to AEs, and 31 (39%) patients reported an AE considered possibly/probably related to study drug. Most commonly reported AEs during the study were headache (20% patients), pharyngolaryngeal pain (15%), nasopharyngitis (14% vomiting (13%), diarrhea (10%) and upper respiratory tract infections (10%). Concl:Sapropterin (5, 10 and 20mg/kg/day) reduces blood Phe levels in PKU patients through 22 weeks of treatment with an acceptable safety profile.

2231/W

Dose-related effect of sapropterin dihydrochloride (sapropterin) on blood phenylalanine (Phe) in patients with phenylketonuria (PKU). *M. Wasserstein[1], B. Burton[2], D. Grange[3], C. Harding[4], M. Lipson[5], N. Longo[6], L. Waber[7], C. Whitely[8], J. Wolff[9], J. Bebchuk[10], A. Dorenbaum[11], G. Vockley[12].* 1) Mount Sinai Schl Med,New York,NY; 2) Child Mem Hosp,Chicago,IL; 3) St Louis Child Hosp,St Louis,MO; 4) Oregon Health & Sci U,Portland,OR; 5) Kaiser Permanente,Sacramento,CA; 6) U Utah,Salt Lake City,UT; 7) Child Med Ctr,Dallas,TX; 8) U Minnesota Med Ctr,Minneapolis,MN; 9) U Wisconsin,Madison,WI; 10) Statistics Collaborative Inc.,Washington,DC; 11) BioMarin Pharmaceutical Inc.,Novato,CA; 12) Child Hosp,Pittsburgh,PA.

Intro:Sapropterin, an oral formulation of tetrahydrobiopterin, can decrease blood Phe levels in patients with PKU. We report the effects of 3 sapropterin dose levels on blood Phe in PKU patients who previously responded to sapropterin. Methods:80 patients(≥8yrs) with PKU and elevated blood Phe(≥600μmol/L), who had relaxed/abandoned a Phe-restricted diet entered the forced-dose titration phase of an open-label study and received 3 consecutive 2-wk courses of sapropterin, 5,20 and 10mg/kg/day (od). Mean(SD) change from Wk 0 in blood Phe level was calculated at Wks 2, 4 and 6 after 5,20 and 10mg/kg/day respectively, and analyzed using a longitudinal model (subjects served as their own controls). Results:Subjects were 98% Caucasian, 59% male, with mean(SD) age of 20.4(9.6)yrs. Mean(SD) decreases in blood Phe from Wk 0 at Wks 2, 4 and 6 after treatment with 5,20 and 10mg/kg/day were -100(295), -263(318) and -204(303)μmol/L respectively. Mean change in blood Phe was related to dose, shown by a statistically significant difference in effect when comparing doses ($p<0.01$ for all pairwise comparisons). Proportion of subjects with ≥30% decrease from Wk 0 in blood Phe was 25%, 55% and 46%, for 5,20 and 10mg/kg/day respectively. All dose levels were well tolerated (Randolph et al.) with no apparent relationship between dose and safety profile. Concl:In this forced-dose titration phase, sapropterin (5,10 and 20mg/kg/day) effectively reduced blood Phe in subjects with PKU in a dose-related manner with an acceptable safety profile. 20 mg/kg/day produced significantly greater decreases in blood Phe than lower doses.

2232/W

Occurrence of Bone Crises in Adolescents on Enzyme Replacement Therapy for Gaucher Disease. *P.S. Kishnani, J. Mackey, B.G. Crissman.* Department of Pediatrics, Division of Medical Genetics, Duke University Medical Center, Durham, NC.

Introduction: Children with Gaucher disease (GD) are at risk for developing irreversible bone complications during a critical period of growth. The standard of care for type 1 GD is enzyme replacement therapy (ERT) with imiglucerase, which has been shown to reduce the frequency of bone crises (BC) in patients with type 1 GD[1] and improve other manifestations of bone disease. However, due to the rapid growth and increased metabolic needs that occur during puberty, a static dose of ERT may leave adolescents susceptible to complications due to an inadequate dose. We report the effects of dose of ERT with imiglucerase on the occurrence of BC in otherwise stable adolescents with type 1 GD. Methods: Medical records of 5 adolescents with type 1 GD managed at our center were reviewed. Results: All cases (3M, 2F) began ERT prior to puberty (median age 16.6y, range: 13-20y) at an initial dose of 60U/kg/2wks with good response to ERT. The total ERT dose of 2 male patients remained static over time due to effectiveness noted in hematological, visceral, and growth parameters. However, following the onset of puberty both patients developed BC requiring hospitalization, by which time ERT doses had decreased to 27 and 49U/kg/2wks. Both patients' dosages were reinstated at 60U/kg/2wks and no further BC occurred. Based on this experience, ERT doses in 3 subsequent adolescents have been adjusted to maintain adequate levels (45-60U/kg/2wks) throughout onset of puberty. All cases have passed onset of puberty without occurrence of BC; doses have since been reduced. Conclusions: In this case series adolescents who experienced a reduction in ERT dose during puberty were susceptible to BC, while those whose dose per body weight was maintained based upon these observations have not suffered from BC. These data suggest that during adolescence, a period of high physiological stress, rapid growth and increased metabolic demands, GD patients require close, regular monitoring and individualization of ERT dose based upon all clinical manifestations of GD in order to prevent skeletal complications such as BC. Larger studies are needed. [1]Charrow J, et al. Clin.Genet. 71:205-211, 2007.

2233/W

Endothelial dysfunction improved by l-arginine supplementation in the patient with Noonan syndrome, moyamoya-like vascular changes, and antiphospholipid syndrome. *Y. Akita, S. Yatsuga, J. Nishioka, Y. Koga.* Dept Pediatrics & Child Health, Kurume Univ Sch Medicine, Kurume, Japan.

We report a 12-year-old Japanese female with Noonan syndrome who had antiphospholipid syndrome and moyamoya-like vascular changes. She presented choreic movements in her face and extremities. She manifested clinical features that resemble those of Turner syndrome and has a normal karyotype. Tests for anticardiolipin antibody and lupus anticoagulant were positive. Magnetic resonance angiography revealed occlusion of bilateral internal carotid arteries and moyamoya-like vascular changes in the basal ganglion region. Moyamoya-like vascular changes are characterized by collateral vessels formation in the basal cerebral vasculature caused by chronic thrombosis or stenosis. We evaluated endothelial function in the patient by flow-mediated vasodilation (FMD) and found a significant decrease vs controls. We started l-Arginine supplementation therapy in the patient. l-Arginine is known to be an important precursor of nitric oxide (NO), which improved endothelial dysfunction in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke) in our study. In this case, l-Arginine supplementation may reduce damage of focal brain ischemia by increasing microcirculation in cerebral blood flow(CBF).

2234/W

Intrathecal enzyme therapy in mucopolysaccharidosis I cats reduces storage throughout the brain. *M. Haskins[1], S. Walkley[2], J. Rhodes[1], P. O'Donnell[1], C. Bryan[1], N.M. Ellinwood[3], R. Cahayag[4], A. Cheng[4], C. Henschel[4], C.A. O'Neill[4], J. White[4], C. Vite[1].* 1) Sch Vet Med, Univ Pennsylvania, Philadelphia, PA; 2) Albert Einstein, Col Med, Bronx, NY; 3) Dept An Sci, Iowa State Univ, Ames, IA; 4) BioMarin Pharmaceutical, Inc., Navato, CA.

Mucopolysaccharidosis (MPS) I is a lysosomal storage disease caused by deficient activity of alpha-L-iduronidase (IDUA). The most common subtype has severe mental retardation associated with storage of central nervous system (CNS) glycosaminoglycans (GAGs). An orthologous cat model of MPS I has widespread storage of CNS GAGs. Approved clinical therapy for MPS I is weekly intravenous enzyme replacement with recombinant human IDUA (Aldurazyme®;ALD). To determine if intrathecal administration of ALD could alter CNS lesions, we injected 8 adult MPS I cats with ALD (0.1 mg/kg in Elliots B®, 0.5 mL/kg) and 4 adult MPS I cats with vehicle (equivalent mL/kg), each treated 3 times, 4 days apart. Prior to each injection, cerebrospinal fluid (CSF) was collected from the cysterna magna, immediately followed by slow bolus injection of ALD or vehicle. Three cats given ALD and one given vehicle developed abnormal posture with lowered forelimbs, almost resting on their elbows. One set of cats (2 ALD and 1 vehicle) was terminated 2 days post last injection, a second set 28 days post last injection, and two remaining sets will be terminated at 2 and 4 months. MPS I cats, both untreated affected and those treated with vehicle, had 1% (±1.0) of normal cat brain IDUA activity and a 72-fold (±22) above normal GAG concentration across 4 brain regions. Two days post the last ALD treatment, MPS I cats had a 4-fold (±0.6) increase in IDUA activity compared to normal and only a 4.1-fold (±1.5) increase in GAG concentration compared to normal, along with reduced immunostaining for GM2 and GM3 ganglioside and unesterified cholesterol compared to control. After 28 days, treated MPS I cats had 22% (±7.6) IDUA activity compared to normal and only 2.4-fold (±2.4) the GAG concentration compared to normal cats. Histological evaluation and quantitation of brain gangliosides is ongoing. IDUA was not detected in CSF or blood from 2-28 days post-treatment.

2235/W

Acidic amino acid tag enhances response to enzyme replacement in mucopolysaccharidosis type VII mice. *S. Tomatsu[1], A.M. Montaño[1], T. Nishioka[1], M. Gutierrez[1], C. Vogler[2], W.S. Sly[3], T. Oguma[4].* 1) Dept Pediatrics, Ped Res Inst, St Louis Univ, St Louis, MO; 2) Department of Pathology, Saint Louis University School of Medicine, St. Louis, MO, USA; 3) 4Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, MO, USA; 4) Daiichi Pharmaceutical CO., Tokyo R&D Center, Tokyo, Japan.

Enzyme-replacement therapy is an established means of treating lysosomal storage diseases (LSD). Infused therapeutic enzymes are targeted to lysosomes of affected cells by interactions with cell-surface receptors that recognize carbohydrate moieties, such as mannose and mannose 6-phosphate, on the enzymes. We have tested an alternative, acidic oligopeptide-based targeting system for delivery of enzymes to tissues, especially bone and brain, in a murine mucopolysaccharidosis type VII (MPS VII) model. This strategy is based upon tagging a short peptide consisting of acidic amino acids (AAA) to N terminus of human β-glucuronidase (GUS). The pharmacokinetics, biodistribution and the pathological effect on MPS VII mouse after 12 weekly infusions were determined for recombinant human untagged and tagged GUS. The tagged GUS was taken up by MPS VII fibroblasts in a mannose 6-phosphate receptor-dependent manner. Furthermore, the AAA-tagged enzyme had five times more prolonged blood clearance compared with the untagged enzyme. The tagged enzyme was delivered effectively to bone, bone marrow, and brain in MPS VII mice and was effective in reversing the storage pathology. The storage in osteoblasts was cleared similarly with both enzyme types. The tagged enzyme reduced storage in cortical neurons, hippocampus, and glia cells. A highly sensitive method of tandem mass spectrometry on serum, first reported here, indicated that the concentration of serum dermatan sulfate and heparan sulfate in mice treated with the tagged enzyme decreased more than that in mice treated with the untagged enzyme and were nearly normalized. These preclinical studies suggest that this AAA-based targeting system may enhance enzyme-replacement therapy for certain human LSDs.

2236/W

Intrathecal delivery of iduronate 2-sulfatase to the CNS of cynomolgous monkeys. *A.R. Garcia, J. Pan, A. Stronge, M. Tonini, M. Alessandrini, C. Neal, J. Lieb, Y. Lu, M. Wiles.* Preclinical Research, Shire Human Genetic Therapies, Cambridge, MA.

Hunter syndrome, or Mucopolysaccharidosis (MPS) II, is an inherited X-linked disorder caused by a deficiency of the lysosomal enzyme iduronate 2-sulfatase (I2S), resulting in the accumulation of undegraded glycosaminoglycans (GAG). In contrast to the attenuated form, the severe form of MPS II adversely affects CNS function. Intrathecal (IT) injection of I2S in cynomolgous monkeys was performed to examine the effect of dose on distribution in the CNS. Eleven normal monkeys received 3 monthly IT bolus injections of 3 mg (n=3), 30 mg (n=3), 100 mg (n=2), or 150 mg (n=3) I2S via an implanted lumbar port/catheter assembly; 3 monkeys served as vehicle controls. Brain and spinal cord were collected 24 hr after the final injection. I2S levels were determined by a sensitive activity assay. Distribution of human I2S within the CNS was verified by immunohistochemistry (IHC). All IT injections were well tolerated with no visible adverse reaction. At the 3 mg dose there was no increase in I2S activity in the brain compared to endogenous levels measured in controls. At 30, 100, or 150 mg doses, I2S activity was significantly elevated (4X, 8X, and 10X of endogenous levels, respectively). IHC for I2S revealed a dose dependent delivery of enzyme throughout the brain. At the 3 mg dose, I2S staining was seen in meningial and some glial cells but not neurons. At higher doses, many cerebral neurons, meningial, glial, and perivascular cells were stained more intensely for I2S. Deep penetration of enzyme in cerebral neurons from layer I (surface) near the meninges to layer VI (near the deep white matter) was seen by I2S IHC. Neuronal I2S staining was equivalent between frontal, middle, and rear sections of the brain. Similarly, brain I2S activity was evenly distributed along the brain's rostro-caudal axis. In summary, repeated IT injection of I2S results in dose dependent, widespread delivery to many cell types of the CNS and in deep penetration of the enzyme in cerebral cortex. Therefore, IT injection of I2S may represent a useful approach for the treatment of CNS manifestations of MPS II.

2237/W

Long-term Correction of PKU in the *Pah*^{enu2} mouse by mutant and chemically modified forms of Phenylalanine Ammonium Lyase. *P. Laipis[1], J. Embury[1], W. Zeile[1], C. Henschel[2], S. Bell[2], P. Fitzpatrick[2], R. Zori[3], C. O'Neill[2], L. Tsuruda[2].* 1) Dept. Biochemistry and Molecular Biology, Univ. Florida College Medicine, Gainesville, FL; 2) BioMarin Pharmaceutical Inc., Novato, CA; 3) Dept. Pediatrics, Univ. Florida College Medicine, Gainesville, FL.

Phenylketonuria (PKU) is the most frequent disorder of amino acid metabolism (~1 in 10^4 births) in populations of European origin. PKU patients accumulate phenylalanine (Phe) to abnormally high concentrations due to low or absent phenylalanine hydroxylase enzyme (PAH) activity. High Phe exposure results in symptoms ranging from mild cognitive impairment to severe mental retardation. Although deleterious effects can be minimized by a Phe-restricted diet instituted at birth, most adult PKU patients are poorly compliant leading to cognitive and behavioral deficits. Alternate therapies would be valuable, especially for possible treatment of Maternal PKU Syndrome. A recombinant phenylalanine ammonium lyase (rPAL) was modified by addition of multiple polyethylene glycol molecules (PEG) and used as an enzyme substitution therapy for PKU (rAvPAL-PEG). Treatment of BTBR *Pah*^{enu2} mice, an animal model of PKU, with rAvPAL-PEG resulted in long-term correction of Phe levels (~ 6 months). Male PKU mice administered weekly subcutaneous injections of either wild-type or a mutated form of rAvPAL-PEG rapidly reduced serum Phe levels into the physiological range. After 6-8 weekly injections, serum Phe levels stabilized at or near physiological range for the entire week. This pharmacodynamic effect was maintained even when injections ceased for 2-4 weeks. Longer pauses in treatment (~10 weeks) resulted in a loss of the stable weekly physiologic Phe levels; this effect could be re-induced by reinitiating rAvPAL-PEG administrations. Mice were healthy, showed increased body weight and exhibited no adverse effects. We previously reported histological abnormalities in brains of PKU mice and their reversal by a gene therapy vector expressing PAH; similar studies are in progress with rAvPAL-PEG treated mice. These results suggest that rAvPAL-PEG is a viable therapeutic option to reduce blood Phe in PKU patients.

2238/W

Enzyme Replacement Therapy in 18 Older, Severely Affected Patients with Pompe Disease. *D.L. Marsden[1], A. van der Ploeg[2].* 1) Genzyme, Cambridge, MA; 2) Erasmus Medical Center, Rotterdam, NL.

Background: Pompe disease, due to a deficiency in lysosomal acid-αglucosidase (GAA), results in progressive skeletal muscle weakness and respiratory insufficiency leading to substantially decreased quality of life and often early death. Clinical trials in infants showed that enzyme replacement therapy (ERT) was safe and effective. There are currently limited outcomes data in older patients. We reviewed physician reported outcomes of severely affected juvenile and adult patients treated with recombinant human GAA. **Methods**: Physician reports of outcomes for 18 juvenile and adult patients with severe Pompe disease enrolled in an extension phase of an early clinical trial (3) or a compassionate use program (15) were reviewed. Mean age at ERT initiation was 30.8 ± 14.3 years (N=18); treatment duration ranged from 8 to 75.6 months. At baseline, all patients were wheelchair bound. 17 patients required respiratory assistance by invasive (N=9), non-invasive (N=7), combined invasive/non-invasive (N=1) ventilation. They received a starting dose of 10 mg/kg weekly or 20 mg/kg bi-weekly ERT infusions. **Results**: Most patients showed signs and symptoms of advanced stage Pompe disease prior to ERT. 10 patients demonstrated improvements in respiratory function. Motor function improved for 13 of 18 and stabilized in the remaining 5; no declines in muscle strength or tone were noted. 15 of 16 patients reported positive improvements in quality of life since commencing ERT. Treatment was well-tolerated, with only one report of a mild transient infusion-associated reaction during the first infusions. **Conclusions**: ERT for juvenile and adult patients with severe Pompe disease is associated with gains in respiratory and motor function. Intervention earlier in the disease course was associated with greater improvement. Overall, patients were satisfied with their treatment and reported positive improvements in their quality of life regardless of the magnitude of clinical gains or baseline disease involvement. For rare diseases, all forms of clinical information, including physician reported outcomes, can provide meaningful outcomes data for clinical decision making.

2239/W

Management of hypersensitivity reactions in mucopolysaccharidosis type II (Hunter disease). *E. Miebach, G. Schulze Frenking, M. Beck.* Klinikum der Joh Gutenberg University Mainz, Mainz, Germany.

MPS II is an X-linked multisystemic metabolic disorder, caused by the deficiency of the lysosomal enzyme iduronate-sulfatase. Enzyme replacement therapy with Elaprase® is now available for affected patients. In our institution, 21 Hunter patients are under treatment with this enzyme preparation. In 8 of these patients an anaphylactoid reaction was observed, mainly in children with neurological involvement. The symptoms occurred between the 5th and 6th infusion. Most of the patients developed flush, urticaria and coughing during the infusion. Drug reactions can be caused by an IgE mediated anaphylaxis or by no-IgE mediated anaphylactoid response. Both reactions are clinically indistinguishable. In general, reaction time varies from 5 minutes to 4 hours after exposure. 1%-20% of patients may experience biphasic anaphylaxis with a recurrence of symptoms after a period of recovery. In our patients, the drug reactions have been treated according a regimen that was adapted from the Guidelines of "Joint task force on practice parameters for adults" (J Allergy Clin Immunol, 2005. 115(3 Suppl 2): S483): 1. Stopping the inciting antigen until recovering. 2. Monitoring of cardiopulmonary status (vital signs), classifying the severity. 3. Pharmacologic therapy (antihistamins, corticosteroids) in all cases. 4. Intravenous volume (saline solution) application in case of hypotension. 5. Oxygen, inhalation with bronchodilators if needed. 6. Further observation, at least for 8 hours in case of moderate or severe reaction. 7. Adapted infusion rate for the following week(s). Using this regimen, no severe reaction with need of resuscitation occurred in our patients receiving enzyme replacement therapy. For further evaluation determination of antibodies is needed.

2240/W

Intrathecal Enzyme Replacement Therapy in a child with mucopolysaccharidosis VI and symptomatic spinal cord compression. *M.V.R Munoz[1], D. Horovitz[2], T. Vieira[1], R. Costa[1], L. Vedolin[1], S. Fagondes[1], L. Jardim[1], J. Llerena[2], R. Giugliani[1].* 1) Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil; 2) Centro de Genética Medica, Instituto Fernandes Figueira/FIOCRUZ - Rio de Janeiro, Brazil.

In MPS VI, deficiency of Arilsulfatase B and subsequent glycosaminoglycan storage can cause spinal cord compression within the cervical meninges. Surgical treatment carries a high risk of morbidity and mortality. As intravenous enzyme replacement therapy (ERT) is not likely to cross the blood-brain barrier, we investigated the use of intrathecal recombinant human Galsulfase(IT rhASB) in an MPS VI patient with spinal cord compression. To our knowledge, IT therapy has not been attempted previously for MPS VI. Purpose: To evaluate the safety and efficacy of IT rhASB for spinal cord compression caused by cervical meningeal storage in a MPS VI child. Methods: We assessed a 7 year-old child with MPS VI, presenting important spinal cord compression, at baseline with clinical, neurological and biochemical evaluations, 12 minute walk test (12MWT) and conventional MRI and diffusion tensor imaging (DTI) studies of the CNS. He was monitored for changes in these parameters during 4 IT infusions of rhASB administered monthly via lumbar puncture (LP). Patient received 1.5 mL of rhASB dilluted on 3.0 mL of Elliotts B solution at each infusion. The patient had never received intravenous ERT. Results: No adverse events were observed. After 3 infusions he presents improvements in cord compression signs, improved sensibility tests, ability to rise from his bed and to walk longer distances when aided. There were no clinically significant changes in serum chemistries or CSF protein, glucose, or cell count. 12MWT does not present clinically significant improvement so far, despite several important neurological changes. Systemic effects have also been preliminarly observed, especially reduction in liver and spleen. Further evaluation is ongoing, and available results will be presented. Conclusions: These preliminary results suggest that IT rhASB appears to be a safe new therapy for spinal cord compression for this MPS VI patient.

2241/W

Urinary GAG behavior and clinical correlation in three patients with MPS I-Scheie during irregular enzyme replacement therapy. *M.V. Munoz-Rojas, T. Vieira, A. Federhen, L. Pinto, K. Lazzaroni, M. Burin, J. Coelho, R. Giugliani.* Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil.

Introduction: MPS I is caused by L-iduronidase deficiency and subsequent glycosaminoglycan (GAG) storage in organs and tissues and above normal urinary (u)excretion levels. Clinical trials with laronidase ERT have shown decreased urinary excretion levels in patients with MPS I after laronidase ERT introduction. The standard laronidase dosage is 0.58 mg/ kg, weekly on a regular basis. Purpose: To report the behavior of uGAG concentration during standard dosing, on ERT irregular infusion time intervals, in three MPS I - Scheie patients, with self reported clinical correlation. Methods: uGAG concentration on the first void, was assessed for all patients, prior to laronidase infusion when one or more weekly infusions had been missed, independently of the reason for missing an infusion. Any adverse event, occurred since last infusion, as well as any report on clinical improvement or worsening was captured. Results: All three patients are on ERT for over 2 years and all have several uGAGs analysis showing lower GAGs concentration when compared with pre-treatment levels. Patient 1, who has received standard dose ERT but usually in an every-other-week interval, revealed uGAG levels within normal limits on all occasions and reports no clinical worsening or complains. Patient 2 and 3, have received standard dose ERT on a weekly basis although with infusion gaps of several weeks; Both reveal uGAG levels increase although patient 2 reports only a few mild complains mainly on sleeping and breathing worsening while patient 3 refers important worsening with intercurrent infections, abdominal distension, fatigue and sleep apnea symptoms. Conclusions: ERT seems to play an effective role in decreasing the concentration of uGAGs, which in turn, may correlate at some extent with somatic clinical status. Although Laronidase standard dose and regimen is established, inter individual response may exhibit a substantial difference and it might reflect that individual adjustments may be possible for result optimization.

2242/W

Clinical Benefit Of Treatment with Alglucosidase Alfa in Infants and Children with Advanced Pompe Disease. *M. Nicolino[1], B. Byrne[2], C. Spencer[2], J. Levine[3], N. Leslie[4], E. Wraith[5], P. Kishnani[6].* 1) University Hopital Debrousse, Lyon France; 2) Shands Hospital at the University of Florida, Gainsville, FL; 3) Children's Hospital, Boston, MA; 4) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 5) Royal Manchester Hospital, Manchester, UK; 6) Duke University Medical Center, Durham NC.

Introduction. Pompe disease is caused by a deficiency of acid alpha glucosidase (GAA). Severe GAA deficiency manifests during infancy with muscle weakness/hypotonia, cardiomyopathy and severe motor delay. Most patients die from cardio-respiratory failure by 2 years of age. **Methods.** An open-label, multinational, multicenter study in patients with cardiomyopathy, minimal residual GAA (<1%) and age >6 to 36 months. Human recombinant GAA (alglucosidase alfa) was administered IV at 20 mg/kg/qow. **Results.** Twenty one patients (10M:11F) were enrolled; 7 were already ventilated. Median age at treatment was 13 months (range: 3.7-46.1); median duration of treatment was 124 weeks (range: 1-172). Treatment with alglucosidase alfa reduced the risk of death by 79% (p=0.0009) and the risk of death or invasive ventilation by 58% (p=0.02) when compared to an untreated historical cohort (n=86). LVMI improved in 17 patients (81%). Motor gains occurred in 13 patients (62%). Over 80% of patients maintained normal growth parameters. Six deaths occurred, none related to alglucosidase alfa. Eleven patients (52%) had infusion-associated reactions (IARs), all managed successfully. Anti-rhGAA antibodies developed in 95% of patients (non inhibitory by in vitro testing). A trend towards decreasing titers was observed with continued treatment for >52 weeks. **Conclusions.** In spite of the late age at initiation of treatment and the advanced stage of disease progression at baseline, results of this study indicate that alglucosidase alfa significantly prolongs survival and invasive ventilation-free survival in patients with Pompe disease when compared to a similar untreated historical cohort. A previous study (Kishnani et al. Neurol 2007) established that treatment at an early age, prior to irreversible muscle tissue damage, may optimize the clinical response.

2243/W

The Challenges of Treating Patients with Hunter Syndrome and CNS Disease with Enzyme Replacement Therapy (ERT): A Case Report. *A. Paras, R. Katz, B.K. Burton.* Division of Genetics, Children's Memorial Hosp, Chicago, IL.

A 7 yo old boy with a severe phenotype of Hunter syndrome began treatment with idursulfase(Elaprase) 9/06. He was given premedication at home with diphenhydramine 1mg/kg. Extreme agitation and anxiety were noted during the initial infusions. During several, ↑BP was noted, even prior to starting Elaprase. Diazepam 0.2mg/kg home pretreatment was added and was effective for several weeks. A few weeks later, ↑BP recurred but this time after Elaprase was initiated and it was accompanied by facial flushing and ↑HR. The infusion rate was slowed and IV diphenhydramine was given with resolution of symptoms. During the following weeks, the infusions was run at a reduced rate and no further symptoms were observed. Two months later, there was an infusion-related reaction (IRR) with mottling of the skin, ↑BP(193/115), ↑HR, and trembling. The infusion was interrupted and IV diphenhydramine given. Since then the patient has received prednisolone 1mg/kg the day before infusion and solumedrol 1mg/kg immediately before infusion along with his other premeds. On one occasion he was extremely irritable, agitated and combative during an infusion for no apparent reason. VS were normal. There was no response to slowing of the infusion, IV diphenhydramine or diazepam. The infusion had to be terminated early. Except for that one occasion, he has done well. He has had a good response to ERT with increased activity, decreased diarrhea and decreased hepatosplenomegaly on PE.

Patients with MPS II with CNS involvement present unique challenges in administering ERT, including the fact that they are often non-verbal and cannot describe symptoms of IRR's. In addition, they may have problems with agitation and combativeness, requiring medication to make the infusion experience acceptable. In this case, we had difficulty initially distinguishing between the patient's situational hypertension and the hypertension accompanying his IRR. Nonetheless, we were able to overcome these hurdles to enable him to benefit from this important therapy.

2244/W

Increased risk of osteopenic fractures in elderly patients with Gaucher disease. *N. Weinreb, L. Costantini.* Univ Research Foundation, Coral Springs, FL.

Bone disease is a common cause of morbidity in Gaucher disease (GD). 60% of adult patients (pts) have decreased bone mineral density (BMD). However, in elderly pts, statistically "normal" BMD may not indicate functionally normal bone strength. Here we report serial dual energy x-ray absorptiometry (DXA) scores and fracture (Fx) occurrence for 29 GD pts age 57-88 (9M, 20F) treated with enzyme replacement therapy (ERT) with imiglucerase, often in conjunction with bisphosphonates. **Results:** Lumbar (L) spine Z-scores were mostly normal during 9.2 ± 4.9 y on ERT: median -0.50; mean (SD) -0.05 (0.41); centiles 10-90: -1.811.21. Nonetheless, new Fx occurred in 13 (44.8%) pts in the hip (6), spine (4), or other location (11), or at multiple sites (7). Fx occurred in 5/8 (62.5%) pts with splenectomy v 8/21 (38.1%) pts without and in 7/20 (35%) N370S homozygotes v 6/9 (67%) with other genotypes. 10y Fx probability: 42% (CI 17-55). L spine T-scores were < -1 for 18/29 pts, indicating persistent osteopenia/osteoporosis in 62%, and < -2.68 in 25%. New Fx occurred in 8/15 (53.0%) pts with T-scores ≤ the median of -1.60 v 5/14 (35.7%) with T-scores > -1.60. Pts with low T scores were predominantly women (13/15F v 7/14M) and had higher use of bisphosphonates (86.7% v. 35.7%). Fx were not restricted to pts with the lowest T-scores. Findings were similar for femoral neck T-scores. **Conclusions:** Normal DXA Z-scores in elderly GD pts do not indicate low Fx risk. Low T-scores, as seen in our pts, do confer an important Fx risk. The 42% 10y Fx probability in our elderly GD pts is higher than expected for comparably aged individuals without GD. Because other risk factors such as Fx history, nutritional status, and concurrent illness were similar in our pts regardless of Fx status, cumulative pathophysiology from long-standing, untreated GD may independently increase Fx risk. Treatment with ERT may allow pts to achieve normal Z-scores. However, starting ERT late in life, even in conjunction with bisphosphonates, may not reverse established osteopenia/osteoporosis sufficiently to avoid future Fx. Therefore, detection of bone loss in young pts and early therapeutic intervention to achieve and maintain normal BMD is an important component of GD management.

2245/W

36-Months on Treatment: Open-Label Phase I/II Long-Term Study of Enzyme Replacement Therapy (ERT) with Gene-Activated Human Glucocerebrosidase (GA-GCB) in Patients with Type 1 Gaucher Disease. *A. Zimran[1], M. Phillips[1], G. Altarescu[1], K. Bhirangi[2], R. Mensah[2], D. Elstein[1].* 1) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Shire Human Genetic Therapies, Inc., Cambridge, MA.

AIM: To evaluate the long-term safety and clinical activity of Gene-Activated human glucocerebrosidase (GA-GCB), a novel ERT for patients with type 1 Gaucher disease. **BACKGROUND:** GA-GCB is produced in a human cell line using proprietary gene-activation technology, with and has an identical amino acid sequence to the naturally occurring human enzyme. **METHODS:** Ten of 11 patients who completinged the Phase I/II study enrolled in the long-term extension study. One patient discontinued treatment for reasons unrelated to GA-GCB. During the first 12 months of treatment with GA-GCB patients received 60U/kg GA-GCB every other week. At or after Month 12, all patients qualified (Therapeutic Goals; Semin Hematol. 2004) for a step-wise dose reduction from 60U/kg to 45U/kg (13 weeks) and then to 30U/kg. **RESULTS:** Preliminary data up to Month 30 on treatment with GA-GCB are included in this abstract. Month 36 data will be presented. Safety results up to Month 30: GA-GCB was generally well tolerated at all doses administered. To date, no drug-related serious adverse events have been reported. The majority of were mild to moderate. Two patients experienced an infusion-related adverse event during study extension, without interrupting treatment. Notably, no patients developed anti-GA-GCB antibodies up to Month 30. There were statistically significant increases in hemoglobin from baseline (mean increase from baseline = 2.17g/dL; mean percent change of 18.7%; from baseline) and in platelet counts (mean increase from baseline = 85.8 x 103/mm3; mean percent change of 154.0%). At Month 24, MRIs showed statistically significant decreases in the mean percent change of spleen and liver volume from baseline (by 70.9% and 26.9%, respectively). **CONCLUSION:** GA-GCB was generally well tolerated and demonstrated clinical activity in disease parameters in these adult patients with type 1 Gaucher disease. These results have led to the development of Phase III clinical trials that will enroll adults and children.

2246/W

***N*-acetylmannosamine therapy for podocytopathies and other kidney disorders due to hyposialylation.** *E. Klootwijk[1], I. Manoli[1], D. Hickey[1], C. Ciccone[1], D. Darvish[2], D. Krasnewich[1], W.A. Gahl[1], M. Huizing[1].* 1) MGB, NHGRI, NIH, Bethesda, MD; 2) HIBM Research Group, Encino, CA.

We created knock-in mice with a M712T missense mutation in *GNE*, encoding the key enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase (Gne/Mnk). Homozygous mutant ($Gne^{M712T/M712T}$) mice, deficient in sialic acid synthesis and glycoprotein sialylation, died before postnatal day 3 (P3) and exhibited severe hematuria, proteinuria and significantly abnormal glomerular structure. Ultrastructural findings included segmental splitting of the glomerular basement membrane (gbm) and effacement of the podocyte foot processes. Biochemical analysis of the mutant mice kidneys revealed decreased Gne-epimerase enzyme activity and deficient sialylation of the major podocyte sialoprotein, podocalyxin, after sialylated proteins were isolated using the sialic acid-specific lectin *Limax Flavus Agglutinin* (LFA). In contrast, overall kidney protein glycosylation, assessed by periodic acid-Schiff staining, was normal at age P2. Nor were significant differences detected in the expression of the podocyte marker podocin, the mesangial cell markers alpha smooth muscle actin and desmin, the endothelial cell marker Pecam-1, or the gmb component laminin beta-1. Oral administration of the sialic acid precursor *N*-acetylmannosamine (ManNAc) to the pregnant mothers allowed survival of 43% of the $Gne^{M712T/M712T}$ pups beyond P3. Survivors exhibited improved renal histology, increased sialylation of podocalyxin, and increased Gne/Mnk protein expression and Gne-epimerase activities. These findings establish this $Gne^{M712T/M712T}$ knock-in mouse as the first genetic model of podocyte injury due to hyposialylation. Moreover, the results support evaluation of ManNAc, a simple and well-tolerated intervention, as a treatment for renal disorders involving proteinuria and hematuria due to podocytopathy and/or segmental splitting of the gbm. Candidate disorders include Alport's syndrome, minimal change nephrosis, focal and segmental glomerulosclerosis, glomerulonephritis and other forms of idiopathic nephritic syndrome.

2247/W

Modulation of translation termination in dystrophin. *P.S. Lai, G.G. Xiong, P.P. Lim, S.K.H. Tay, P.S. Low.* Dept Pediatrics, National Univ Singapore, Singapore 119074.

Mutations in the gene encoding for muscle protein, dytrophin, cause an X-linked disorder called Duchenne Muscular Dystrophy (DMD). Some compounds like aminoglycosides have been shown to suppress premature stop codons, permitting translation to continue to normal termination of the transcript. In this study, we report the development of a cell-based expression assay which allows the investigation of the effects of aminoglycosides in modulating premature termination of translation from dystrophin stop codons. Constructs containing mutation cassettes derived from patients involving three types of stop codons, namely UGA, UAA and UAG, were cloned and transfected into mammalian HEK293 cells and readthroughs were then measured via expression of a fluorescent-tagged marker. Using this assay, four aminoglycosides were tested at varying concentrations of up to 2.5 mg/ml and at four different timepoints of treatments. It was found that using this cell-based assay system, translation readthroughs could be detected for all the three types of stop codons with G418 (2.4 mg/ml) showing highest expression initially up to 48 hours but after 72 hours of treatment, gentamicin (1.0 mg/ml), tobramycin (2.4 mg/ml) and paromomycin (2.5 mg/ml) result in readthroughs ranging between 42% to more than 70%. Thus, gentamicin, paromomycin and tobramycin, which are clinically approved for use as antibiotics, exhibited a higher efficiency in nonsense suppression compared to G418. UGA stop codon was most susceptible to the induced readthroughs compared to UAG or UAA codons. Aminoglycosides and other similar pharmacological compounds may offer an alternative strategy for therapy in DMD if the specificity, efficiency and level of readthroughs can be further improved.

2248/W

In vivo reduction of storage cells and glycosphingolipid accumulation in a mouse model of a generalized glycosphingolipid storage disease using a new inhibitor of glucosylceramide synthase. *S. Barnes[1], Y. Sun[1], D. Copeland[2], K. McEachern[2], C. Siegel[2], G. Grabowski[1].* 1) The Divistion and Program in Human Genetics, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, OH; 2) Genzyme Corporation, Framingham, MA.

Substrate reduction therapy seeks to abate aberrant lysosomal accumulation of glucosylceramide (GC) through inhibition of glucosylceramide synthase. Here, a murine model of Gaucher disease (4L/PS-NA) was used as a model system. 4L/PS-NA has an acid β-glucosidase (GCase) V394L/V394L (4L) point mutation combined with a hypomorphic (6% wild-type) expression of the mouse prosaposin transgene (PS-NA). The combined deficiencies of GCase and prosaposin resulted in accumulation of several glycosphingolipids, including GC, lactosylceramide and globotriaosylceramide. A ceramide analog, C9, was a potent inhibitor (IC50 ~20 nM) of glucosylceramide synthase. In this study, 3 wk old 4L/PS-NA mice received C9 orally for 12 wks. These mice showed reduced number of storage cells and CD68 positive staining in the spleen, liver and lung compared to age-matched untreated control animals. No effect was seen in the brain and spinal cord. Lipid analysis revealed decreased levels of lactosylceramide and globotriaosylceramide with more moderate effects on accumulated glucosylceramide in visceral tissues. These results indicated that the synthesis pathway is inhibited, but the residual GCase mutant enzyme activity in these tissues was not sufficient to clear accumulated GC. This study demonstrates that substrate reduction therapy with ceramide analogues inhibition of glucosylceramide synthase represents an alternative approach for treating the visceral pathology in severe variants of glycosphingolipid storage diseases.

2249/W

Post-marketing surveillance of miglustat in patients with Type 1 Gaucher disease (GD1). *B. Bembi[1], D. Hughes[2], B. Schwierin[3], C.E.M. Hollak[4].* 1) Unità Operativo Dipartimentale, Istituto per l'Infanzia, "Burlo Garofolo", Trieste, Italy; 2) Royal Free and University College Medical School, London, UK; 3) Actelion Pharmaceuticals Ltd, Allschwil, Switzerland; 4) Academic Medical Center, Amsterdam, The Netherlands.

IS^3 is a non-interventional post-marketing surveillance programme aimed at enhancing awareness of safety precautions and stimulating appropriate monitoring during miglustat use in patients with GD1.

From March 2003 to 9 March 2007, information was available on the first 98 GD1 patients (60% female) prescribed miglustat across 11 European countries (45 centres).

Overall exposure to miglustat represented a cumulative period of 147 patient-years, with a median exposure (range) of 17.9 (1.3-107.7) months. Mean patient age (SD) was 44.3 (15.9) years. At baseline, 65 patients (66%) had previously been treated with enzyme replacement therapy, with a median duration (range) of 64.0 (1.0-176.0) months. Baseline neurological assessments were available in 89 patients (91%), amongst whom 23% displayed one or more neurological manifestations (17% tremor, 9% neuropathy, 12% memory problems, 13% cognitive abnormalities). Fifty-three percent of patients had bone manifestations at baseline, the most frequent being osteopenia (41%), bone pain (28%) and avascular necrosis (16%). During follow up, no safety signals were reported in 48 patients (49%). Twenty-three patients (23.5%) discontinued, most frequently due to gastrointestinal disturbances (14 patients); most of these cases occurred during the first 6 months of treatment. New tremor was reported in 13 patients (13%), and memory problems occurred in 7 patients (7%). Bone pain was observed in 5 patients, two of whom exhibited skeletal symptoms at baseline.

In conclusion, long-term miglustat therapy was well tolerated in patients with GD1. Most gastrointestinal side effects occurred during the first weeks of treatment. No new safety concerns were identified.

2250/W

The Pharmacological Chaperone AT1001 Reduces Globotriaosylceramide Substrate Levels in Fabry Transgenic Mice and Increases α-Galactosidase A levels *in vitro*, *in vivo* and in Healthy Volunteers. *R. Khanna, E.R. Benjamin, R. Soska, H.H. Chang, A. Schilling, Y. Lun, S.A. Sitaraman, D.J. Palling, D.J. Lockhart, andK.J. Valenzano.* Amicus Therapeutics, 6 Cedar Brook Drive, Cranbury, NJ 08512.

Fabry disease is an X-linked lysosomal storage disorder caused by inherited mutations in α-galactosidase A (GLA). Mutations in GLA lead to reduced catabolism and consequent lysosomal accumulation of the natural substrate, globotriaosylceramide (GL-3), which contributes to disease pathology. It has been shown that the pharmacological chaperone, AT1001 (migalastat hydrochloride), can increase mutant GLA (R301Q) levels both *in vitro* and *in vivo*. In the current study, the effect of AT1001 on GL-3 levels was tested using GLA deficient mice that express the mutant R301Q human transgene (R301Q GLA Tg/KO). Daily oral administration of increasing doses of AT1001 (10, 30, 100 and 300 mg/kg) to R301Q GLA Tg/KO mice for 2 or 4 weeks resulted in a dose-dependent and significant increase in GLA levels in disease-relevant tissues (skin, heart and kidney). Importantly, tissue GL-3 levels were significantly reduced in all three organs after 4 weeks of treatment. The ability of AT1001 to affect other mutated forms of GLA was also evaluated in lymphoid cell lines derived from male Fabry patients (missense mutations leading to both classic and variant phenotypes). A majority of the tested cell lines showed a response (1.5- to 20-fold increase in GLA levels; EC50 values from 600 nM to greater than 1 mM). In a Phase 1 clinical study in healthy male volunteers, AT1001 was generally well-tolerated at all doses with no serious adverse events. Oral administration of AT1001 at 50 or 150 mg twice daily for 7 days resulted in a dose-related increase in GLA levels in white blood cells that persisted for 7 days after drug withdrawal. Collectively, these data indicate that AT1001 merits further evaluation as a treatment for patients with Fabry disease.

2251/W

Prevalence of polyneuropathy in adult type 1 Gaucher disease (GD1): a multinational prospective observational study. *L. Marodi[1], M. Biegstraaten[2], I.N. Van Schaik[2], E. Mengel[3], M. Petakov[4], C. Niederau[5], P. Giraldo[6], D. Hughes[7], M. Mrsic[8], A. Mehta[7], C.E.M. Hollak[2], the 018 Study Group.* 1) Department of Pediatrics, University of Debrecen, Debrecen, Hungary; 2) Academic Medical Centre, Amsterdam, The Netherlands; 3) Universitaets Kinderklinik, Mainz, Germany; 4) Institute of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia; 5) Klinik fur Innere Medizin, Universitat Essen, Duesseldorf, Germany; 6) Miguel Servet University Hospital, Zaragoza, Spain; 7) Royal Free Hospital, London, UK; 8) University Hospital Centre, Department of Hematology, Zagreb, Croatia.

GD1 has traditionally been categorized as non-neuronopathic. However, some cases of polyneuropathy (PNP) have been reported and also in patients exposed to miglustat. Since there is no definite explanation, a multinational (7 countries, 8 centres) prospective, observational study has been set up to establish the prevalence and incidence of PNP in GD1. This study was set up under the auspices of the European Working Group on Gaucher Disease. Diagnosis of PNP was based on compatible neurological signs and/or symptoms and abnormal electrodiagnostic studies. A standardised protocol was used in all centres. An independent central assessor adjudicated PNP diagnosis. Secondary endpoints included the 2-year incidence of PNP and other parameters (neuropsychological status, organ involvement, skeletal manifestations, laboratory measurements and quality of life). 103 GD1 patients were enrolled; either untreated (n=17) or treated with enzyme replacement therapy (n=86). Mean age±SD was 42.6±14.5 years (53% female). Eleven patients were diagnosed with sensory or sensory/motor axonal PNP (10.7%, 95%CI, 5.5-18.3%). This prevalence is significantly higher than in the general population (0.12 to 3.6%). Patients with PNP were older than those without PNP (mean±SD, 61.1±10.3 vs. 40.4±13.4, respectively). Further investigations will focus on the relation with disease severity, and other factors associated with PNP. These findings prompt awareness of this new co-morbidity in GD1, and suggest that careful questioning and, in case of suspected PNP, further examination by an experienced neurologist is needed.

2252/W

Miglustat improves function and enhances β-galactosidase activity in a patient with juvenile GM1 Gangliosidosis: A pharmacologic chaperone effect? *C.P. Morgan[1,2], D.R. Adams[3], C.J. Tifft[1,2].* 1) Children's National Medical Center, Washington, DC; 2) Genetics of Development and Diseases Branch, NIDDK, NIH, Bethesda, MD; 3) Human Genetics Branch, NHGRI, NIH, Bethesda, MD.

GM1 gangliosidosis, caused by a deficiency of lysosomal β-galactosidase, is a neurodegenerative disorder with a broad clinical spectrum reflecting the degree of residual enzyme activity. Miglustat, an imino sugar, competitively inhibits glucosylceramide synthase, the first step in glycosphingolipid synthesis, and can reduce the synthesis of GM1 ganglioside. Imino sugars have also been shown to act as molecular chaperones with a number of acid hydrolases, including β-galactosidase (Tominaga *et al*, 2001; Yam G. *et al*, 2006). Here we report a patient with juvenile GM1 gangliosidosis who showed rapid clinical improvement following treatment with miglustat. The patient is an 18-year-old male with precocious development until age 5 when he developed deterioration of expressive and receptive language and gait disturbance. After a 7 year diagnostic odyssey, juvenile GM1 gangliosidosis was confirmed. He continued to decline, and by age 16 he had lost all speech skills and was non-ambulatory. After 3 years of miglustat therapy he has regained the ability to speak in sentences and short paragraphs although remains dysarthric, and has gained some tentative ambulatory skills limited by hip dysplasia. The patient's fibroblasts were incubated with miglustat for up to 4 days at concentrations of 5, 25, and 50μM which is within the range of plasma concentrations in treated patients. We found that under these conditions β-galactosidase activity was increased 35-45% in treated versus untreated cell lysates ($p<0.01$). We hypothesize that the increase in enzyme activity may result from a pharmacologic chaperone effect of miglustat. Studies are underway in additional juvenile GM1 patients to further characterize the effects of miglustat.

2253/W

Miglustat in Niemann-Pick type C disease (NP-C): results of 24 months' treatment. *M. Patterson[1], D. Vecchio[1], H. Prady[2], L. Abel[3], J.E. Wraith[2].* 1) Dept Neurology, Columbia Univ, New York, NY, USA; 2) Royal Manchester Children's Hospital, Biochemical Genetic Unit, Manchester, UK; 3) Department of Optometry and Vision Sciences, University of Melbourne, Australia.

NP-C is an inherited neurodegenerative disorder characterized by an intracellular lipid-trafficking defect and pathological storage of glycosphingolipids. Miglustat, a small iminosugar molecule, prevents accumulation of GSLs by reversibly inhibiting glucosylceramide synthase. Due to its ability to cross the blood-brain barrier, miglustat has the potential to treat NP-C. Adults and juveniles (n=29, age ≥12 years) were randomized to either miglustat 200 mg t.i.d. (n=20) or standard of care (n=9) for 12 months. In addition, 12 children (age 4-12 years) received miglustat at a dose adjusted for body surface area. All patients were then given miglustat for a second year. Horizontal saccadic eye movement (HSEM) velocity was the primary endpoint. Juveniles/adults receiving miglustat for 12 and 24 months were compared by ANCOVA using baseline and center as covariates. 19 adults/juveniles (mean±SD age 24.6±9.1 years) and 10 children (7.2±2.5 years) completed the 24-month study. Although an increase (worsening) from baseline in HSEM-α was seen at last value in adults/juveniles treated for 12 or 24 months, the increase was smaller in the 24-month group (treatment difference, -0.594; 95%CI -2.078, 0.889). The pattern in children was comparable with the adult/juvenile 24-month group. A higher proportion of patients had stable or improved swallowing capacity in the 24-month than in the 12-month group, and patients in the 24-month group showed a more favorable change on the Standard Ambulatory Index. The most common AEs were diarrhea (64%), weight loss (68%), tremor or aggravated intention tremor (57%), and abdominal pain (54%). The safety profile was similar in both treatment groups. In conclusion, miglustat may slow disease progression in NP-C. A collaborative project on NP-C natural history will help to further understand interventional trial outcomes. The safety/tolerability of miglustat 200 mg t.i.d. in NP-C was consistent with that seen in type 1 Gaucher disease, where half this dose was administered.

2254/W

The pharmacological chaperone AT2101 increases β-glucocerebrosidase levels in macrophages and lymphoblasts derived from Gaucher patients. *C.W. Pine[1], B.E. Ranes[1], F. Insinga[1], K. Ludwig[1], G.A. Grabowski[2], N.J. Weinreb[3], G.M. Pastores[4], D. Gruskin[5], P. Kaplan[6], H. Do[1], D.J. Lockhart[1], B.A. Wustman[1].* 1) Amicus Therapeutics, Cranbury, NJ; 2) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) Univ. Research Foundation for Lysosomal Storage Diseases Inc, Northwest Oncology Hematology Associates, Coral Springs, FL; 4) Departments of Neurology and Pediatrics, New York Univ. School of Med., New York, NY; 5) Departments of Human Genetics and Pediatrics, Emory Univ. School of Med., Atlanta, GA; 6) Section of Metabolic Diseases, Children's Hospital of Philadelphia, Univ. of Penn School of Med., Philadelphia, PA.

Gaucher disease (GD) is caused by a deficiency of β-glucocerebrosidase (GCase). Deficient GCase activity leads to symptoms such as anemia, thrombocytopenia, hepatosplenomegaly, bone necrosis, infarcts, osteoporosis and in some cases, neuropathic disease. The pharmacological chaperone AT2101 selectively binds and stabilizes N370S-GCase in the ER and increases its trafficking to the lysosome. To evaluate the effects of AT2101 on different GCase variants, we conducted an ex vivo response study using macrophages and EBV-transformed lymphoblasts. Plasma was also screened for potential biomarkers associated with inflammation, bone metabolism, multiple myeloma and neurodegeneration. The study was conducted on samples from 53 patients enrolled at 5 sites in the United States. Results: The study included 26 males and 26 females with type I GD, and one male with type III GD. Patients ranged in age from 7 to 83 years; 50 of 53 patients were receiving enzyme replacement therapy and blood was drawn prior to enzyme infusion. Analysis of 40 markers showed elevated chitotriosidase activity, TRACP 5b, PARC, IL-8, IL-17, VEGF, MIP-1α and α-synuclein and reduced bone-specific alkaline phosphatase levels in some patients. Incubation with AT2101 increased GCase levels in macrophages or lymphoblasts derived from 52 of 53 patients (mean=2.6-fold, range:1.4- to 8.6-fold) with mutant alleles including N370S, L444P, 84GG, R163X, Y212H, Y135X, V394L, R120Q, A190E, L324P, IVS2(+1), del 136T, F216Y, L174F, A90T, R463C, G202R, K79N and complex B exon 9/10.

2255/W

Experience with laronidase in a bone marrow-transplanted patient with severe pulmonary disease. *V. Valayannopoulos[1], J. De Blic[2], N. Mahlaoui[3], B. Stos[4], A. Chabli[5], F. Jaubert[6], P. de Lonlay[1], A. Fischer[3].* 1) Metabolic Unit, Necker-Enfants Malades Hosp, Paris, France; 2) Pediatric Pneumology; 3) Immuno-Hematology; 4) Pediatric Cardiology; 5) Biochemistry Laboratory; 6) Pathology Laboratory.

Background: Mucopolysaccharidosis type I or Hurler's disease (MPS I-H) is a severe multi-organ lysosomal disease, which untreated is rapidly fatal. Long-term survival has occurred in children with MPS I-H after successful bone marrow transplantation (BMT). Laronidase, a recombinant human alpha-L-iduronidase, safely and effectively alleviates many systemic manifestations of this disease.Case Report: We describe a 14 years old MPS I-Hurler patient that underwent BMT twice, with his heterozygous twin. His clinical course was initially similar to other MPS I-BMT patients, but he developed 5 years ago, a progressive respiratory failure with life-threatening pulmonary hypertension. His pulmonary disease was multifactorial: bone disease responsible for thoracic and spinal deformities; infiltration of upper airways and sleep hypoventilation; interstitial infiltration of lungs with storage material in alveoli. Clinically he was polypneic, fatigable and wheelchair-bound. Methods and Results: We treated this patient for 24 months so far, with continuous oxygen therapy for pulmonary hypertension, nocturnal non-invasive ventilation and enzyme replacement therapy. The patient's clinical condition improved dramatically. He can now stand and walk alone and climb stairs without significant fatigue or dyspnea. Pulmonary hypertension improved and oxygen therapy during daytime was discontinued. His thoracic CT-scan improved and an upper airways and tracheal endoscopy showed decreased obstruction. Urinary glycosaminoglycans decreased over 50 percent.-Conclusion: Enzyme replacement therapy may be an interesting option for treating MPS I BMT patients who develop severe respiratory complications. Further studies should be done to assess the contribution of ERT in other visceral symptoms occurring in these patients.

2256/W

A new therapeutic approach to the treatment of Gaucher Disease: mechanism of action of the pharmacological chaperone AT2101 and Phase I trial results. *B.A. Wustman[1], R. Khanna[1], D.J. Palling[1], A.C. Powe[1], J.J. Flanagan[1], C.W. Pine[1], R. Soska[1], L. Pellegrino[1], K.J. Valenzano[1], A. Marian[2], R. Demnati[3], D.J. Lockhart[1], H.V. Do[1].* 1) Biology, Amicus Therapeutics, Cranbury, NJ; 2) MDS Pharma Services, Lincoln, NE; 3) MDS Pharma Services, Montreal, Quebec.

Gaucher Disease is a lysosomal storage disorder caused by genetic mutations that lead to reduced β-glucocerebrosidase (GCase) activity. While many GCase variants are catalytically competent, the mutations often destabilize the enzyme and/or impair exit from the endoplasmic reticulum (ER). We have developed a new therapeutic approach for the treatment of genetic diseases using small molecules called pharmacological chaperones. In this study, we used co-crystallization, thermal stability, radiolabeled pulse-chase and subcellular fractionation methods to study the effects of the pharmacological chaperone AT2101 on GCase. We found that AT2101 selectively binds and stabilizes GCase at neutral pH, thereby preventing premature degradation via ERAD and/or facilitating passage through the ER quality control system and restoring proper protein processing and trafficking to the lysosomes. Mutant GCase (N370S) is stable in lysosomes for at least 3 days after AT2101 is removed from cells and has a higher specific activity than N370S GCase from untreated cells. AT2101 also increases enzyme levels for other GCase variants including L444P, R463C, L174F, F216Y, F331S, G202R, V394L, D409H, and D409V in patient-derived cell lines or using heterologous expression systems. We then evaluated the effects of AT2101 on GCase levels in mouse models and human subjects. Treatment of L444P knock-in mice with AT2101 resulted in a dose-dependent increase in L444P GCase levels in liver, spleen, lung and brain. In single and repeat-dose Phase 1 clinical trials with a total of 72 healthy volunteers, AT2101 was well tolerated with no serious adverse events. In the repeat-dose study, a dose-dependent increase in GCase levels (up to ~3.5 fold) was observed during the 7 day treatment period, and enzyme levels remained elevated for more than a week after removal of the drug. AT2101 is currently being evaluated in Gaucher patients in Phase 2 clinical trials.

2257/W

Therapeutic potential of a generalized stress response. *R. Deering[1], S. Purvis[1], G. Dong[1], J. Keefer[1], K.D. Smith[1,2].* 1) Dept of Human Genetics, Johns Hopkins Univ, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

In response to various stresses, cells mount several evolutionarily conserved pathways, comprising the generalized stress response (GSR), that enhance cell survival and homeostasis. Histone deacetylase inhibitors (HDACi) have been used to alleviate the symptoms of a variety of diverse Mendelian and complex disorders. It seems unlikely that non-specific HDACi activity would elicit specific responses related to each specific genetic abnormality. Also, similar responses are induced by several non-HDACi. We hypothesize that both HDACi and non-HDACi activate a GSR to enhance cell survival and homeostasis. We investigated HDACi 4-phenylbutyrate and trichostatin A and non-HDACi hydroxyurea and sulforaphane (confirmed by HDAC activity assays) in sickle cell disease (SCD) and X-linked adrenoleukodystrophy (XALD). In SCD, symptoms are due to mutations in β-globin and are lessened by increased levels of HbF. XALD is associated with increased levels of very long chain fatty acids (VLCFA). Beneficial downstream drug responses (HbF levels increase in SCD and VLCFA decrease in XALD) were observed with both HDACi and non-HDACi and were dependent on drug-induced mitochondrial biogenesis, a common stress response. The evolutionarily conserved GSR involves transcriptional upregulation of genes that regulate reactive oxygen species, sense and repair DNA damage, are molecular chaperones, degrade proteins, and regulate fatty acid, lipid, and energy metabolism. The drugs tested increased transcription levels of genes involved in the major stress response systems: unfolded protein response, heat shock response, MAPK cascades, mitochondrial biogenesis, and antioxidant response. Inhibiting stress activated protein kinases blocked drug-induced mitochondrial biogenesis. These data support the hypothesis that triggering a GSR is a universal response to these drugs. Thus, enhanced cell survival and improved cellular homeostasis, rather than specific changes in gene expression responsive to each distinct disorder, may provide protection in a variety of genetic disorders. These drugs may have therapeutic potential for a spectrum of disorders with mild cellular phenotypes.

2258/W

Treatment of Pompe Disease with the Pharmacological Chaperone AT2220: Mechanistic Studies and Phase 1 Clinical Results. *J.J. Flanagan, X. Wu, A.C. Powe, R. Khanna, R. Soska, W. Liang, E.R. Benjamin, D. Palling, S. Sitaraman, B.A. Wustman, K.J. Valenzano, D.J. Lockhart, H.V. Do.* Amicus Therapeutics, Cranbury, NJ.

Pompe disease is a genetic disorder caused by mutations in acid α-glucosidase (GAA). GAA deficiency leads to lysosomal glycogen accumulation and results in progressive skeletal muscle weakness, reduced cardiac function, respiratory insufficiency, and CNS impairment. GAA is synthesized as a precursor glycoprotein and requires protein and carbohydrate processing to yield the mature lysosomal enzyme. We are developing a new therapeutic approach for the treatment of genetic disorders by using small molecules called pharmacological chaperones to selectively bind and stabilize mutant proteins. In this study, we show that the pharmacological chaperone AT2220 (1-deoxynojirimycin HCl) significantly increases GAA specific activity in patient-derived fibroblasts. Using transient expression in COS7 cells, multiple GAA missense mutations showed increased activity in response to AT2220. Moreover, all responsive GAA mutations showed improved processing which is indicative of increased protein trafficking. Administration of AT2220 to wild-type mice increased GAA activity in multiple tissues affected in Pompe disease. In cardiac and skeletal muscles from these animals, GAA processing also improved after AT2220 treatment. The above results indicate that AT2220 increases protein trafficking, processing and cellular activity for wild-type GAA and a number of GAA missense mutations. AT2220 was evaluated in two Phase 1 clinical trials. In a single ascending dose study, 32 individuals received oral doses of 50, 150, 300, or 600 mg AT2220 or placebo. AT2220 was shown to be highly orally bioavailable, with linear pharmacokinetics and a plasma half-life of 7 to 8 hours. In a multiple ascending dose study, 24 individuals received oral doses of 50, 150, or 450 mg/day AT2220 or placebo for 7 days. In both studies, there were no drug-related serious adverse events and the drug was generally well tolerated. Collectively, these data indicate that AT2220 merits further evaluation as a treatment for Pompe disease.

2259/W

Therapeutic dosage of valproic acid may not increase survival motor neuron protein in fibroblasts from patients with spinal muscular atrophy type I and II. *. Gunadi[1], T.H. Sasongko[1], S. Yusoff[1], A.H. Sadewa[3], R. Sutomo[4], M.J. Lee[1], M. Matsuo[2], H. Nishio[1].* 1) Department of Genetic Epidemiology, Kobe University Graduate School of Medicine, Kobe, Japan; 2) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; 3) Department of Biochemistry, Gadjah Mada University School of Medicine, Yogyakarta, Indonesia; 4) Department of Pediatrics, Gadjah Mada University School of Medicine, Yogyakarta, Indonesia.

Homozygous absence of the survival motor neuron 1 gene (*SMN1*) is the most frequent cause of spinal muscular atrophy (SMA). Clinical severity may be modified by the presence of the *SMN2* gene, almost identical to *SMN1*. Administration of valproic acid (VPA), a well-known drug for epilepsy, is a potential treatment of SMA, since it has been reported to increase the full-length (FL) *SMN2* expression. VPA may have splicing modulation activity, as well as histone deacetylase (HDAC) inhibitor activity. However, it is controversial whether it can increase the *SMN2* expression in SMA patients. In this study, we analyzed *SMN2* transcripts and SMN protein levels in SMA fibroblasts cultured in the medium with therapeutic concentration of VPA. Fibroblasts from two SMA patients lacking *SMN1* were used. One patient was an SMA type I (2 *SMN2* copies) and the other one was an SMA type II patient (3 *SMN2* copies). Semi-quantitative RT-PCR analysis showed neither significant difference in *SMN2* splicing patterns nor in total *SMN2* transcript amounts between mock and VPA-treated fibroblasts from both patients. Western blotting analysis also demonstrated that SMN protein levels were not changed at various concentrations of VPA. In conclusion, VPA within therapeutic dosage may not increase *SMN2* transcripts and SMN protein levels in fibroblasts from patients with SMA type I and II. Our study did not test the VPA effects on fibroblasts from SMA type III and IV patients, although there have been reported that VPA ameliorate their symptoms. Further studies are necessary to select SMA patients who will respond to therapeutic dosages of VPA.

2260/W

Mechanisms Underlying Potentiator Activation of CFTR. *L.C. Pyle, A. Ehrhardt, L. Fan, J. Fortenberry, W. Wang, K. Nowotarski, K. Varga, M. Sthanam, J.P. Clancy, E.J. Sorscher, S.M. Rowe.* Cystic Fibrosis Research Center, UAB, Birmingham, AL.

Small molecule modulators of CFTR overcome gating defects of surface localized mutant channel and are being developed as therapies for cystic fibrosis. Although these agents have entered clinical testing, their mechanism(s) of action are poorly understood. CFTR activation requires PKA-regulated phosphorylation of the regulatory domain (R-D), followed by ATP dependent gating mediated by the two nucleotide binding domains. We have established a gel-shift method by which phosphorylation of isolated R-D (residues 635-836) can be monitored. The potentiator P1 does not induce phosphorylation of the R-D (4% of forskolin response, n=7, P=NS). Unexpectedly, two potentiators, P8 and P10, confer robust phosphorylation of the R-D (P8: 32% of forskolin response, n=8, P<0.005, P10: 37% of forskolin response, n=8, P<0.005), which is inhibited by the PKA inhibitor H89. Neither P8 nor P10 increased cellular cAMP. The results suggest compartmental inhibition of CFTR-associated phosphotases (eg. PP2A) or phosphodiestereases (eg. PDE4) as an underlying mechanism. We next evaluated CFTR potentiators in two ΔF508 CFTR polarized epithelia models, CFBE41o- and Fisher rat thyroid cells stabily transduced with ΔF508 CFTR. Cells were studied after low temperature (27ºC x 48 hrs) or chemical correction of ΔF508 CFTR misprocessing. Total short-circuit current (I_{sc}) was determined following serial addition of potentiator, forskolin, and genistein. P1 both directly activated CFTR and potentiated forskolin mediated I_{sc} (potentiation being the predominant effect (9.8 vs 1.5 μA/cm^2, n=12, P<0.05), a unique observation in CFBE41o- cells), while P8 and P10 conferred activation of CFTR without potentiation. Our findings suggest agents that do not phosphorylate the R-D may be better suited to rescue endogenous cAMP mediated CFTR activation. These studies provide a means to biochemically and functionally categorize novel CFTR modulators. Understanding the mechanism underlying activation or potentiation of CFTR may allow a more rational approach to the rescue of ion transport caused by particular CFTR mutations.

2261/W

The Evaluation of Three Novel Small Molecule Classes Identified Through Quantitative High-Throughput Screening (qHTS) as Potential Chaperones for Gaucher Disease. *D.J. Urban[1], W. Zheng[2], O. Goker-Alpan[1], E. Goldin[1], J. Inglese[2], C. Austin[2], E. Sidransky[1].* 1) Medical Genetics Branch, National Human Genome Research Institute, NIH Bld 35 Rm1A100, 35 Convent Drive, Bethesda, MD 20892-3708 USA; 2) NIH Chemical Genomics Center, National Human Genome Research Institute, NIH 9800 Medical Center Drive, MSC 3370 Bethesda, MD 20892-3370 USA.

Gaucher disease is an autosomal recessive lysosomal storage disorder caused by mutations in the glucocerebrosidase gene. Most identified mutations are missense mutations, where the reduced enzyme activity may be due to misfolding. It has been proposed that chaperone therapy with small molecule inhibitors could be used to correct the defect. Quantitative high throughput screening (qHTS) was successfully used to rapidly identify three structural series of potent, selective, non-sugar glucocerebrosidase inhibitors. These included sulfonamides, quinolines and triazines. In order to characterize the mechanism of action for these compounds and to determine their selectivity profiles, we performed enzyme kinetic assays using four different lysosomal hydrolases. We found that the glucocerebrosidase inhibitors identified in our screening were highly selective for glucocerebrosidase and not the other related hydrolyses. Structure activity relationship data was used to select compounds with high activity, which were evaluated further using both enzyme and cell-based assays. Using fibroblast cell lines from patients homozygous for N370S, we found that compounds from two identified structural series increased the activity of mutant glucocerebrosidase by 40-90%. In addition, confocal microscopy using antibodies against glucocerebrosidase demonstrated enhanced lysosomal co-localization in the treated N370S lines, indicating chaperone activity. These novel small molecules have potential as leads for chaperone therapy for Gaucher disease, and this paradigm promises to accelerate the development of leads for other rare genetic disorders.

2262/W

THALIDOMIDE THERAPY IN A PATIENT WITH THALASSEMIA MAJOR. *L. Aguilar.Lopez[1], J.L. Delgado-Lamas[1], B. Rubio[1], F.J. Perea[2,3], B. Ibarra[2,3].* 1) Servicio de Hematologia, Hospital de Especialidades UMAE, CMNO, IMSS, Guadalajara, Mexico; 2) Division de Genetica, Centro de Investigacion Biomedica de Occidente, CMNO, IMSS Guadalajara Mexico; 3) Doctorado en Genética Humana, Universidad de Guadalajara, Guadalajara, Mexico.

The β thalassemia is characterized by a deficiency or absence of β globin chains. The homozygote state (Thalassemia Major), has high blood transfusion requirements since early age. We describe a 21 years old women with β Thalassemia Major diagnosed at 5 months of age. In 1997, the biochemical studies showed high HbF levels (62.3%) and HbA2 of 3.61%, the genotype was identified as -28 A-C/Cd 39 T-C. She had chronic blood transfusions, every 2 or 3 months, with an iron overload. She was splenectomized at the age of five years. She had received chelation therapy (Desferoxamina) with different time intervals. Her hemoglobin levels without transfusion were as low as 2.9 g/dL. The patient was received at the hematology service of the Hospital de Especialidades in Dcember 2001 with 4.0 g/dL, when she initiated the thalidomide therapy (100 mg per day), the first hemoglobin increase was observed after three moths to 7 g/dL, since then she has the thalidomide therapy uninterruptedly and never was transfused again with hemoglobin levels between 7.6 to 10.6 g/dL and almost 100% of HbF. She is at present in good health conditions with hemoglobin values of 10.2 g/dL. To our knowledge this is the first report of a Thalassemia Major patient treated with thalidomide with such a great results at the hemoglobin levels and general good health conditions. The molecular and physiological effects of the thalidomide must be deeply investigated, since it is know the angiogenic effect in cancer, the benefic effect in thalassemic patients could be mediated by its gene modulator effect however the true mechanism require to be investigated.

2263/W

Identification of compounds with ability to induce read-through of nonsense mutations by high throughput screening. *L. Du[1], R. Damoiseaux[2], J. Goldstine[3], J.M. Pollard[1], H. Feng[1], C.H. Lai[1], M. Ambrose[1], R.A. Gatti[1,3].* 1) Department of Pathology and Laboratory Medicine, The David Geffen School of Medicine at UCLA,CA,90095; 2) Molecular Shared Screening Resources, Department of Pharmacology, UCLA, CA,90095; 3) Department of Human Genetics, The David Geffen School of Medicine at UCLA, CA,90095.

A large number of common genetic diseases result from nonsense mutations which introduce premature termination codons (PTCs) into coding sequences and cause the formation of either no protein or truncated non-functional protein. It has been known that certain compounds can influence the fidelity of stop-codon recognition and induce read-through of PTCs mutations, which allows translation of a full-length normal protein. In many cases, the read-through-induced protein might be at least partially functional, even if it contains a wrongly-incorporated amino acid. Considering that large numbers of genetic disorders are caused by PTC mutations, the read-through of PTCs might be exploited as a potential treatment strategy. In this study, we successfully developed a high throughput PTT-ELISA screening assay (HTS) for identifying novel PTC read-through compounds using Ataxia-telangiectasia (A-T) as a genetic disease model. This PTT-ELISA assay is based on a coupled transcription/translation reaction (PTT) that uses plasmid templates containing prototypic Ataxia-telangiectasia mutated (ATM) mutations, patterned after disease-causing mutations in A-T patients. The PTT-ELISA assay has been validated for a fully-automated 384-well robotic platform and is adaptable for large-scale screening of chemical libraries. As proof of principle, we screened ~37500 compounds and identified several low-molecular-weight compounds with potential PTC read-through activity in vitro; these compounds were subsequently confirmed by manual testing. Ex vivo ELISA experiment showed that one compound could induce ATM protein in ATM deficient cells containing PTC mutation.

2264/W

Preparing for treatment of familial dysautonomia with kinetin: improved mRNA splicing in FD carriers. *M. Leyne[1], G. Gold-von Simson[3], J. Mull[1], L.M. Rolnitzky[4], D. Berlin[3], Y.T. Chen[1,2], L. Liu[1], R.S. Shetty[1,2], F.B. Axelrod[3], S.A. Slaugenhaupt[1,2].* 1) Center for Human Genetics Research, Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Department of Pediatrics, New York University School of Medicine, New York NY; 4) Division of Biostatistics, New York University School of Medicine, New York NY.

Familial dysautonomia (FD) is caused by mutations in *IKBKAP*. The most common mutation disrupts mRNA splicing and reduces IKAP protein expression below the threshold required for proper development of the sensory and autonomic nervous systems. As part of an NINDS sponsored drug screen, kinetin was found to promote normal splicing and increase expression of normal mRNA and protein in FD cells, suggesting it as a potential therapeutic agent. Prior to initiating clinical trials of kinetin in FD patients, we evaluated *IKBKAP* expression in peripheral blood samples obtained from 45 FD patients, 26 FD carriers, and 24 non-carriers. Estimated mean *IKBKAP* mRNA levels, expressed as amount relative to the non-carrier average, were 0.23 in FD patients and 0.58 in carriers. Interestingly, comparison of *IKBKAP* mRNA levels of the 22 FD patients with related carriers enrolled in the study showed a strong correlation, suggesting genetic influence on splicing efficiency. Next, kinetin was given orally to 29 FD carriers who were divided into 5 cohorts. Cohort doses were determined from the modified Fibonacci dose escalation scheme (e.g., x, 2x, 3.3x, 5x, 7x; x = 3.14 mg/kg/d). After the first single dose, serum kinetin levels were determined at 30 min, 1 hr, 2 hr, 6 hr, 12 hr, and 24 hr. Each volunteer took the same daily dose for a one-week period. Blood was sampled prior to the first kinetin dose and after the last dose for measurement of *IKBKAP* mRNA levels. Maximum serum concentration was achieved in 1-2 hours. In cohort 5 concentrations remained at therapeutic levels (> 10 μM) for 6 hours. After 7 days of a single daily dose, expression of wild-type to mutant mRNA ratios increased and the percent positive change of *IKBKAP* mRNA correlated with serum kinetin levels. These exciting results support initiation of trials in FD patients.

2265/W

Cannabinoid therapeutic testing of a Friedreich ataxia mouse model. *R. Mouro Pinto, S. Al-Mahdawi, M.A. Pook.* CCCB/BICGP, Division of Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge, UB8 3PH, UK.

Friedreich ataxia (FRDA) is an autosomal recessive disease causing degeneration in the central and peripheral nervous system, cardiomyopathy, skeletal abnormalities and increased risk of diabetes. It is caused by deficiency of the mitochondrial protein frataxin. The genetic mutation found in 98% of FRDA chromosomes is the unstable hyperexpansion of a GAA triplet repeat in the first intron of the *FXN* gene. There is currently no effective treatment for FRDA. A GAA repeat expansion mutation-based transgenic mouse model of FRDA has been developed. The mice exhibit both intergenerational and age-related somatic instability of the GAA repeat, with prominent expansions detected in the cerebellum. In addition, a decreased level of frataxin expression was achieved, which is accompanied by mild oxidative stress. The neurological phenotype of these mice includes a progressive coordination defect, as measured by decreased rotarod performance, and vacuolar pathology within large neurons of the dorsal root ganglia. However, the degree of impairment does not extend to overt ataxia. The antioxidant activity of cannabinoids such as Δ^9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) indicates that they may be effective in preventing and/or treating the development of neurodegenerative disorders such as FRDA. Thus, the potential neuroprotective effect of such cannabinoids is being investigated on the FRDA mouse model available. CBD has been administered in two doses - 10 and 20 mg/kg - over a 3 month period (6-9 and 3-6 months of age respectively). A 20mg/kg dose of CBD:THC (1:1) is currently being administered to test the potential benefits of combining CBD with THC (3-6 months of age). Data will be presented on functional studies (locomotor coordination and activity analysis), together with histological and biochemical analysis to determine more subtle effects, i.e. presence of vacuoles in the DRG, levels of oxidative stress, and aconitase and mitochondrial respiratory chain complex activities.

2266/W

Bioaminergic deficits in Rett syndrome : from pathophysiology to clinical trials. *L. Villard[1], E. Dura[1], J. Mancini[2], A. Moncla[1,3], J.C. Roux[1].* 1) Inserm U491, Faculte de Medecine de La Timone, Marseille, France; 2) Dpt of Pediatric Neurology, La Timone Children's Hospital, Marseille, France; 3) Dpt of Medical Genetics, La Timone Children's Hospital, Marseille, France.

Rett Syndrome (RS) is a severe neurological disorder with an incidence of about 1/10,000 female births. It is caused by mutations in the *MeCP2* (methyl-CpG binding protein 2) gene located on the X chromosome. The MeCP2 protein is believed to play a pivotal role in silencing other genes. RS girls exhibit a number of neurodevelopmental defects associated to autonomic dysfunctions. Because a significant proportion of deaths in RS may be caused by sudden respiratory arythmia, we have investigated breathing dysfunction in *Mecp2*-deficient mice. We have shown that adult *Mecp2*-deficient mice have erratic breathing with highly variable respiratory rhythm and frequent apneas probably due to reduced norepinephrine content and a drastic decrease in the number of tyrosine-hydroxylase (TH) expressing neurons in the medulla. We subsequently developed a pharmacological protocol to treat *Mecp2*-deficient animals when they start to manifest breathing problems. We have shown that treating these mice with a norepinephrine reuptake inhibitor (desipramine) significantly improves their respiratory rhythm during several weeks. This treatment significantly extends the lifespan of the treated animals up to twice the lifespan of untreated animals. We recently identified a cellular mechanism to explain this efficiency, mainly characterized by an increase of the number of TH-expressing neurons in the medulla. These results suggest that pharmacological stimulation of the noradrenergic system could be useful in RS. We are currently starting a phase II clinical trial with RS patients in France using desipramine.

2267/T

Correlation of phenylalanine levels with intellectual outcome and executive functioning in patients with phenylketonuria. *L.V. Furtado[1], N.L. Cantor[2], S.L. Ernst[3], J.B. Fulton[2], N. Longo[1,3].* 1) Dept Pathology, Univ Utah ARUP Laboratories, Salt Lake City, UT; 2) Primary Children's Medical Center, Salt Lake City, UT; 3) Dept Pediatrics, Univ Utah ARUP Laboratories, Salt Lake City, UT.

Background: Phenylketonuria (PKU) is characterized by elevated phenylalanine levels that can impair brain development and functioning. It is treated with a diet restricted in phenylalanine. It is unclear whether there are certain periods in which phenylalanine levels have a stronger effect on psychometric cognitive measures. Here we correlate phenylalanine levels at specific ages to subsequent or concomitant intellectual and executive functioning in patients with PKU. Methods: Phenylalanine levels at time of diagnosis, time at which therapy was initiated, and average phenylalanine levels for different periods (<1 year, 1-3 years, 3-5 years, 5-10 years, 10-18 years, >18 years) were correlated to the results of psychometric testing (Wechsler Intelligence Scale for Children (WISC-III, WISC-IV); Wide Range Achievement Test (WRAT-3, WRAT-4); Wechsler Adult Intelligence Scale (WAIS-III); and Children's Category Test (CCT-2)) in 52 patients with PKU over 5 years of age (average age 14.4±5.9 years, range 5.6-30 years). Data were analyzed by regression analysis, using $p<0.05$ as level of significance. Results: In our group of patients, the highest phenylalanine level at diagnosis did not correlate with later IQ or functional outcome. By contrast, there was a negative correlation between the time at which dietary treatment was initiated and later reading and spelling scores. Average plasma phenylalanine levels at ages 3-5 years and 5-10 years negatively correlated with average final IQ and verbal performance, while no significant correlation was noted with other age periods. Part of the correlation in the 5-10 year old group could be explained by the known relationship between phenylalanine levels and performance at time of testing. Conclusions: Time at which therapy is initiated and phenylalanine levels between 3 and 10 years of age negatively correlated with long-term measure of intellectual functioning and with performance on verbal academic measures in patients with PKU.

2268/T

Idursulfase Replacement Therapy in 2 Infants with Hunter Syndrome. *D. Viskochil, C. Ashurst, I. Hung, J. Carey, S. Bleyl, N. Longo.* Dept Pediatrics, Div Med Gen, Univ Utah, Salt Lake City, UT.

Mucopolysacharidosis II (MPS II) is an X-linked lysosomal storage disorder caused by a deficiency in iduronate-2-sulfatase. Based on the success of a double-blind, placebo-controlled clinical trial (Muenzer J, et al., 2006, Genet Med 8; 465-473), the Federal Drug Administration recently approved the administration of idursulfase (Elaprase™) for individuals with MPS II. The 2 primary endpoints of the trial were the distance covered in a 6-minute walk and change in predicted forced vital lung capacity, which essentially excluded children less than 5 years of age. We have initiated idursulfase replacement therapy in 2 infants with MPS II. Case 1 presented to our service at 8 months of age with gibbus. Additional findings included macrocephaly, mild facial gestalt, a salmon-colored, pebbly skin patch on his thorax, and intermittent otitis media/upper respiratory infections. He had a prior history of hernia repair at 1 month of age, but did not have hepatosplenomegaly, cardiac defects, or joint contractures. Case 2 presented at 8 months of age with hepatomegaly and hearing loss. He also had macrocephaly, mild facial gestalt, anterior beaking of the 2nd lumbar vertebra, and recurrent otitis media. Idursulfase has been provided for a total of 37 weeks for case 1 with interruption of therapy for 10 weeks due to lack of insurance coverage, and 20 consecutive weeks for case 2. Response to therapy was noted by decreased urinary glycosaminoglycans (GAGs) in both cases. Both developed transient skin rashes during infusion on the 5th week, which resolved by decreasing the infusion rate. Subsequently, over time and with diphenylhydramine pretreatment, we have increased the rate of infusion to the standard dosing (0.5 mg/kg administered step-wise over 3 hours). Both have tolerated the enzyme infusions with only mild hypersensitivity reactions, without respiratory compromise. We conclude that idursulfase therapy over the short-term is effective and safe in children less than 5 years of age. Long-term studies of infants treated with weekly infusions will be important to evaluate the potential prevention of complications due to an accumulation of GAGs in MPS II.

2269/T

Over expression of Klotho (KL) gene in fibroblast cell line of Hutchinson-Gilford progeria syndrome (HGPS) does not rescue the phenotypes of HGPS cells. *L. Wang[1,2], N. Zhong[1,2,3].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Peking University Health Science Center, Beijing, China; 3) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

HGPS is a premature senile disease of children caused by de novo mutation of LMNA gene that encodes a nuclear envelope protein of lamin A/C. The lamin A/C is an important component involved in nucleus membrane skeleton structure. As a new gene related with aging process, KL could prolong the life of mice and also lead to, if deleted, similar symptoms in mice with HGPS features such as growth depletion, lipopenia, adermotrophia etc. In this study, we have investigated whether KL gene could ameliorate the abnormal phenotype of HGPS cells. An expression plasmid was constructed with a full length of coding sequence of KL gene, followed by transfected into three HGPS skin fibroblast cell lines which were determined to carry a G208G mutation. Parameters on cell morphous, levels of protein expression and cell cycle were detected by use of confocol, western-blot and FACS, respectively, to present the effects of KL-encoded protein on HGPS cells. Our data showed that there was no significant improvement on morphous abnormality of HGPS cells nucleus membrane as well as effective reduction on the expression of progerin, the defect lamin A protein observed in HGPS cells. Neither were distinct changes of cell cycles found between KL-treated and untreated HGPS cells. Our results indicated that KL gene does not rescue the pathogenic alterations in the development of HGPS.

2270/T

An effect of bone marrow transplantation for a 15-year-old patient with Mucopolysaccharidosis (MPS) IVA in Japan. *Y. Chinen[1], Y. Higa[1], T. Higa[1], N. Hyakuna[1,2], T. Ohta[1].* 1) Dept Pediatrics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 2) Dept Pediatrics, Okinawa Prefectural South Medical Center, Okinawa, Japan.

Mucopolysaccharidosis IVA (MPS IVA; Morquio A disease) is an autosomal recessive lysosomal storage disorder that is caused by defective N-acetylgalactosamine-6-sulfate sulfatase (GALNS). A progressive skeletal dysplasia is commonly observed among the MPS IVA patients. We describe a patient with MPS IVA diagnosed at 14 years of age. At 8 years of age he had odontoid dysplasia with atlanto-axial subluxation and underwent the surgical operation of atlanto-axial fixation. He had twice correction osteotomy for valgus knee. He had mild corneal clouding, glaucoma, mild tricuspid insufficiency, snoring loudly and moving with a wheelchair. At 15 years 8 months of age he received related-donor bone marrow transplantation (BMT). After BMT, the enzyme activity of GALNS in white blood cells increased to 73.6 nmol/mg protein/17hr from 2.8, bone mineral density at L2-4 to 0.539 g/cm2 from 0.374, the concentration of uronic acid in urine decreased to 23.5 mg/g creatinine from 34.6. He could walk for100 meters after correction osteotomy for valgus knee. Glaucoma and snoring loudly disappeared, but the shape of vertebral deformity and hypermobility of joints were unchanged three years after BMT.

2271/T

Underexpression of the GABAergic system in the fragile X syndrome: a novel target for treatment? *F. Kooy[1], C. D'Hulst[1], P.P. De Deyn[2].* 1) Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Neurochemistry and Behavior , Univ Antwerp, Belgium.

In the recent past, we have demonstrated decreased expression of 7 out of 18 known subunits of the GABAA receptor in cortex of fragile X mice, including the major isoforms, using real time PCR. To investigate whether the entire pathway was evolved, We measured mRNA levels of enzymes responsible for GABA synthesis (GAD), transport (GAT1-4) and degradation (SSADH) in the fragile X mouse model and found approximately 50%; under expression of the main components of GABA metabolism, transport and degradation As $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian brain, implicated in anxiety, depression, epilepsy, insomnia and learning and memory, processes also disturbed in fragile X patients, we argue that an overall dysfunction of the GABAergic system has neurophysiologic and functional consequences that might relate to the behavioural phenotype associated with fragile X syndrome. This hypothesis is supported by western blotting and electrophysiological findings from other groups. We propose a model demonstrating the involvement of the GABAergic system in epilepsy (decreased presence of specific subunits), in sleeping problems (through interactions of the $GABA_A$ receptor with the master circadian clock and melatonin) and in behavioural problems (through the influence of GABA on the HPA-axis mediated stress response). We postulate that the well described $GABA_A$ receptor pharmacology might open new powerful opportunities for treatment of the behavioural and epileptic phenotype associated with fragile X syndrome.

2272/T

Direct Brain Delivery of Iduronate 2-Sulfatase Reduces Glycosaminoglycan Accumulation and Improves Histopathology in the CNS and Peripheral Tissue of Hunter Mice. *Y. Lu, J. Pan, AR. Garcia, A. Stronge, M. Tonini, C. Neal, J. Lamsa.* Preclinical Research, Shire HGT, Cambridge, MA.

Hunter syndrome, or mucopolysaccharidosis (MPS) II, is an X-linked inherited disorder caused by the deficiency of the enzyme iduronate 2-sulfatase (I2S), which is involved in the lysosomal catabolism of the glycosaminoglycans (GAG) dermatan and heparan sulfate. To evaluate the effect of I2S on GAG accumulation and CNS pathology, we injected I2S (0.1mg) or vehicle directly into the right striatum of the Hunter mouse brain. We found that a single injection of I2S caused an improvement of histopathology of the brain and liver, comparing wild-type, vehicle treated, and I2S treated Hunter mice. Specifically, in the brain we found a reduction of abnormally high lysosomal activity in microglial, meningial and perivascular cells using LAMP-1 immunostaining; decreased glial fibrillary acidic protein (GFAP) immunostaining with reduced astrocyte cell size and its processes, possibly reflecting a reduction of inflammation in the CNS; and reduced intracytoplasmic vacuolization in Purkinje cells. In the liver, we found that central I2S delivery caused a significant increase in I2S levels as measured by ELISA. We also found a significant decrease of LAMP-1 staining in hepatocytes, sinusoidal cells and connective tissues; reduction of GAG accumulation, to a degree similar to wild type controls; and a marked reduction of intracytoplasmic vacuolization in all hepatocytes. Our data demonstrate that CNS I2S delivery is effective in reducing GAG accumulation and improving histopathology in the brain. It also indicates that central delivery of I2S not only distributes to, and affects the brain, but also peripheral organs. These data suggest that I2S injected directly into the CNS is able to improve the histopathological markers described here and provides a basis for establishing levels of I2S effective in improving biochemical and histological markers of MPS II in future studies.

2273/T

Expression, purification and evaluation of activities of human EGF-IL-18 fusion protein. *J. Lu[1], Z.J. Zheng[1], J.H. Pan[1], Y. Peng[1], Y. Bai[1, 2].* 1) Dept Medical Genetics, Wenzhou Medical Col, Wenzhou, Zhejinag, China; 2) Dept Celluar and Structural Biology, UTHSCSA, San Antonio, Texas, USA.

We report here the expression, purification, and in vitro and in vivo analysis of activities of EGF-IL-18 fusion protein. The epidermal growth factor (EGF) and Interleukin-18 (IL-18) cDNA was fused together and cloned in an expression vector. The recombinant EGF-IL-18 fusion protein was processed and then purified. The resulting EGF-IL-18 fusion protein was shown to be able to induce IFNγ expression and secretion in KG-1 cells, and promote PBMNC proliferation. This fusion protein also stimulated activation of CD4+ T cells, and increased the percentage of B and NK cells in PBMNC challenged with tumor antigens. Moreover, EGF-IL-18 fusion protein could induce significant tumor regression in SMMC-7721-xenografted Balb/c nude mice when administered together with peritumoral injection of X-Ray-irradiated NK-92 cells. The present observation indicates a promising therapeutic approach against cancer.

2274/T

Effectiveness of intrathecal rhIDU in deep brain structures in MPS I dogs. *A. Chen[1], M. Passage[2], S. Le[2], C. Vogler[3], P. Dickson[2].* 1) Neurology, Harbor-UCLA Med. Ctr., Torrance, CA; 2) Pediatrics, LA Biomed at Harbor-UCLA, Torrance, CA; 3) Pathology, St. Louis Univ. School of Med., St. Louis, MO.

Introduction: Intrathecal (IT) recombinant human α-L-iduronidase (rhIDU) has been shown to reduce mean brain glycosaminoglycans (GAGs) to normal levels in MPS I dogs.[1] In this study, we examined functional neuroanatomical regions including deep structures following treatment with IT rhIDU.

Methods: MPS I dogs were treated monthly with 3-4 doses of 1.08 mg IT rhIDU (n=5). Normal dogs (n=5) and untreated MPS I dogs (n=2) were also studied. Sections ≈0.5 - 1 cm^3 were evaluated from superficial neuroanatomical regions (rostral forebrain and cerebellum), from deeper structures (hippocampal formation, basal ganglia/thalamus), and from brainstem. Samples were assayed for GAG using an Alcian blue dye binding method.

Results: Superficial regions: rostral forebrain GAG was 2.36±0.543 μg/mg in normal dogs; 8.05±1.07 μg/mg untreated MPS I dogs; 3.51±0.660 μg/mg, monthly IT-treated MPS I dogs. Cerebellum: 2.67±0.327 μg/mg, normal; 7.59±0.658 μg/mg, untreated MPS I dogs; 3.95±0.849 μg/mg, IT-treated. Deep regions: basal ganglia: 3.51±0.599 μg/mg, normal; 5.40 μg/mg (n=1), untreated; 4.76±0.903 μg/mg, IT-treated. Hippocampal formation: 3.30±0.396 μg/mg, normal; 5.93±0.170 μg/mg, untreated; 3.91±0.391 μg/mg, IT-treated. Brainstem: 3.73±1.10 μg/mg, normal; 6.49±2.14 μg/mg untreated , 5.36±0.740 μg/mg IT-treated. Pathology is pending.

Conclusion: GAG storage in untreated MPS I dogs was similar among different functional neuroanatomical regions. GAG storage reduction with IT rhIDU was better (48-56%) in the superficial regions of the brain, as compared to deeper regions and brainstem (12-34%). There may be regional differences in the efficacy of IT rhIDU in the MPS I dog brain.

1. Dickson P., et al. Molec. Genet. Metab. 91 (2007) 61-8.

2275/T

Enzyme Replacement Therapy for MPS II: Developing a Pre-medication Protocol. *M. Descartes[1], J. Franklin[1], T.L. Hanvey[2].* 1) Dept Genetics, Univ Alabama, Birmingham, Birmingham, AL; 2) Children's Hospital of Alabama, Birmingham, AL.

MPS II is an X-linked, lysosomal disease that is caused by deficiency of iduronate-2-sulfatase (I2S). Elaprase® (Idursulfase), the first product for the treatment MPS II was approved in July 2006. Infusion reactions are commonly reported in patients on Elaprase. We report the management approach of a patient with persistent infusion reactions. Our patient was diagnosed with MPS II at 4 years of age and started on Elaprase at 4.3 years. The patient received Elaprase at 1mg/kg/dose once per week. The Elaprase was prepared and administered following manufacturer recommendations. Our patient's first infusion-related adverse event occurred on his fourth infusion. He developed general malaise and fever after the infusion. The patient was being pre-medicated with acetaminophen PO and diphenhydramine IV. He developed flushing, whelps, and irritability on the fifth infusion. The infusion was stopped, methylprednisolone IV administered, and infusion re-started at a slower rate. With each infusion the patient had nausea, emesis, flushed face, and pruritic rash. During the sixth infusion, ranitidine IV was given in addition to diphenhydramine due to a rash. The premedication regimen was then changed to include methylprednisolone IV. The patient developed a rash but resolved with additional diphenhydramine. With infusion eight, the premedication regime was changed to include PO prednisone to be given 2 hours prior to infusion. The IV methylprednisolone pre-infusion was stop and acetaminophen PO and diphenhydramine IV were given upon patient arrival. The maximum flow rate was also decreased. Irritability, gagging and rash were noted. The symptoms resolved by stopping the infusion and by giving additional diphenhydramine. With the ninth infusion, coughing and crying were noted. With the tenth infusion, the patient remained irritable. These last symptoms resolved untreated. This patient transitioned to care at a local hospital due to the time traveled (over 4 hours one way each week). The family was anxious for care closer to home. This patient's response to the infusions represents the difficulty in determining the best pre-medication protocol for these patients.

2276/T

Treatment of MPS I dogs from birth with intrathecal and intravenous rhIDU. *N.M. Ellinwood[1], E.M. Snella[1], J. Jens[1], K.L. Kline[2], J. Parkes[2], J. Wengert[2], M. Passage[3], S. Le[3], P. Dickson[3].* 1) Dept Animal Sci, Iowa St Univ, Ames, IA; 2) Dept Vet Clin Sci, Iowa St Univ, Ames, IA; 3) Dept Pediatrics, LA Biomed at Harbor-UCLA, Torrance, CA.

Introduction: Recombinant human α-L-iduronidase (rhIDU) is used as enzyme replacement therapy for mucopolysaccharidosis (MPS) I. Antibodies develop in treated patients that may reduce the efficacy of treatment. Previous research in gene therapy has shown natural tolerance in MPS I mice treated from birth.[1] **Methods:** MPS I dogs received 0.58 mg/kg/week IV rhIDU (n=5) or 2.0 mg/kg/week (n=1). Two low dose IV dogs received 1.74 mg rhIDU every three months via intrathecal injection (n=2). Serum samples for specific, anti-iduronidase IgG antibodies were analyzed by ELISA. **Results:** The pups were treated beginning at age 1 day (n=2), 4-8 days (n=3), and 25 days (n=1). Two pups infused on the first day of life (when natural pup mortality is high) died following one treatment. No adverse events occurred in the dogs treated after 4 days of age. After 10-24 weeks of treatment, serum anti-iduronidase antibody levels were < 1 OD unit/ul in all treated pups (tolerance cut-off is 20 OD units/ul). No immune suppressive therapy was used in the pups. In contrast, 8 adult normal and MPS I dogs receiving IV rhIDU developed a mean antibody titer of 149 OD units/ul (range 60.2-377.9) after 12 weeks of treatment.[2] Treated pups also had a more normal appearance to the cranium and had less toe-splaying than untreated littermates. **Conclusion:** MPS I pups beginning IV rhIDU treatment between 4 and 25 days of age did not develop anti-iduronidase antibodies after 10-25 weeks of treatment, consistent with immune tolerance to therapy. This may have important implications for MPS I patients beginning treatment early in life.

1. S.D. Hartung, et al., Molec. Ther. 9 (2004) 866-875.
2. E. Kakkis, et al., PNAS 101 (2004) 829-834.

[supported by The National MPS Society, Inc., The Ryan Foundation, the Sanfilippo Children's Research Foundation, ISU, CIAG, and the NIH (NS054242 and RR002512)].

2277/T

Treating symptomatic spinal cord compression with intratechal enzyme therapy in three Brazilian patients with MPS: What has happened so far. *R. Giugliani[1], D. Horovitz[2], L. Jardim[1], R. Costa[3], S. Fagondes[3], T. Vieira[1], A.B. John[3], L. Vedolin[4], J. Llerena[2], M.V.R. Munoz[1].* 1) Medical Genetics Serv, Hosp Clinicas Porto Alegre, Porto Alegre, RS, Brazil; 2) Instituto Fernandes Figueira, FIOCRUZ, Rio de Janeiro, RJ, Brazil; 3) Pulmonary Diseases Service, Hospital de Clínicas, Porto Alegre, RS, Brazil;; 4) Neuroradiology Department, Hospital Mãe de Deus, Porto Alegre, RS, Brazil.

BACKGROUND: In MPS, deficiency of specific enzymes can cause spinal cord compression due to storage of glycosaminoglycans within the cervical meninges. In 2005, we used intrathecal infusions of recombinant human α-L-iduronidase to treat a MPS I adult patient with spinal cord compression (P1) and recently we conducted the use of IT-ERT in pediatric patients with MPS I (P2) and MPS VI (P3). To our knowledge, these were the first MPS I adult, the first MPS I child and the first MPS VI patient who received IT-ERT. METHODS: The patients underwent a series of 4 monthly courses of IT-ERT (specific enzyme dilluted on Elliotts B solution). Patient P1 performed follow-up evaluations at 12 and 18 months post-IT ERT, including 12 MWT, pulmonary function exams, imaging studies and complete neurological examination. P2 and P3 performed the same evaluations as P1 on baseline and on the immediate follow- up, but were not able to perform reliable pulmonary function tests nor 12 MWT. Pre-infusion CSF pH of P2 and P3 were measured. RESULTS: Neurological symptoms improved on all cases. On patient P1, after an initial improvement, it was noticed on the 12-month evaluation an evidence of neurological worsening; after 18 months this worsening was evident also on pulmonary function tests, especially on pulmonary diffusion.Pre-infusion CSF pH were above 8,0 on both cases. CONCLUSIONS: This procedure seems to be a safe treatment for spinal cord compression in MPS. We speculate that, after an initial set of monthly infusions, a protocol with longer intervals between infusions (2 to 4 times a year) could be enough to maintain the clinical benefits. Further studies are required.

2278/T

Bone mass in children with Type I Gaucher Disease treated with low dose imiglucerase. *R. Heitner[1], J.M. Pettifor[2], S. Lipshitz[3].* 1) Pediatrics, Johannesburg Hospital University Witwatersrand South Africa; 2) Paediatrics;MRC Mineral Metabolism Research Unit. Chris Hani Baragwanath Hospital University Witwatersrand South Africa; 3) Linksfield Clinic South Africa.

Purpose:To assess bone mass in children with Type I Gaucher Disease treated with low dose imiglucerase over a period of 4.5-15 years. The haematological manifestations and organomegally of the disease are well managed with enzyme replacement therapy (ERT)using imiglucerase.Data shows that long term, irrespective of the dose used(15-60u/kg.body mass)there is normalization of the haematological and organ parameters. The effect of the dose on bone disease is less clear. High dose ERT is recommended to normalize bone mineral density(BMD)in patients with Type I Gaucher Disease. Previous studies have all been done in adults. **Methodology**: BMD was measured in 10 patients (age range 7-20 years)who had been on treatment for 4.5 to 13 years on a dose of 10u/kg.body mass fortnightly.BMD was measured using DXA (Discovery W Hologic Inc) at the lumbar spine and proximal femur and at the distal 1/3 and ultradistal forearm in 5 patients over the age of 18y using the same machine.Z-scores for each site were calculated using the manufacturers reference values. **Results**:Besides one patient who started on treatment after developing clinical bone pathology,none of the children complained of bone pain,had bone crises,pathological fractures or bone remodelling changes on MRI scan or standard radiology. Normal growth was achieved in all patients.Mean BMD Z score at the total hip was 0.44+0.94, at the spine -1.64+0.82,and at the distal 1/3 forearm -2.54+0.96. BMD Z score at the hip was inversely correlated with age.This association was not found at the spine.There was an almost significant inverse relationship between the age of the subject and BMD Z score at the lumbar spine (R^2=0.4.p= 0.054)This relationship was not noted at other sites. **Conclusion** Low dose imiglucerase in children appears to preserve bone mass at the hip.This is possibly not so at the spine or distal 1/3 radius.Further longitudinal studies are required. In resource poor countries low dose imiglucerase may provide a means of preserving bone integrity.

2279/T

Enzyme Replacement Therapy (ERT) in females with Fabry disease: an update from FOS - the Fabry Outcome Survey. *D.A. Hughes[1], M.A. Barba-Romero[2], P.B. Deegan[3], A. Linhart[4] on behalf of the FOS Research Group.* 1) Royal Free and University College Medical School, London, UK; 2) Albacete University Hospital, Albacete, Spain; 3) Addenbrooke's Hospital, Cambridge, UK; 4) Charles University, Prague, Czech Republic.

Fabry disease is an X-linked lysosomal storage disorder characterized by deficient activity of the enzyme α-galactosidase A. Signs and symptoms of Fabry disease are observed in both hemizygous males and heterozygous females and include neuropathic pain, cardiac symptoms, disturbances in renal function and stroke. Data from FOS, an international database of patients with Fabry disease, were analyzed to examine the effect of long-term ERT in heterozygous females with Fabry disease. In March 2007, 1356 patients were registered with FOS, comprising 558 adult males, 572 adult females and 226 children. The mean age at FOS entry for adult females was 43.7 years and the mean age at which ERT was started was 45.3 years in these patients.

Following 3 years of ERT, neuropathic pain - assessed using the brief pain inventory (BPI) - was improved (BPI pain at its worst, 4.15 [2.34-5.96] versus 4.92 [3.68-6.17] at baseline, mean [95% CI], n = 13, p = n.s.). Similarly, QoL - assessed using the EQ-5D questionnaire - was improved after 3 years of ERT (EQ-5D score, 60.7 [50.5-69.9] versus 58.1 [46.4-69.8] at baseline, mean [95% CI], n = 12, p = n.s.). A decrease in left ventricular mass (LVM) was observed after 3 years of ERT (LVM indexed for height, 46.3 [34.4-52.3] g/$m^{2.7}$ versus 50.9 [41.0-61.0] g/$m^{2.7}$ at baseline, mean [95% CI], n =16, p = n.s.). Renal function - assessed by measuring glomerular filtration rate (GFR) - remained stable over 3 years of ERT (GFR, 65.2[61.2-70.3] ml/min/1.73 m^2 versus 67.5 [63.5-71.4] ml/min/1.73 m^2 at baseline, mean [95% CI], n = 30, p = n.s.).

These data suggest the beneficial effects of long-term ERT on clinically significant aspects of Fabry disease in heterozygous females. Early diagnosis and prompt therapeutic intervention may lead to further clinical benefit in female patients with Fabry disease.

2280/T

Agalsidase alfa reduced cardiac mass in Fabry disease patients with left ventricular hypertrophy. *C. Kampmann[1], A. Linhart[2], R. Schiffmann[3], R. Devereux[4].* 1) University Children's Hosp, Mainz, Germany; 2) Charles University, Prague, Czech Republic; 3) National Institutes of Health, Bethesda, MD, US; 4) Weill Cornell Medical College, NY, NY, US.

Left ventricular hypertrophy (LVH) is a common finding in Fabry disease. This retrospective study was conducted to assess the effect of agalsidase alfa (Replagal®) (Shire HGT, Cambridge, MA, US), 0.2 mg/kg, every other week) on left ventricular mass index (LVMi) in male and female Fabry patients with baseline LVH. All 45 adult patients (34 male, 11 female) had participated in clinical trials and/or had received at least 3 years of commercial treatment with agalsidase alfa. Serial echocardiograms were obtained at baseline and at 1 and/or 3 years of treatment, and were assessed in a blinded fashion by a single investigator (RD). LVMi change from baseline was evaluated with a sign-rank test. At baseline, 14 patients had LVH (mean LVMi=55.4±5.7 g/$m^{2.7}$; in the remaining 31 patients, mean LVMi=39.1±6.5 g/$m^{2.7}$). In 9 baseline LVH patients who had 1-year data, LVMi had declined by 9.2±7.9 g/$m^{2.7}$ (P=0.008), and in 10 patients who had 3-year data, LVMi had declined by 5.1±7.5 g/$m^{2.7}$ (P=0.037). In 28 patients without baseline LVH, a small increase in LVMi was observed after 1 year of treatment (3.6±5.7 g/$m^{2.7}$, P=0.002), but the average LVMi remained well within the normal range. In 26 patients without baseline LVH, a small and statistically insignificant increase in LVMi was seen after 3 years (2.1±7.9 g/$m^{2.7}$, P=0.27). Although no untreated patients were included in this study, another group of male and female Fabry patients had serial examinations in a separate natural history study. These patients with baseline LVH demonstrated an increase in LVMi of 6.0±13.3 g/$m^{2.7}$ (n=13; P=0.17) and 20.3±11.7 g/$m^{2.7}$ (n=8; P=0.008) in LVMi after 1 and 3 years, respectively. In contrast, LVMi in natural history patients without baseline LVH did not change after 1 year (-0.8±3.7 g/$m^{2.7}$, n=10, P=0.32) and increased after 3 years (4.8±5.0 g/$m^{2.7}$, n=21, P<0.001). In conclusion, treatment with agalsidase alfa for 1 or 3 years reduced LVM in Fabry disease patients with baseline LVH.

2281/T

Long-term weekly dosing of idursulfase in the treatment of mucopolysaccharidosis II (MPS II, Hunter syndrome). *J. Muenzer[1], E. Wraith[2], M. Beck[3], R. Giugliani[4], P. Harmatz[5], C.M. Eng[6], A. Vellodi[7], R. Martin[8], U. Ramaswami[9], M. Calikoglu[1], S. Vijayaraghavan[2], A.C. Puga[4], B. Ulbrich[9], M. Shinawi[6], M. Cleary[7], S. Wendt[3].* 1) University of North Carolina, Chapel Hill, NC, US; 2) Royal Manchester Children's Hospital, Manchester, UK; 3) University of Mainz, Mainz, Germany; 4) Medical Genetics Service, HCPA/UFRGS, Brazil; 5) Children's Hospital, Oakland, CA, US; 6) Baylor College of Medicine, Houston, TX, US; 7) Great Ormond Street Hospital, London, UK; 8) St. Louis University, St. Louis, MO, US; 9) Cambridge University Teaching Hospitals, Cambridge, UK.

MPS II is an X-linked lysosomal storage disorder caused by a deficiency in iduronate-2-sulfatase. A recent 1-year, double-blind, placebo-controlled clinical trial of enzyme replacement therapy with idursulfase (Elaprase®, Shire HGT, Cambridge, MA, US) showed that both weekly and every other week (EOW) dosing of idursulfase (0.5 mg/kg) significantly improved the primary endpoint (a composite comprising sum of the ranks of changes in percent predicted forced vital capacity (%FVC) and distance walked in 6 minutes (6MWT) compared to placebo, with the magnitude of the clinical benefit being larger in the weekly compared with the EOW group (P = 0.13). This trial has been continued as an open-label extension study designed to evaluate the long-term safety and efficacy of weekly dosing of idursulfase (0.5 mg/kg). All patients who completed the double-blind study (n = 94) enrolled in the extension study and were treated with idursulfase at 0.5 mg/kg weekly. Changes in absolute and %FVC, 6MWT, and organ size were continued to be monitored in the open-label extension study. Safety was assessed continuously during the study by monitoring treatment emergent adverse events and by periodic determination of anti-idursulfase antibodies in blood samples. First year open-label efficacy and safety results will be presented.

2282/T

Long-term phenotypic correction of murine Hemophilia A and immunological differences of bioengineered FVIII variants delivered by helper-dependent adenoviral vectors. *V. Cerullo[1], M.P. Seiler[1], R. Garcia[1], C. Clarke[1], R.J. Kaufman[4,2], S.W. Pipe[3], B. Lee[1,2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Pediatrics and Communicable diseases, University of Michigan; 4) Biological Chemistry and Internal Medicine, University of Michigan.

Bioengineering of the factor VIII (FVIII) molecule has produced variants that overcome secretion and/or inactivation in vitro, but their value for in vivo gene therapy has not been evaluated. We tested six modified FVIII variants for in vivo efficacy by expressing them from helper dependent adenoviral (HD-Ad) vectors. We constructed wild type (WT), B-domain deleted (BDD), a point mutant for improved secretion (F309S), a variant with a partial B-domain deletion for improved secretion (N6), a combination of the point mutant and partial B-domain deleted variant (F309N6), and an inactivation resistant (IR8) FVIII variant. All constructs expressed functional protein after injection of high dose HD-Ad. However, activity improved from 20-50% with WT, to nearly 100% with the N6 and F309N6 variants. Interestingly, mice treated with N6 showed long-term FVIII activity for 64 weeks with reduced anti-FVIII antibody titer. Importantly, the N6 and F309N6 vectors resulted in therapeutic levels of FVIII activity after a 50% lower viral dose, indicating that transgene modification itself can considerably improve the dose efficacy of HD-Ad; a key impediment to clinical application. In summary, bioengineering of the FVIII molecule may be an attractive measure to augment the safety profile of HD-Ad gene therapy for hemophilia A.

2283/T

Functional model systems for congenital ichthyosis: Basic and long way to therapy. *K.M. Eckl[1], S. Torres[1], S. de Juanes[3], D. Metze[2], P. Krieg[3], H.C. Hennies[1].* 1) Cologne Ctr Genomics, Univ Cologne, Div Dermatogenetics, Köln, Germany; 2) Univ Muenster, Dermatology, Münster, Germany; 3) DKFZ, Div Eicosanoids, Heidelberg, Germany.

In the last four years several new genes for autosomal recessive congenital ichthyosis (ARCI) were identified. However, still only little is known about the pathophysiology of this clinically and genetically heterogeneous group of severe disorders of keratinization. To investigate the role of proteins involved in the development of ARCI, we have established 3D organotypic skin models (epidermis equivalents) with primary keratinocytes and fibroblasts. Here we were able to analyse histopathologically and immunohistochemically the structure of the 3D model, especially the suprabasal layers including the stratum corneum. To stratify the effects of inactivation of different genes involved in ARCI, primary keratinocytes from healthy donors were transfected with siRNA to knock down specific genes. This was done for *TGM1*, *ALOX12B*, *ALOXE3*, *ABCA12*, *Ichthyin,* and *FLJ39501*. We found the typical histopathologic features seen in patient samples. Quantitative RT-PCR analysis was performed in samples from transfected keratinocytes and 3D models showing knock-down rates of 95% on average. Analysis of keratinocytes from patient biopsies demonstrated the impact of passage number, mutation type etc. We used double knock downs for *ALOX12B* and *ALOXE3*, which code for subsequent members of the same pathway, to investigate the effect of intermediate products. Importantly, these samples showed still knock-down efficiencies of 80% and 99%. We compared our results in humans (patients and 3D models) with those from 12R-LOX-deficient mice by expression profiling and qRT-PCR analyses. Knock-down efficiency did not always reflect phenotypical/histological findings. Since knock-down efficiencies were high even after seven days, we are increasing the differentiation period to study time-dependent changes in hyperkeratosis and forming a marked stratum corneum. Our models clearly mimick congenital ichthyosis and give us the chance to establish approaches for therapy under controlled and identical, patient independent conditions.

2284/T

Metabolically Biotinylated Helper Dependent Adenovirus: a new and rapid approach for targeting of High-Capacity Adenoviral Vector. *A. Erez, V. Cerullo, M. Seiler, C. Clarke, M.A. Barry, B. Lee.* Dept Human Molecular Genetics, Baylor Col Medicine, Houston, TX.

Developing cell-targeting vectors is an important goal in gene therapy. Metabolic biotinylation of first-generation adenoviral vectors for cell targeting has already been shown. However, there are several advantages in using helper-dependent adenoviral vectors; HD-Ad contain only the noncoding termini of the viral genome, can deliver large DNA fragments of up to 36 Kb into target cells, elicit reduced toxicity and generate prolonged transgene expression in vivo. We constructed a novel metablically biotinylated Helper Virus (Fib2102) to package HD-Ad vectors. Co-infection of a helper-dependent packaging cell line with the fiber-modified helper virus and various HD-Ad constructs would allow the production of fiber-modified HD-Ad expressing different transgenes, obviating the requirement to fiber-modify each individual transgene-expressing HD-Ad. We modified the c-terminus of the Ad5 fiber by addition of a 70 amino acid biotin acceptor peptide (BAP) in our helper virus backbone by homologous recombination. Adenovirus particles bearing the BAP were metabolically biotinylated during vector production by the endogenous biotin ligase to produce covalently biotinylated virions. The resulting biotinylated vectors can be used to transduce different cell type receptors by conjugation to specific biotinylated antibodies. In particular, we tested whether a biotinylated HD-Ad generated with this system expressing the LacZ transgene could transduce chondrocytes in vitro and in vivo. We found that a fiber-modified HD-Ad coupled to the chondrocyte specific α-10 integrin antibody was more efficient at transducing chondrocytes than vectors bearing wild type fiber . We show here the novel construction of a fiber-modified helper adenovirus which can be used to propagate high titers of fiber-modified HD-Ad. When coupled to cell-specific antibodies, it improves transgene expression both in vitro and in-vivo. This study demonstrates progress in retargeting strategies for helper-dependent vectors more specifically for low transducing cell types.

2285/T

Correction of arginase deficiency with a helper-dependent adenovirus expressing mouse arginase I. *C. Gau[1], G. Lipschutz[1], J. Livesay[1], V. Cerullo[2], B. Lee[2], W. Grody[1], S. Cederbaum[1].* 1) UCLA, Los Angeles, CA; 2) Baylor College of Medicine, Houston, TX.

Purpose: Arginase I (AI) deficiency is characterized by episodes of hyperammonemia and neurodegeneration. In contrast to the human disease, in which patients survive into adulthood, the current mouse model of AI deficiency dies by 14 days with no visible signs of neurodegeneration. Our goal is to prolong the survival of AI deficient mice with a helper-dependent adenovirus expressing arginase I (Hd-AV mAI) specifically in the liver, in order to examine the effect of AI loss in other organs. *Methods:* An Hd-AV vector expressing mouse AI under a liver specific promoter was created. 1-4 day old pups from an $AI^{+/-}$ cross were injected in the superficial temporal vein with virus. Viral expression was examined by RT-PCR, and mice were analyzed for arginase activity, serum ammonia levels, and tissue amino acid levels. *Results:* We have doubled the life expectancy of the AI knockout mice to 27 days by injection with Hd-AV mAI. The viral DNA was detected in all tissues assayed, but the mRNA was detected only in the liver. Death at 27 days correlated with a loss of total viral DNA. Arginase activity assays showed that Hd-AV mAI injected knockout mouse livers have approximately a third of the activity of heterozygotes at 15 days, and their ammonia levels are normal. Saline-injected knockout mice at this age are on the verge of dying and have elevated ammonia levels. In addition, arginine and ornithine levels in the livers of the injected knockout mice were similar to those of saline injected heterozygous mice. By 26 days, the arginase activity in the Hd-AV mAI injected knockout mouse livers had dropped to less than 10% of heterozygote livers. Ammonia levels of the injected knockout mice began increasing between days 25 and 26, suggesting the cause of death to be similar to that of uninjected knockout mice. *Conclusions:* We have shown that the phenotype of the arginase I deficient mouse can be corrected using a viral vector expressing AI at 30%; of its normal activity, and that death in the injected knockout mice is due to the loss of AI expression.

2286/T

Characterization and AAV2/8-mediated gene therapy for maternal PKU syndrome in a PKU mouse model. *E.J. Lee, H. Kim, J.W. Park, J.O. Choi, E.S. Park, H.Y. Park, S.C. Jung.* Department of Biochemistry, School of medicine, Ewha Womans University, Seoul, Korea.

Phenylketonuria (PKU) is an autosomal recessively inherited metabolic disorder caused by a deficiency of phenylalanine hydroxylase (PAH). The accumulation of phenylalanine leads to severe mental and psychomotor retardation, and uncontrolled female patients present maternal PKU syndrome. Recently, we reported the cognitive outcome of biochemical and phenotypic reversal of PKU mouse model, Pahenu2, by the AAV 2-mediated gene delivery of a human PAH transgene. However, the therapeutic effectiveness had been limited only in male PKU mice. In this study, we generated pseudotyped rAAV2/8-hPAH vector and infused into female PKU mice via the hepatic portal vein or the tail vein. Two weeks after injection, the complete coat color change to black was observed in female PKU as in male. The PAH activities in the liver increased to 65-70% of wild-type in female PKU mice, to 90% in male PKU. Plasma phenylalanine level in female PKU mice decreased to normal value. In addition, the offsprings of the treated female PKU mice can completely overcome the maternal PKU syndrome. The crown-rump length and body weight of fetuses from treated female PKU mice were recovered to the wild type values. Also, the spontaneous abortion rate of treated female PKU mice was normalized. These results indicate that recombinant AAV2/8 mediated gene therapy might be a promising therapeutic strategy for PKU.

2287/T

Balloon Occlusion Catheter-Based Delivery of HDAd into the Nonhuman Primate Liver Results in Stable, High Level Transgene Expression with Minimal Toxicity. *P. Ng[1], G. Stapleton[2], M. Law[2], D. Palmer[1], Y. Zuo[1], M. Finegold[3], A. Beaudet[1], C. Mullins[2], N. Brunetti-Pierri[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatric Cardiology, Baylor College of Medicine, Houston, TX; 3) Department of Pathology, Baylor College of Medicine, Houston, TX.

Helper-dependent adenoviral vectors (HDAds) hold tremendous potential for liver-directed gene therapy because they can mediate long-term transgene expression without chronic toxicity. Due to a nonlinear dose-response, high doses are required to achieve hepatic transduction resulting in dose-dependent acute toxicity. To overcome this obstacle, we have developed in baboons a method to achieve efficient hepatic transduction with low dose of HDAd. A sausage-shaped balloon occlusion catheter was percutaneously positioned in the inferior vena cava of baboon 1 to occlude hepatic venous outflow. $1x10^{11}$ vp/kg of HDAd expressing the baboon a-fetoprotein (bAFP) marker was injected via a percutaneously placed hepatic artery (HA) catheter and left to dwell within the liver for 15 min before balloon deflation. As controls, $1x10^{11}$ vp/kg were administered to baboon 2 and 3 by peripheral intravenous and HA injection respectively without balloon occlusion. All procedures were well tolerated. Mild and transient transaminitis was observed for all animals. Importantly, a high level of AFP was achieved in baboon 1 that was 10-fold greater than baboons 2 and 3 and this high level has been sustained to date (at least 420 days). To distinguish between procedure-related versus vector-mediated toxicity, baboon 4 underwent the balloon procedure but was mock injected with saline and similar mild transaminitis was observed suggesting that the mild hepatotoxicity was procedure and not vector related. Reduction of the occlusion time from 15 to 7.5 min did not affect the level or duration of AFP. Therefore, the therapeutic index of HDAd can be significantly improved by delivering the vector preferentially into the liver using a minimally invasive balloon catheter technique and this may be a first step towards clinical application of HDAd for liver-directed gene therapy.

2288/T

AAV based site-specific integration mediated gene therapy in the hereditary tyrosinemia type I (HT1) mouse model. *Z. Wang[1], T. Storm[2], M. Finegold[3], M. kay[2], M. Grompe[1].* 1) Oregon Stem Cell Center, Oregon Health & Science University, Portland, OR; 2) Department of Pediatrics and Genetics, Stanford University, Stanford, CA; 3) Texas Children's Hospital, Houston, TX.

Recombinant adeno-associated virus (AAV) vectors are mostly episomal and rarely integrate into the host genome. Integration occurs randomly throughout the genome. For therapy of genetic diseases, an integrating vector with site-specificity would be ideal. An AAV vector in which a human fumarylacetoacetate hydrolase (Fah) expression cassette is flanked by ~ 1 kb of homology to rDNA in the region of the I-Ppo site was generated. We tested the hypothesis that an AAV genome can be targeted to this location by homologous recombination. **RESULTS**: Adult $Fah^{-/-}$ mice injected with a dose of $3x10^{11}$ particles (high dose) gained weight after NTBC withdrawal while control mice died after 4-6 weeks. 10^9 AAV (low dose) rescued $Fah^{-/-}$ mice after initial weight loss, followed by weight gain. The hepatocytes of weight-stabilized mice were serially transplanted into secondary $Fah^{-/-}$ recipients. All recipients displayed weight gain after transplantation indicating stably integrated Fah expression cassette. DNA from completely repopulated animals was analyzed by Southern blot. Junction fragment analysis indicated that about 50% of integration events were site-specific in the rDNA locus in AAV8 injected mice and less in AAV2 mice. Considering the polymorphysisms in the rDNA repeats, majority of the integration events could be site-specific integration. The sequence results of the junction fragment generated by site-specific PCR exactly match the predicted junction sequence. The similar results were obtained in neonatal mice. Next, coinjection of AAV with Adeno-IPpoI show that no clear enhancement of the already high percentage of site-specific integration was seen. Dose dependent comparison study between AAV2-rDNA-Fah and AAV2-Fah showed that AAV2-rDNA-Fah can rescue the Fah-/- mice with about 1/30-1/10 dose of AAV2-Fah. Thereby, AAV-rDNA is superior to the regular AAV vector with high percentage site-specific integration and with low rescue dose, providing a new, clinical oriented strategy for genetic disease therapy.

2289/T

Overwhelmingly activated CNS immunity in MPS IIIB mice and significantly delayed neurological disease progression by immune suppression. *H. Fu[1,2], J. Etter[1], H. Auer[1,2], C. Wang[1], J. DiRosario[1].* 1) Center for Gene Therapy, CCRI; 2) Dept. Pediatrics, OSU.

Mucopolysaccharidosis (MPS) IIIB is characterized by progressive and severe neurological manifestations. No treatment is available for MPS IIIB. The mechanism of neuropathology in MPS IIIB is not well understood, though the characteristic pathology is lysosomal accumulation of heparan sulfate (HS), and recent studies suggest cascades of pathology and inflammation secondary to HS storage in MPS IIIB mouse brain. The present study investigates the roles of the CNS immunity in MPS IIIB neuropathology. First, using gene expression microarrays, we observed significant upregulation of an overwhelming number (>50) of immune related genes in 6-month-old MPS IIIB mouse brain, involving broad range of immune cells and molecules. The only 3 down-regulated immune genes were associated with acute immune responses. We have confirmed many of these altered gene expression patterns by RT-PCR, western blot and immunohistochemistry, including CD86, CD52, CD45, CD22, C4, Lfi30, and S100. We also saw upregulation of >60, and down-regulation of <30, immune transcripts in blood, with limited overlap of altered transcription (6↓, 1↓) between brain and blood. These suggest that the involvement of CNS immune responses in MPS IIIB is broad. Based on these findings, we treated MPS IIIB mice with daily oral administration of low dose prednisolone, to determine whether suppressing the immune response has beneficial impacts. Animal behavior tests demonstrated that the treatment significantly improved the ability of MPS IIIB mice to find a hidden platform in water maze and the latency on an accelerating rotarod. Not surprisingly, prednisolone did not result in the correction of lysosomal storage in any tissues. We demonstrate for the first time that immunosuppression alone can significantly slow the CNS disease progression, and immune responses do indeed contribute greatly to the neuropathology of MPS IIIB. We strongly believe that CNS immunity should be given serious consideration in future therapeutic development, though there is still much to be learned about the role of the immune response in MPS IIIB.

2290/T

Prevalence of Gastrointestinal Symptoms and Effects of Enzyme Replacement Therapy with Agalsidase alfa in a Cohort of Young Fabry Patients. *R.V. Parini[1], F. Santus[1], P. Desveaux[2], G. Pintos-Morell[3], U. Ramaswami[2].* 1) Metabolic Diseases Unit, Department of Paediatrics, San Gerardo Hospital, Monza, Milano, Italy; 2) Paediatric Metabolic Unit, Addenbrooke's Hospital, Cambridge, UK; 3) Dept. of Paediatrics, University Hospital Germans Trias i Pujol, Badalona, Spain.

Gastrointestinal (GI) symptoms are frequent in Fabry Disease. Our aim was to assess the prevalence of GI symptoms and the effect of Enzyme Replacement Therapy (ERT) with agalsidase alfa in a young population with Fabry disease followed in 3 metabolic centers.Number and frequency of GI symptoms were analysed in 41 patients (20 males and 21 females) less than 21 years of age. 17 (13 males and 4 females) are on ERT and have been treated for at least 1 year. 29/41 patients (70%) had GI symptoms more than once a week. Most frequent GI symptoms were abdominal pain (27), diarrhoea (13), constipation (11) nausea (8), bloating (9) and vomiting (5). 2 patients had all 6 symptoms. 19/29 patients (65%) had more than one symptom. No patient had gastritis, haemorrhoids, ulcer or pancreatitis. **Treated subgroup:** At baseline, 12/17 (70%) had GI symptoms and 11/12 (92%) had symptoms more than once a week. Data after 12, and 24 months of ERT show a reduction in frequency of GI symptoms. The number of patients with GI symptoms fell from 12 at baseline to 9 and 5 at 12 and 24 months respectively. Number of patients with GI symptoms more than once per week fell from 11 at baseline to 3 and 1 at 12 and 24 months. **Conclusion** 70% of our young patients with Fabry disease had GI symptoms. They had a clear benefit from ERT with agalsidase alfa. Favourable effects appear early after starting treatment and are sustained.

2291/T

Enzyme replacement therapy in children with Fabry disease: current practice as reported in FOS the Fabry Outcome Survey. *U. Ramaswami[1], G. Pintos-Morell[2], G. Kalkum[3], R. Parini[4], M. Beck on behalf of the FOS investigators[3].* 1) Paediatric Metabolic Unit, Addenbrooke's Hospital, University of Cambridge, UK; 2) Department of Paediatrics, German Trias i Pujol Hospital, Badalona, Spain; 3) Department of Paediatrics, University Children's Hospital, Mainz, Germany; 4) Clinica Pediatrica, Università Milano Bicocca, Monza, Italy.

Fabry disease (FD) is an X-linked lysosomal storage disorder characterized by deficient activity of the enzyme α-galactosidase A. Signs and symptoms of this condition are already present in childhood and include neuropathic pain, gastrointestinal disturbances and hypohidrosis, which can severely impact upon quality of life. Data from FOS, an international database of patients with FD, were analyzed to compare disease severity - as assessed using a modified version of the Mainz Severity Score Index (FOS-MSSI) - and the proportion of children and adults receiving treatment. In February 2007, 1329 patients were registered with FOS, comprising 551 men, 558 women and 220 children (102 boys, 118 girls). In total, 80.9% of men and 57.3% of women had received enzyme replacement therapy (ERT). ERT was administered to 45.9% of boys under 10 years of age and 72.3% of boys aged 10 years or older. Similarly, 34.1% of girls under 10 years of age and 49.4% of girls aged 10 years or older had received ERT. Prior to receiving ERT, median FOS-MSSI scores were 16.5 (10-90th percentile, 6.0-33.5; n = 498) and 9.5 (0.0-24.0; n = 503) in adult males and females, respectively. In boys and girls under 10 years of age, median FOS-MSSI scores were 5.0 (0.0-12.5; n = 33) and 2.5 (1.0-16.5; n = 36), respectively. Boys and girls aged 10 years or older had median FOS-MSSI scores of 8.0 (1.0-16.5; n = 57) and 7.5 (0.0-17.0; n = 72), respectively. These data indicate that, while a significant number of adults with FD are receiving ERT, young patients (< 10 years old) are less likely to be on treatment, despite exhibiting signs and symptoms of FD. This may be due to the false perception that the symptoms of FD do not represent a significant disease burden in these patients, as FOS-MSSI scores are often lower in children than in adults.

2292/T

Adenoviral mediated gene delivery rescues a neonatal lethal murine model of mut0 methylmalonic acidemia *mut*^{0}. *R.J. Chandler, C.P. Venditti.* Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Methylmalonic acidemia (MMA) is an autosomal recessive metabolic disorder caused by mutations in the mitochondrial matrix localized enzyme, methylmalonyl-CoA mutase (MUT). Patients presenting with a severe phenotype (mut0 MMA) typically present in crisis in the first 24-48 hours and can perish despite intervention. Survivors display a well-recognized phenotype of extreme metabolic instability and secondary complications throughout life. Elective liver transplantation has been successfully used as an adjunctive treatment in severely affected patients and can prevent frequent decompensations but does not normalize methylmalonic acid levels. Potential alternatives to liver transplantation, inherently limited due to donor organ supply, are hepatocyte-directed therapies to restore methylmalonyl-CoA metabolism. We have used Mut -/- mice, an animal model of severe mut0 MMA that displays uniform neonatal lethality in the first 48 hours of life, to assess the efficacy of viral gene delivery as a potential therapy for MMA. A bi-functional adenovirus that independently expresses both the Mut gene as well as GFP was used to deliver these genes via direct injection of newborn Mut-/- pups. None of the mutants that received the intramuscular injection survived until weaning; all Mut-/- pups died within the first 48 hours of life. However, Mut-/- newborn pups treated by intrahepatic injection with the same virus were rescued, with 44% of the injected Mut-/- pups surviving beyond weaning (day 15). Methylmalonyl-CoA mutase mRNA and protein were present in the rescued mutants, and metabolite levels were decreased. The results demonstrate that adenoviral mediated, hepatic methylmalonyl-CoA mutase expression can rescue the Mut-/- pups from neonatal lethality. These experiments represent the first successful viral gene delivery in any lethal organic acidemia murine model and provide proof of principle for liver directed gene delivery approaches in methylmalonic acidemia. *Mut*$^{-/-}$*mut*^{0}*Mut*intramuscular.

2293/T

Assay system for evaluation of allele-specific gene silencing by RNA interference (RNAi). *H. Hohjoh[1,4], Y. Ohnishi[1,2,3,4], Y. Tamura[1], M. Yoshida[1], K. Tokunaga[2].* 1) National Institute of Neurosci, NCNP, Tokyo, Japan; 2) Dept Hum Genet, Univ Tokyo, Tokyo, Japan; 3) JSPS Research Fellow; 4) equally contributed to this work.

Allele-specific gene silencing by RNA interference (ASP-RNAi) is an advanced application of RNAi technique and is therapeutically useful for specifically inhibiting the expression of alleles associated with diseases without suppressing the expression of their corresponding wild-type alleles. To realize such allele-specific gene silencing by RNAi, the design and assessment of small interfering RNA (siRNA) duplexes conferring allele-specific gene silencing is vital, but is also difficult. We developed an assay system with mutant and wild-type reporter alleles encoding the Photinus and Renilla luciferase genes for assessment of allele-specific gene silencing by RNAi, and selected competent siRNA duplexes conferring ASP-RNAi against mutant allele carrying double nucleotide substitutions related to familial Alzheimer's disease. In this study, we focused on the human Prion Protein (PRNP) mutant alleles carrying various single nucleotide substitutions, and attempted to improve ASP-RNAi against the single nucleotide mutations by using the system described above. From the data, it was suggested that introduction of one base mismatch into siRNAs and shRNAs could yield enhancement of discrimination between the mutant and wild-type alleles in allele-specific gene silencing, and more interestingly that the introduced mismatches that conferred marked improvement of ASP-RNAi, were intensively present in a portion of the guide siRNA element, corresponding to the 'seed region' in microRNAs. Therefore, it is possible that disturbance of base pairing in the corresponding 'seed region' as well as the central position (participating in determination of cleavage site in target mRNAs) of guide siRNA element could greatly influence discrimination of target mutant alleles from wild-type alleles.

2294/T

Early experience with intrathecal rhIDU for spinal cord compression in MPS I patients. *P. Dickson[1], V. Muñoz-Rojas[2], R. Giugliani[2], D. Naylor[3], A. Chen[3], M. Passage[1], S. Le[1], A. Victoroff[1], A. Mlikotic[4].* 1) Dept Pediatrics, Harbor-UCLA Medical Ctr, Torrance, CA; 2) Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; 3) Dept Neurology, Harbor-UCLA Medical Ctr. Torrance, CA; 4) Dept Radiology, Harbor-UCLA Med Ctr. Torrance, CA.

Intrathecal enzyme replacement therapy with recombinant human α-L-iduronidase (rhIDU) reduces brain glycosaminoglycan (GAG) levels to normal and spinal meninges GAG levels by 57-70% in treated MPS I dogs.[1] Three patients with MPS I (Hurler-Scheie and Scheie types) and spinal cord compression age 13-39 y received 1.74 mg IT rhIDU monthly for 3-6 doses. One subject was enrolled in an ongoing clinical trial of IT rhIDU; two were treated off-study. One off-study patient was reported previously.[2] All subjects showed improvements in the signs and symptoms of spinal cord compression. Improvements included reduction in lower extremity pain in 2/2 subjects, improvement in numbness/tingling in 2/2 subjects, newfound ability to move the right leg in 1, improvement of pain and temperature asymmetries in 2/2, improved strength and range of motion in 1, and disappearance of ankle clonus in 1. MRI and evoked potentials did not change. Six-minute walk test improved for 1 subject and decreased for 1 subject; the third subject is non-ambulatory. IT rhIDU was well-tolerated by the subjects. There was one SAE (pneumonia), felt unlikely to be related to IT rhIDU. Other related AE were mild and/or self-limited. A CSF leukocytosis developed in 1 subject (peak 37 WBC) with no meningeal signs and responded to oral steroids. The subject also experienced elevation of CSF opening pressure which resolved. All CSF anti-iduronidase antibody titers were < 1 OD unit/μl. IT rhIDU appears to alleviate some of the signs and symptoms of spinal cord compression and is well-tolerated by subjects. Further study is needed, and a clinical trial is underway.

1. Dickson P., et al. Molec Genet Metab 2007
2. Giugliani R., et al. Abstract ASHG 2005.

2295/T

Nitisinone (OrfadinR) reduces the massive fractional excretion of homogentisic acid in alkaptonuria patients. *M. Kayser, W. Introne, K. O'Brien, I. Bernardini, R. Kleta, W. Gahl.* Human Biochemical Genetics Section, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Alkaptonuria (AKU), a rare metabolic disorder of impaired tyrosine catabolism, is due to deficiency of homogentisic acid oxygenase. An organic compound, homogentisic acid (HGA), accumulates and binds to connective tissue causing darkened urine, darkened cartilage (ochronosis), joint destruction, and cardiac valve deterioration. Homogentisic acid is actively secreted through organic anion transporters in the renal tubules at levels 3-4 times the glomerular filtration rate. In AKU patients, mean plasma levels of HGA are 6.6 ug/ml and urinary HGA excretion averages 4.2 grams per day, more than 100 times normal. Nitisinone (NTBC), a potent reversible inhibitor of p-hydroxyphenylpyruvic acid dioxygenase, was shown in two separate, small studies to reduce urine homogentisic acid excretion in AKU patients up to 95%. We measured urine and plasma HGA levels in 42 AKU patients enrolled in either a natural history or a long-term treatment trial evaluating the clinical efficacy of nitisinone. Plasma HGA, measured using an HPLC/UV method, was 0.355 ug/ml (0.148-0.815) in the 6 patients receiving nitisinone and 5.65 ug/ml (2.62-11.2) in the 36 patients not receiving nitisinone. and Urine HGA, measured using a stable isotope dilution GC/MS technique, was 9.9 mg/dl (1.13-24.8) in the nitisinone-treated patients and 255.4 mg/dl (42-585.9) in those not receiving nitisinone. The average fractional excretion of HGA was 276% (90-520) in the nitisinone-treated patients vs 422% (80-1328) in those not receiving nitisinone. In conclusion, nitisinone reduces the filtered load of HGA, resulting in decreased tubular secretion through the organic anion transporter systems and, consequently, decreased urine HGA excretion.

2296/T

Mapping Accessible Sites in Rod Opsin Transcripts for Post Transcriptional Gene Silencing Therapy. *R.T. Taggart[1,2], E.H. Yau[1,2], T.A. Kolniak[1,2], M.C. Butler[1,2], J.M. Sullivan[1,2].* 1) Ophthalmology, SUNY Buffalo, Buffalo, NY; 2) VA Western NY Medical Center, Buffalo, NY.

More than 140 different rhodopsin gene mutations are associated with autosomal dominant retinitis pigmentosa. To accommodate this diversity we employed a mutation independent strategy to reduce both the normal and mutant rhodopsin transcripts in heterozygous carriers with post transcriptional gene silencing agents (PTGS) while providing a modified rhodopsin transcript that is resistant to the PTGS agent. A major limitation in finding effective PTGS agents is the identification of accessible sites within the cellular mRNA. We utilized in silico predictive methods (m-fold & s-fold) and a novel reverse transcriptase based PCR method to map accessible ribozyme sites (MARS). The accessible sites were confirmed by competitive hybridization and studies of in vitro cleavage of transcripts by ribozymes in cell lines stably expressing rhodopsin and a reporter secreted alkaline phosphatase (SEAP). In silico analysis identified 10 candidates among 236 potential ribozyme cleavage sites (NUH). MARS analysis identified 22 sites including those predicted by in silico studies. The eight accessible regions of the rhodopsin transcript included 30 potential ribozyme cleavage sites. Four additional ribozyme sites were chosen, either from previous efficacy studies or because they resided within inaccessible regions. 34 sites were evaluated for ribozyme cleavage using a SEAP bicistronic reporter system. 18 of 34 sites showed significant knockdown of SEAP expression ($p<0.01$) with five of these sites providing robust knockdown of SEAP measures. Five ribozyme constructs were identified as lead candidates for further optimization prior to animal studies. By combining mRNA accessibility analysis with a cell based in vitro approach five very promising ribozymes were identified for human rhodopsin. As rod opsin has many mutations responsible for retinal degenerations, a robust ribozyme targeted against rhodopsin mRNA is of therapeutic interest.

2297/T

Mosaicism for a PKD1 gene mutation revealed through family genetic analysis for a living related donor transplant for autosomal dominant polycystic kidney disease (APKD). *P.W. Lunt[1], C. Dolling[1,2], Y. Patel[3], L. Meredith[4], Athena Diagnostics[5], A. Gardner[6], A. Connor[7], C. Dudley[7].* 1) Clin. Genetics Dept, St Michaels Hosp, Bristol BS2 8EG, UK; 2) (now at) W.Midlands Reg.Genet.Service, Birmingham Women's Hosp, Birmingham B15 2TG, UK; 3) Nat.Genet.Ref.Lab.(Manchester), St.Mary's Hosp., Manchester M13 0JH, UK; 4) Inst.Med.Genet., Univ.Hosp.of Wales, Cardiff CF14 4XW, UK; 5) Athena Diagnostics, Worcester, Mass. USA; 6) Bristol Genet. Labs.,Southmead Hosp., Bristol BS10 5NB, UK; 7) Richard Bright Renal Unit, Southmead Hosp., Bristol BS10 5NB, UK.

The 25yr-old HLA-matched sister of a 28yr female with renal failure due to APKD, inherited from their mother, wished to donate a kidney to her sister. Renal ultrasound and MRI scans were clear, but under age 30 yrs leave a 15% residual risk of APKD; too high to be considered as a donor. Mutation in two genes can cause APKD; 85% PKD1 on 16p; 15% PKD2 on 4q. Linkage analysis with close flanking markers was uninformative at PKD2 gene, but indicated a shared maternal allele at PKD1 gene. As PKD2 mutation would also tend to give a milder phenotype, this result suggested the clinically unaffected sister could share a PKD1 mutation, and be unsuitable as a donor. Full gene sequencing (Athena Diagnostics) was undertaken, revealing a nonsense mutation (Glu313X) in exon 5 of PKD1 gene in the affected sister and in the mother. Surprisingly, this was absent in DNA from the potential donor sister. However, the mutation appears at reduced dosage in the 50yr mother, who has multiple renal cysts, but is otherwise clinically asymptomatic. Renal scans in the grandparents had been normal, and on DNA testing neither carries the PKD1 mutation. The apparent conflicting genetic results are explained by somatic and germline mosaicism for the PKD1 mutation in the mother, which was confirmed on quantitative analysis of her leukocyte and buccal cell DNA. Recognition of this enabled a successful fully-matched sibling renal transplant. We recommend that mosaicism be considered in apparent 2-generation APKD families, and highlight the necessity for mutation identification in families considering related living donor transplantation.

2298/T

Significant correction of disease after postnatal administration of recombinant EDA in canine X-linked ectodermal dysplasia. *M.L. Casal[1], J.R. Lewis[1], E.A. Mauldin[1], A. Tardivel[2], K. Ingolde[2], M. Favre[3], F. Paradies[3], S. Demotz[3], O. Gaide[4], P. Schneider[2].* 1) School of Veterinary Medicine, Univ of Pennsylvania, Philadelphia, PA; 2) Department of Biochemistry, Univ of Lausanne, Switzerland; 3) Apoxis, SA, Switzerland; 4) Department of Dermatology and Venerology, Univ of Geneva, Switzerland.

X-linked hypohidrotic ectodermal dysplasia (XLHED) in man (MIM #305100; defect in ED1), developmental defect, is characterized by sparse or absent hair, missing and/or malformed teeth, and hypoplastic eccrine glands. There is significant morbidity and mortality in affected children due to hyperthermia caused by their inability to sweat and an increased risk of respiratory tract infection. Tooth abnormalities include delayed primary and secondary dentition and poor occlusion, conical tooth crowns (peg teeth), and oligodontia, which lead to difficulties with mastication, growth retardation, and speech impairment. The canine model of XLHED was used to study the developmental impact of EDA on secondary dentition, since the dog has an entirely brachyodont, diphyodont dentition similar to humans, and as opposed to mice that only have permanent teeth (monophyodont dentition). Also, clinical signs in XLHED humans and dogs are virtually identical, whereas several are missing in the murine equivalent. In our model, the genetically missing EDA was compensated for by post-natal intravenous administration of soluble recombinant EDA. Shirmer tear testing was used to measure lacrimation; mucociliary clearance was examined to assess pulmonary function; and a modified iodine-starch test was used to evaluate sweating. Untreated XLHED dogs have an incomplete set of conically shaped teeth similar to those seen in human patients with XLHED. After treatment with EDA, significant normalization of adult teeth was achieved in 4 of 5 XLHED dogs. Moreover, treatment restored normal lacrimation and resistance to eye and airways infections, and improved sweating ability. These results not only provide a proof of concept for a potential treatment of this orphan disease, but also demonstrate an essential role of EDA in the development of secondary dentition.

2299/F

Mitochondrial DNA mutations and polymorphism in idiopathic asthenozoospermic men of Indian origin. *R. Kumar[1], A. Bhat[2], R.K. Sharma[3], R.N.K. Bamezai[2], R. Dada[1].* 1) Anatomy, AIIMS, NEW DELHI, DELHI, India; 2) SCHOOL OF LIFE SCIENCES, JNU, New Delhi India; 3) army R and R hospital, delhi cantt.India.

Rakesh Kumar1, Audesh Bhat2, Sharma R K3, R N K Bamezai2,Dada R1 Department of Anatomy, AIIMS1, School of life sciences, JNU2, Army R & R Hospital New Delhi. India Background: Studies on sperm function especially motility turned attention to the possible role of sperm mitochondria in male infertility. There are no introns between genes but all exons, so every change in mitochondrial DNA (mt DNA) is potentially lethal to cellular respiration. During spermatogenesis sperms require energy for biosynthetic processes and motility. Inhibition of sperm OXPHOS and rearrangements to the mt. DNA genome can affect sperm function. As copy number of mitochondrial genome in sperm is far less than somatic cells, therefore slight damage to the mitochondrial genome results in impaired sperm function and infertility with less severe effect on other tissues and systems. Aims: The aim of this study was to identify point mutations which may be associated with human male infertility. Methods: The whole mitochondrial genome was isolated form sperm and blood and mutations were screened with the help of DNA sequencing. The semen and blood samples analyzed were obtained from 25 oligoasthenospermic idiopathic infertile men and 20 controls. Results: G to A transition was detected in ND4 gene at nucleotide position 11719 in sperm DNA of 19 cases and only 14 from blood DNA. Though this is a non-synonymous change, the aminoacid remaining the same. The polymorphism A750G, A4769G and A8860G has been found in all the semen as well blood DNA of the cases but only in 12 controls. A750G, A4769G are non-synonymous changes but A8860G polymorphism in ATPase 6 gene changes aminoacid threonine to alanine. Though A8860G is a known polymorphism in the Indian Subcontinent but it needs a relook as its frequency seems to be more in infertile men than in controls. Conclusion: Further studies are in progress to see the effect of these mutations on the ATP depletion and Reactive oxygen species (ROS) production.

2300/F

Male infertility due to congenital bilateral aplasia of the vas deferens: how should couples undergoing IVF/ICSI be offered genetic counseling? a case report. *Y.D. Nobre[1], M.B. Toralles[2], I. Gomy[3].* 1) Surgery/Urology, University of Sao Paulo, Ribeirao Preto, Brazil; 2) Medical Genetics, Federal University of Bahia, Brazil; 3) Medical Genetics,University of Sao Paulo,Ribeirao Preto,Brazil.

Couples attempting IVF/ICSI,whereas there is male infertility due to obstructive azoospermia,should be offered genetic counseling and testing for mutations in the CFTR gene,which causes both cystic fibrosis(CF)and congenital bilateral aplasia of the vas deferens(CBAVD).C-BAVD occurs in about 2% of infertile males and most of them are compound heterozygotes for mutations in the CFTR gene,more often the 5T allele in one copy and a CF mutation in the other one.In 20% of cases,CBAVD does not seem to represent a mild form of CF as these patients may have urinary tract anomalies.In those cases without renal anomalies and no CFTR mutations are found,the sweat test is useful to distinguish CBAVD patients. We present a case of a Brazilian infertile couple whereby the man had obstructive azoospermia with no further symptoms. Both deferens ducts were absent whereas both testis were topic. Testis biopsies showed normal spermatogenesis. Pelvis MRI revealed no seminal vesicles. Renal and urinary tract ultrasound showed no anomalies.Karyotype and hormones were normal.Genetic analysis for the most common CFTR mutations was carried out in the couple and no mutations were found.The sweat test was positive in the man and negative in the wife.Thus we indirectly recognized a low CFTR protein as the cause of CBAVD,which allowed us to offer the couple a more suitable genetic counseling.As the couple consented to undergo IVF/ICSI,we estimated the risk of a child with cystic fibrosis as 1/44 and the risk of a male child with both cystic fibrosis and CBAVD as 1/88. We also explained the higher risk of birth defects and genomic imprinting disorders with IVF/ICSI.Although preimplantation genetic diagnosis(PGD)is possible in order to select those embryos free of mutations,we do not know the type of mutation this couple may carry,and,as there are more than a thousand mutations in CFTR gene,it would be reasonable to ponder the benefits of avoiding disease transmission with the medical risks and the financial burden of PGD.

2301/F

Polymorphic marker analysis is more informative in polar body vs. blastomere based PGD. *P. Renbaum[1], T. Eldar-Geva[2], B. Brooks[2], E.J. Margalioth[2], E. Levy-Lahad[1], G. Altarescu[1].* 1) Medical Genetics Unit,Zohar PGD Lab Shaare Zedek Medical Center, Jerusalem, Israel; 2) IVF Unit, Shaare Zedek Medical Center, Jerusalem, Israel.

We performed a retrospective analysis of the level of informativity of our complete marker set for 28 diseases for polar body (PB) and Blastomere analysis. Of 319 polymorphic markers flanking 28 disease genes, 90% provided an informative PB assay in all families undergoing PGD, while only 33% of these markers were informative for blastomere analysis. We also analyzed families carrying five different disorders to assess their eligibility for PB vs. Blastomere PGD. Usage of three or more linked markers in conjunction with the gene mutation nearly eliminates misdiagnosis due to ADO, therefore minimal requirements for PGD were set at 4 informative assays flanking the disease gene. Data was divided into families with four or more informative assays (eligible) and families with less than 3 informative assays (ineligible). In 100% of families recruited for PB-PGD at least 4 assays were always informative. Of 12 families with Myotonic Dystrophy, only 5 families (41%) had four informative markers (11 tested) for blastomere PGD. Ten of 16 carriers of FRAXA premutations (62%) were eligible for blastomere PGD (14 markers tested), while only 3 out of 7 CFTR carrier families (42%) were eligible for blastomere PGD (15 markers tested). For Tay Sachs and Non Syndromic Deafness, of 15 and 14 polymorphic markers analyzed (respectively), no family had four fully informative assays (including mutation) for use in blastomere analysis, and a majority of these families had two or less informative assays, making them ineligible for blastomere PGD. Conclusions: PB-PGD enables diagnosis using multiple polymorphic markers for a significantly higher number of families compared to blastomere PGD. In X-linked diseases where only 3 alleles are analyzed, more families have enough informative markers for blastomere PGD analysis. In diseases with founder mutations and couples from isolated ethnic groups it may be more difficult to perform blastomere PGD due to high frequency of identical haplotypes in the couple.

2302/F

Does cryopreservation lead to an increased DNA-fragmentation Index (DFI) in human spermatozoa? *T. Winkle[1], P. Dietl[1], F. Gagsteiger[1,2], S. Köder[1], J. Eckert[3], M. Susa[3], N. Ditzel[1,2].* 1) ReproGen- Ulm, Ulm, Germany; 2) IVF- Zentrum Ulm, Ulm, Germany; 3) Diagnostik- Zentrum Ulm, Ulm, Germany.

Cryopreservation is often used to store human germ cells, especially spermatozoa. After thawing, these spermatozoa are examined in accordance with WHO-criteria as to concentration, motility and morphology before being used for assisted reproduction techniques (ART). However, sperm chromatin integrity is never examined, in spite of the fact that it has been demonstrated that spermatozoa with a high DNA-fragmentation index (DFI) lead to poorer ART outcome. So we examined whether the act of cryopreservation using liquid nitrogen leads to an increased DFI. On the one hand we stained a part of the native semen sample with Propidiumiodide (PI) according to a slightly modified protocol of the Nicoletti assay (Nicoletti et al. 1994) and analyzed the spermatozoa with a fluorescence activated cell sorter (FACS) to obtain the DFI. On the other hand the remaining part of the sample was frozen and stored in the gas phase of liquid nitrogen (-196°C) over night. After thawing the next day, the frozen samples were also stained with PI and measured in the FACS. In this preliminary study we analyzed semen samples of 15 patients so far. Until now we can find a significant correlation between cryopreservation and an increased DFI. According to our results we can say that there is a tendency that cryopreservation increases the DFI in human spermatozoa. Thus new possibilities of reducing the DFI in semen samples should be developed to increase the chances of fertilization for patients especially after cryopreservation of spermatozoa.

2303/F

Analysis of sperm and Blood mitochondrial DNA in pathogenesis of oligoasthenoteratozoospermia. *R. Dada[1], R. Kumar[1], R. Kumar[2], N.P. Gupta[2], R.K. Sharma[3].* 1) Anatomy, AIIMS, New Delhi, Delhi, India; 2) urology, AIIMS, New Delhi, Delhi, India; 3) Army R and R Hospital Delhi Cantt.India.

The mitochondria is the sperm midpiece are the energy generator for mammalian sperm. Sperm require a sufficient supply of adenosine triphosphate (ATP) from mitochondrial oxidative phosphorylation for normal function (delamirande E et al., 1992, Manfredi et al., 1997). In somatic cells, mitochondrial respiratory chain function depends on the coordinated gene expression of both the mitochondrial and nuclear genomes. Sperm count is decreasing at an alarming rate of 2% per annum for last 20 years. This relates to the remarkable decrease of semen quality among healthy young men. Male fertility largely depends on the quality of sperm production. Studies on sperm function especially motility turned attention to the possible role of sperm mitochondria, which produce large quantities of energy for biosynthesis and motility in sperm and are found in high concentration in sperm midpiece. Mitochondria have their own genome, which codes for proteins involved in the respiratory chain and oxidative phosphorylation. Mutation in mitochondrial DNA has been known to cause several neuromuscular diseases, cardiomyopathies but little is known of their role in pathogenesis of spermatogenetic arrest and impaired sperm motility is not known. In the present study we analysed mitochondrial mutation in blood and sperm mitochondrial DNA. The mitochondrial DNA mutation in sperm DNA was higher (32 substitutions) as compared to blood (20 substitutions). A comparison of the sequences of the above genes with a reference sequence revealed a total of 32 nucleotide substitutions in the sperm mtDNA but not in the DNA from the blood cells. Of the 32 substitutions in sperm DNA, 7 were in COI, 12 were in COII, 4 were in ATPase8, and 9 were in ATPase6. This may be due to free radical (ROS) mediated damage. As sperm has just 10-100 copies of mitochondrial genome, mutations in these result in early phenotype manifestation. In such case infertile men with OAT had high mtDNA mutation. The mean sperm count in cases with mtDNA mutation was 2.23 millions /ml. The effects of these mutations to correlate the mitochondrial DNA mutation with phenotype are required. This will aid in providing comprehensive counseling and most adapted therapeutics to the couple.

2304/F

CYTOGENETIC FINDINGS IN WOMEN WITH AMENORRHEA. *R. Baez-Reyes, G. Razo-Aguilera.* Department of Genetics, National Institute of Perinatology, Mexico City, MEXICO.

INTRODUCTION: Amenorrhea is the absence or abnormal cessation of the menses, resulting from ovarian malfunction and the etiology include in some cases chromosomal alterations. OBJETIVE: To study the frecuency of the chromosomal abnormalities(CA) in women referred for counseling and karyotyping with primary amenorrhea(PA) and secundary amenorrhea(SA). METHODS; We report a cytogenetic study of 136 women with primary(87) or secundary(49) amenorrhea was performed at Genetics of Reproduction of the National Institute of Perinatology from January,1997 to June,2007. RESULTS: The frecuency of the CA in primary amenorrhea was 13.2% and secundary amenorrhea was 7.35%. Numerical alterations in the karyotypes observed were: 45,X ; 47,XXX ; X mosaicism (45,X/46,XX ; 45,X/46,XX/47,XXX ; 46,XX/47,XXX ; 46,XX/47,XXX/48,XXXX), Y mosaicism (45,X/46,XY). The presence of 46,XY female condition in 3 cases detected to be associated with PA. The structural chromosomal anomaly were: X,autosomal translocations (X;1, X;4, X;6 and X;8), one reciprocal translocation: t(2;8) and other alterations: 46;Xi(Xq) and mos 45,X/46,Xi(Xq). CONCLUSION: The present study show the importance of the karyotype in the investigation of the causal factor in the evaluation of patients with amenorrhea for a early diagnosis and the possibility of treatment.

2305/F

Polymorphisms in The One-Carbon Metabolism Pathway Genes, Are Associated With Increased Risk For Trisomy 21. *O. Reish[1,2], Y. David[1], E. Manor[3], M. Frydman[2,4], D. Chapman Shimshoni[1].* 1) Medical Genetics Institute, Assaf Harofeh Medical Center, Zerifin,Israel Israel; 2) Sackler School of Medicine,Tel Aviv University, Tel Aviv; 3) The Genetic Institute, Soroka Medical Center and Ben Gurion University, Beer Sheba; 4) The Danek Gertner Genetics Institute, Sheba, Medical Center, Tel Hashomer.

Aim: To evaluate the effect of polymorphisms in the One Carbon Metabolism (OCM) pathway genes, on maternal risk for trisomy 21. The contribution of folic acid was also evaluated. Materials and Methods: Samples included 44 trisomy 21-mothers and 133 controls. All subjects were Jewish, 14 trisomy 21-mothers and 82 control mothers were of Ashkenazi descent. Polymorphisms were analyzed following restriction digest of specific PCR amplicons. Results: Increased risk for trisomy 21 was associated with the Methylene tetrahydrofolate Reductase (MTHFR) 1298C allele (X2 5.6, p=0.009) and MTHFR 1298CC genotype (OR 17.8 95%, CI 2.65-119) among the Ashkenazim. Furthermore, the distribution of genotypic pairs of MTHFR A1298C and C677T alleles (X2 5.153, p=0.001) and pairs of MTHFR A1298C and Methionine synthase reductase (MTRR) A66G alleles (X2 4.68, p=0.09) showed a positive correlation with trisomy 21 pregnancies. In contrast, Methionine Synthase (MS) A2576G polymorphism did not show any association with the risk for trisomy 21 among the Ashkenazi women. In non-Ashkenazi women, the four polymorphisms in the OCM pathway did not show any significant association with the risk for Trisomy 21. Folic acid supplementation significantly reduced the risk for trisomy 21 (OR 4.66 95%, CI 2.24-9.8). Conclusion: This study presents for the first time evidence that MTHFR A1298C polymorphism is a risk factor for Trisomy 21 in Ashkenazi women. The risk for trisomy 21 increases when additional OCM pathway polymorphisms are present in the same individual. Small sample size, non homogeneity in the study group, different genetic background or environmental factors, are possible explanations. Folic acid supplementation reduced the risk for Trisomy 21, probably through replenishment of enzyme activity in the OCM pathway.

2306/F

Systematic review and meta-analyses of preterm birth genetic association studies. *S.M. Dolan[1], M. Merialdi[2], A. Pilar[2], T. Allen[2], B.K. Lin[3], J. Eckardt[9], M.J. Khoury[4], J.P. Ioannidis[5], L. Bertram[6], M. Hollegaard[7], D.R. Velez[8], R. Menon[9].* 1) Albert Einstein College of Medicine; 2) World Health Organization; 3) March of Dimes; 4) Centers for Disease Control and Prevention; 5) University of Ioannina School of Medicine; 6) MassGeneral Institute for Neurodegenerative Disease; 7) Statens Serum Institut; 8) Vanderbilt University; 9) The Perinatal Research Center.

Preterm birth (PB) is a major public health concern with rates over 12% and rising in many parts of the world. Studies reporting associations between single gene variants and PB have been hampered by varying definitions of PB, small sample sizes and population admixture. The challenge of identifying robust associations between genetic variation and susceptibility to PB is enormous. A systematic review and continually updated online field synopsis will provide the cumulative evidence on genetic associations with PB. Such associations can be deemed more robust if they are based on large-scale evidence, extensively replicated, and free of bias. Many criticisms of studies of complex diseases can be avoided if systematic review allows pooling of data and meta-analysis that can reveal true associations. In conjunction with the Human Genome Epidemiology Network (HuGENet), members of the Preterm Birth International Collaborative (PREBIC) conducted a systematic review of the literature on genetic associations in PB. Medline and Embase were searched using a sensitive search strategy to identify all appropriate studies published since 1/1/90. 5421 titles were identified and abstracts reviewed according to a set of inclusion and exclusion criteria. We selected 88 abstracts and obtained full text articles, thereby cataloging all genetic association studies published in the field of PB to date. Where sufficient data exist, we will conduct meta-analyses on specific gene variants. Concurrent with the research, an online summary of the data will be placed online to allow continued updating of data. This work will facilitate research in PB and, like *AlzGene* (www.alzgene.org), can be a model for other fields to integrate cumulative evidence on genetic association studies.

2307/F

Heritability of female reproductive characteristics in a population exposed to polybrominated biphenyls. *K.C. Taylor[1], C.M. Small[1], M.P. Epstein[2], M.L. Terrell[1], M. Marcus[1].* 1) Epidemiology, Emory University, Atlanta, GA; 2) Human Genetics, Emory University, Atlanta, GA.

We investigated whether exposure to polybrominated biphenyls (PBBs), which are hormonally active environmental contaminants, affected the heritability of reproductive characteristics in an exposed population. Using a cohort comprised of 373 families with variable PBB exposure, we estimated the heritability of self-reported age at menarche and menstrual cycle length using the software package MENDEL and further assessed whether such heritability estimates differed among high-exposed and low-exposed families. We limited the menarche analysis to those who reported age at menarche between 9 and 16 years inclusive (N=1045 of 1057 women who reported an age at menarche). Consistent with other studies, age at menarche was heritable in our population (heritability=0.53±0.05). We found evidence of additive genetic effects only (dominance effects were not significant). After stratifying by PBB exposure, heritability was somewhat higher in the low-exposed group than in the high-exposed group (0.63±0.08 vs. 0.46±0.07). For menstrual cycle length, we limited the analysis to premenopausal women who were not using hormonal contraceptives (N=544), and reported having a standard menstrual cycle length (17-43 days) in the past year (N=521 of 544). After controlling for age at interview, we found menstrual cycle length to have an estimated heritability of 0.26 (±0.11). Again, we found evidence of only additive genetic effects and no dominance effects. Heritability of menstrual cycle length was similar in the low-exposed and the high-exposed groups (0.20±0.16 vs. 0.30±0.15). To our knowledge, no other studies have examined heritability of menstrual cycle characteristics. In conclusion, age at menarche was more heritable than menstrual cycle length in this population. PBB exposure may modify the effects of genetic factors on these reproductive outcomes.

2308/F

Polar Body Preimplantation Genetic Diagnosis (PGD) for a de novo mutation in the Duchenne Muscular Dystrophy (DMD) gene: Use of reverse linkage on polar bodies 1 and 2 for confirmation of mutation status in embryos. *G. Altarescu[1], B. Brooks[2], T. Eldar-Geva[2], E. Hadar[2], E.J. Margalioth[2], E. Levy-Lahad[1], P. Renbaum[1].* 1) Medical Genetics Unit, Zohar PGD Lab Shaare Zedek Medical Center, Jerusalem, Israel; 2) IVF Unit, Shaare Zedek Medical Center, Jerusalem, Israel.

We constructed a haplotype based on linked polymorphic markers in a family in which the female proband is a carrier of a new missense mutation in the DMD gene for use in PGD analysis. Single cell diagnosis for PGD requires simultaneous analysis of multiple linked polymorphic markers in addition to mutation analysis in order to reduce misdiagnosis due to allele drop out (ADO). Linkage analysis requires building a family haplotype spanning at least two generations. We present a couple, in which the female was a symptomatic carrier of a new mutation in the DMD gene (T1055G), precluding linkage prior to the PGD cycle. 24 polymorphic markers were identified flanking the DMD gene in a region of less than 2 Mb. Of these, 14 markers (7 intragenic) were found to be informative in this couple, and the maternal and paternal alleles of the proband were identified. Polar bodies 1 (PB1) and 2 (PB2) were biopsied and eight markers together with the familial mutation (mapping in the order DMD-TTTC, DMD-CT, DXS1214, DXS1036, DXS1219, T1055G, DXS1238, DMD-AT, DMD-GAAA) were amplified in a multiplex PCR reaction, followed by hemi-nested fluorescent PCR analysis. The T1055G familial mutation was detected by sequencing and restriction enzyme digestion, and both PB1 and PB2 results were used to link the mutation to the affected maternal allele. Of 7 retrieved oocytes, 4 extruded PB1. Two PB1s were homozygous for all 8 markers and the mutation, each for a different maternal allele, one carried the wild type 1055T sequence, while the other carried the 1055G mutation. 2 of the 4 oocytes fertilized, and analysis of the hemizygous PB2s confirmed the mutation linkage observed in the 2 homozygous PB1s. Both embryos were mutation carriers, and therefore neither was transferable. Concomitant analysis of PB1 and PB2 is a powerful tool for reverse linkage analysis in cases of de novo mutations for maternal autosomal dominant and X-linked disorders.

2309/F

Stillbirth: A multifactorial problem. *E. McPherson, C. Cold.* Marshfield Clinic, Marshfield, WI.

We have reviewed 50 consecutive stillbirths occurring over a 4.5 year period in a community hospital which accepts referrals from the surrounding rural area. In 40 (80%) of cases, a protocol including maternal record review, dysmorphology evaluation, placental pathology, karyotype, autopsy and, when indicated, maternal thrombophilia testing led to identification of at least one cause for fetal death. Since only 2/10 with unknown cause were fully evaluated, it is possible that more complete application of the protocol may have led to more identified causes. Causes of stillbirth are typically classified as fetal, cord/placental, and maternal, but in reality, the welfare of the fetus, placenta and mother are inextricably connected. Overall 16/50 (32%) of stillbirths had more than one causative anomaly. Of the 22 fetuses with major anomalies, 13 also had placental abnormalities contributing to fetal death. Since placental pathology was omitted in 6 cases with known chromosomal abnormalities expected to affect the placenta, the true total may have been greater if complete evaluation was done. Conversely, among the 23 with major placental anomalies, 15 had major fetal anomalies and/or PROM / preterm labor contributing to fetal death. Among 5 cases with well-documented cord constriction, two fetuses had amniotic bands and all 5 cords had preexisting malformations. Of 8 cases in which preterm labor or PROM preceded fetal death, half had cord or placental anomalies, 2 more had borderline small placentas, and 1 had both gastroschisis and placental abruption. The mothers of the stillbirths have a prior history of poor pregnancy outcomes with 38/98 (39%) of previous pregnancies ending in miscarriage or stillbirth. 7/50 mothers have thrombophilia and 4/50 have diabetes. Systematic investigation led to recognition of several families with increased recurrence risk including an inherited chromosome translocation, a family with autosomal dominant partial malrotation predisposing to volvulus, and a family with recurrent extra-long umbilical cord in 3 sibs. In hindsight, the majority of mothers of stillborns have pre-existing risk factors. With more careful prenatal care for high risk women, prevention may become possible.

2310/F

Evaluation of Critical Genetic Variation in Idiopathic Recurrent Miscarriages among South Indian Women- a Genomic and Proteomic Approach. *L. Rao[1], V. Suryanaryana[1], M. Kanakavalli[1], V. Padmalatha[1], T. Raseswari[1], D. Mamata[2], S. Lalji[1].* 1) E409,Centre for Cellular and Molecular Biology, Hyderabad, India; 2) Infertility Institute and Research Centre, Hyderabad, India.

Title: Evaluation of Critical Genetic Variation in Idiopathic Recurrent Miscarriages among South Indian Women- a Genomic and Proteomic Approach Lakshmi Rao1, Venkata Suryanarayana1, Kanakavalli Murthy1, Venkata Padmalatha1, T Raseswari1, Mamata Deenadayal2, Lalji Singh 1 1. Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India 2. Infertility Institute and Research Centre, Hyderabad, India 3. Corresponding author Statement of purpose Recurrent early pregnancy loss (REPL) is defined as three consecutive first-trimester miscarriages. Reasons include increased maternal age, genetic, anatomical, immunological, endocrine and environmental or life style factors like smoking, caffeine-intake, alcohol, drug-intake and stress. We present genomic and proteomic analysis of women with idiopathic REPL to find etiological factors involved. Methods Well-defined REPL subjects (n= 200) and control women (n=99) were included for genotyping studies. For platelet proteome analysis, 25 cases and 10 controls were included. Polymorphisms in detoxification genes (NAT,NAT2,CYP1A1,CYP2D6,SULT1A1,CYP19,aryl hydrocarbon receptor, aryl hydrocarbon receptor repressor), vasoregulatory genes (eNOS,VEGF, anadamide hydrolase), genes involved in blood homeostasis (Prothrombin,Factor V Leiden) were analyzed and their significance was estimated using a two tailed Fisher's P-value at 95% significance level. Platelet proteome-analysis was carried out after isolation and solubilization of platelets. Summary of results: Polymorphisms in CYP1A1 (C4887A and T6235C) showed significant association with REPL as evidenced by logistic regression and haplotype analysis. Novel variants in CYP2D6,anadamide hydrolase,eNOS were associated with REPL. Proteomic analysis of platelets revealed a differential expression pattern in three proteins with approximate molecular masses of 65kDa, and 20kDa which were present in REPL women.

2311/F

Association of Long Polyglycine Tracts (GGN repeats) in Exon 1 of the Androgen Receptor Gene with Cryptorchidism and Penile Hypospadias in Iranian Patients. *R. Radpour[1], M. Rezaee[2], A. Tavasoly[3], S. Solaty[3].* 1) Department of Reproductive Genetics, Reproductive Biomedicine Research Center of Royan Institute, Tehran, Iran; 2) Department of Nanotechnology, Avesina Research Institute, Beheshti University, Tehran, Iran; 3) Department of Urology, Biomedical Research Center of Military University of Medical Sciences, Tehran, Iran.

Hypospadias, located urethral orifice along the ventral side of the penis, and cryptorchidism, failure of the testes to descend into the scrotal sacs, are the two most common congenital malformations in males affecting 0.3-0.7% and 2-4%, respectively, at birth. To study the association of CAG/GGN trinucleotide repeats in the androgen receptor gene with cryptorchidism and hypospadias in Iranian population we performed a case-control study of 76 cryptorchid and 92 hypospadiac (divided into subgroups of glanular, penile, and penoscrotal hypospadias) Iranian males. The length of the CAG/GGN repeat segment was evaluated by using PCR-sequencing in exon 1 and PCR-SSCP in exons 2-8. There were no significant differences in CAG lengths between the cases and controls but GGN numbers were found to be significantly higher (median 24 vs. 22) among both subjects with penile hypospadias (P = 0.018) and those with a history of cryptorchidism (P = 0.001), compared with controls. In addition, the GGN numbers among subjects with penile hypospadias were significantly different, compared with the two other subgroups of hypospadias (P = 0.001). We were able to identify 12 different CAG alleles and 8 different GGN alleles in the cryptorchid group. The mean GGN repeat length increased with the cryptorchidism and penile hypospadias. However, statistical analysis showed no significant differences between the cryptorchidism subgroups (bilateral or unilateral) (P > 0.05). The distribution of GGN allele frequencies was different between cryptorchid men and controls and there was an apparent trend toward a shift to GGN = 22 or GGN > 22 in males with cryptorchidism, but no significant difference was observed with hypospadiac group respect to controls.

2312/F

Comprehensive genetic analyses using a modified whole genome amplification protocol and microarrays to identify genetic disorders and determine embryo implantation from single cells. *W.G. Kearns[1], R. Pen[1], A. Benner[1], E. Widra[2], R. Leach[1].* 1) Shady Grove Center for Preimplantation Genetics, Rockville, MD; 2) Shady Grove Fertility Reproductive Science Center, Rockville, MD.

Purpose: To optimize molecular genetic experimental techniques to successfully amplify DNA from single cells and perform a complex genetic analysis from preimplantation embryos using microarrays.

Design: Prospective study

Methods: A modified whole genome amplification protocol was performed on 185 single cells (3pns, 2pns, white blood cells (wbcs) and cell lines) to optimize DNA extraction and amplification protocols from single cells. We used invariant DNA genomic loci for each chromosome arm to ensure the entire genome was amplified and TaqMan PCR to ensure heterozygous allele amplification. A modified microarray analysis using single nucleotide polymorphisms (snps) was employed to determine total numerical chromosome abnormalities, structural chromosome aberrations, to identify what partner provided the extra aneuploid chromosome, to determine what embryo implanted, to identify epigenetic changes, and to identify single gene or mitochondrial disorders.

Results: Our initial results showed a genomic coverage > 75% with heterozygous allele detection in 60% of cells. The detection rate ranged from 63.9% to 78.1% and a genotype call rate from 42.2% to 48%. Experimental modifications on wbcs and 2pns increased our genomic coverage to > 98% with heterozygous allele detections > 90%. Our detection rate ranged from 95% to 98.1% and a genotype call rate of 95% to 96%.

Conclusion: Using an optimized whole genome amplification protocol and DNA microarrays, we can successfully provide a comprehensive genetic analysis on single cells.

2313/F

Telomere length is increased in women with premature ovarian failure. *K.L. Bretherick[1,2], C.W. Hanna[1,2], M.R. Fluker[3,4], W.P. Robinson[1,2].* 1) Dept of Medical Genetics, University of British Columbia; 2) Child & Family Research Institute; 3) Genesis Fertility Center; 4) Dept of Obstetrics & Gynecology, BC Children's and Women's Hospital; Vancouver, BC, CANADA.

Women with premature ovarian failure (POF) experience menopause before the age of 40, and may therefore be considered prematurely reproductively aged. Rate of reproductive aging may be related to overall rate of aging, a suggestion that is supported by human epidemiologic studies reporting that late child bearing is associated with longer lifespan. We therefore hypothesized that indicators of cellular aging, such as short telomere length, would be more common in women with POF than in controls. DNA was obtained from peripheral blood of POF patients (N=54), control women between the ages of 17 and 55 (control group 1, N= 92), and women who have had a healthy pregnancy after the age of 37 and have not had a miscarriage (control group 2, N=41). Average telomere length was determined by quantitative PCR amplification of the telomeric repeat expressed relative to amplification of a single copy gene. Surprisingly, age-adjusted mean telomere length was significantly longer for POF patients than both control groups (0.968 for POF patients vs. 0.893 for control group 1 and 0.931 for control group 2, p=0.02, two tailed ANCOVA). There is evidence that estrogen exposure may positively affect telomere length, and we have previously reported that haplotype at the estrogen receptor alpha gene (*ESR1*) was significantly associated with risk for POF. However, there was no association between telomere length and genotype at *ESR1* or genotype at a polymorphism in the estrogen receptor beta (*ESR2*) gene in either patient or control group, or in all data combined. Longer telomeres may nonetheless be the result of abnormal hormone exposure in this group, either due to elevated estrogen levels as a result of recruitment of large cohorts of follicles prior to POF onset, or as a result of hormone replacement therapy following diagnosis. Regardless, the increase in telomere length observed in POF patients does not support a pathogenic role for an increased rate of cellular aging in POF.

2314/F

A human/bovine comparative approach to identify transcripts related to oocyte maturation: from fertility to aging. *C. Laperuta[1], C. Carbone[1], M. D'Urso[1], B. Lioi[2], M.V. Ursini[1], M.G. Miano[1].* 1) Institute of Genetics and Biophysics "A. Buzzati Traverso", National Research Council, Naples, Italy; 2) Department of Animal Production Sciences - University of Basilicata, Potenza, Italy.

Decline aging-oocyte competence and premature ovarian failure (POF) are common factors in human female infertility. In both conditions, follicular senescence and ovarian dysfunctions occur at different speed. Understanding how an immature oocyte transforms into an egg during oocyte maturation is critical for knowledge of fertility and decline-oocyte aging. This process guides the achievement of the final competence essential to fertilization and zygote division, through evolutionary conserved nuclear and cytoplasmatic events. The maturation of mammalian oocyte requires a co-ordinated programme of gene expression events that itself regulates the development of ovarian follicles. We studied several cases of POF and using microsatellite analysis we narrowed the extension of an interstitial deletion, which resulted to be located between DXS1187 and DXS1073. In this region mmany candidate gene for this disease are present. Therefore, starting from selected genes involved in POF disorders and oocyte aging, we are carrying out a characterization the expression level of bovine ortologous of genes located in the POF locus during oocyte maturation. The rationale for this analysis was that cow constitutes the best surrogate model for studying reproductive human disorders given the great similarity between the two reproductive systems. We assayed systematically the expression level of each transcript in bovine oocytes at different stage of maturation (immature-MI stage and mature-MII stage) coming from young (12 months old) and older animals (13 years old). Oocyte pools (20-100) were collected by puncturing follicles from ovaries of cows and RNA extraction methods were carried out using high recovery rate methods. Our data identified few transcripts specifically expressed during meiosis I and II. We believe that they may represent a good starting point to investigate on the presence of specific mRNAs in the former maturation stages and assay how they vary during decline-competence in aging oocyte.

2315/F

Augmented androgen production in Polycystic Ovary Syndrome: Genetic assessment in an Indian cohort. *A. Maitra, M.K. Pusalkar, J.S. Gokral, C. Saravanan, P.K. Meherji.* Molecular Endocrinology, National Inst. For Research in Reproductive Health, Mumbai, Maharashtra, India.

Polycystic ovary Syndrome (PCOS) is a common cause of infertility in females, affecting about 5-10% of women worldwide. The syndrome is known to have a complex multigenetic basis. However genetic variations underlying it have still not been defined. Available evidence through in vitro cultures as well as global gene expression profiling of theca cells from PCOS ovaries suggests involvement of promoters of two genes in androgen pathway viz. CYP11A1 and CYP17 in augmentation of androgen production as seen in the syndrome. Present study aims at identifying genetic variants in these promoters vis a vis their association with raised androgen levels. A pentanucleotide repeat (tttta) polymorphism in CYP11A1 and a T>C polymorphism in CYP17 were screened. A cohort of 97 consecutively identified Indian women with PCOS were studied along with 45 age and BMI matched controls. Diagnosis of PCOS was based on the consensus definition specified in Rotterdam Conference (2003-2004). Androgen profile included Testosterone, Androstenedione, DHEAS and 17- Hydroxyprogesterone. Promoters for CYP11A1 and CYP17 were screened by PCR-sequencing from genomic DNA. Testosterone and Androstenedione levels were significantly increased in the PCOS subjects compared to controls ($p<0.05$). The increase was associated with T>C polymorphism in the CYP17 promoter. The polymorphic allele was also significantly higher in frequency in the PCOS group compared to controls. CYP 11A1 and CYP17 were both found to be polymorphic in about 50% of the PCOS cases, which was significant compared to controls. Their association with raised androgens was also seen. The study for the first time reports allelic frequencies of the CYP11A1 and CYP17 promoter variants in an Indian cohort. Association of these variants with PCOS and raised androgens is also highlighted from this screening of a limited, but defined group of women.

2316/F

Mutational analysis of UBE2B in men with dyskinetic spermatozoa. *A. Moore[1, 2], E. Escudier[2], L. Wakselman[1], P. Duquesnoy[1], A-M. Bridoux[1], M. Albert[3], S. Amselem[1], D. Escalier[3].* 1) Inserm U654, Hopital A Trousseau, Paris, France; 2) Inserm U841, Hopital H Mondor, Créteil, France; 3) AP-HP, Hopital Bicetre, Le Kremlin-Bicetre, France.

Several knockout mice have revealed the involvement of factors potentially linked to the ubiquitin/proteasome system in the assembly of sperm flagellar structures in mammals. Among them, mice deleted of the ubiquitin conjugating enzyme Ube2b present a unique sperm flagellar phenotype characterized by an ectopic localization of the longitudinal columns of the fibrous sheath. A similar sperm flagellar phenotype exists in some infertile men, but its molecular basis is still to be identified. The data on Ube2b reported in mice, therefore, prompted us to analyze the orthologous UBE2B gene in those patients. Five patients with a sperm phenotype similar to that of Ube2b-/- mice were investigated. Direct sequencing of all UBE2B coding exons and exon/intron boundaries did not reveal any mutation. The study of further patients is therefore needed to determine whether mutations of UBE2B can concern some men with anomalies of the longitudinal columns of the fibrous sheath.*Ube2bUBE2B.*

2317/F

***SLC26A3* and *CFTR* variants in men with unknown infertility.** *S. Wedenoja[1,2], O. Hovatta[3], J. Toppari[4], C. Holmberg[2], J. Kere[1,3], P. Höglund[1].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; 3) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden; 4) Department of Physiology, University of Turku, Turku, Finland.

A rare autosomal recessive disease congenital chloride diarrhea (CLD) is caused by mutations in the solute carrier family 26 member 3 (*SLC26A3*) gene. It is located on chromosome 7q22-31.1, near the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, and it encodes for an apical epithelial Cl^-/HCO_3^- exchanger. As for duodenum and ductal systems, interaction between the STAS domain of SLC26A3 and the R domain of CFTR stimulates the activity of both transporters, resulting in increased epithelial secretion of HCO_3^- and fluid. SLC26A3 and CFTR show highly similar expression profiles at multiple sites of the male reproductive tract. CLD-males with the homozygous Finnish founder mutation V317del are subfertile, possessing a low concentration, and poor motility and morphology of sperm, high seminal plasma Cl^- with a low pH, and spermatoceles. Although the major cause of male infertility in cystic fibrosis is absence of the vas deferens, a low seminal plasma pH and poor sperm quality emerge. The mildest manifestation of homozygosity, or even heterozygosity, for *CFTR* mutations is male infertility. We aimed to study whether *SLC26A3* variants - alone or together with those of *CFTR* - are associated with unknown male infertility. Direct sequencing of *SLC26A3* exons in men with unknown infertility (n=138) and controls (n=211) revealed 7 novel heterozygous variants: 2 in the promoter and 5 in exons. Among males with unknown infertility, an increased frequency of carriership of any *SLC26A3* variants (p=0.008), or coding variants (n=4) alone (p=0.03) or together with one 5T allele of *CFTR* (p=0.06) emerged. *CFTR* mutations were excluded by appropriate assays. *SLC26A3* variants may be associated with idiopathic male infertility. Functional role of the novel variants is unknown. One infertile man carried, however, both 5T allele of CFTR and V317del mutation for CLD, which both account, in a homozygous form, for male subfertility.

2318/F

Role of dyneins in genetic asthenozoospermia. *D. Zuccarello, A. Ferlin, C. Vinanzi, C. Cazzadore, C. Foresta.* Department of Histology, Microbiology and Medical Biotechnologies, Centre for Male Gamete Cryopreservation, University of Padua, Italy.

The asthenozoospermia (AZS) is a common cause of male infertility characterized by a reduced (progressive motility <50%) or absent sperm motility in fresh ejaculate. The genetic type is a very rare heterogeneous condition. To clarify the role of genetic factors in isolated AZS we analyzed 3 candidate genes for non syndromic AZS, DNAI1 (9p21-p13), DNAH5 (5p15) e DNAH11 (7p21), codifying for 3 proteins belonging to axonemal dyneins cluster, particularly expressed in testis and trachea. In detail, we analyzed 20 exons from DNAI1, 9 out of 79 exons from DNAH5 and 2 out of 82 from DNAH11 in 70 patients affected by isolated AZS. By direct sequencing and DHPLC analysis we have identified 4 heterozygote sequence changes never described: R663C in DNAI1, E1756K and E2666D in DNAH5, and I3040V in DNAH11. Moreover, other 3 known sequence changes were detected: A8S and V335I in DNAI1 and R3004Q in DNAH11. We tested 200 controls (normospermic men) and we found the E1756K with a frequency of 1%; the R3004Q with 2%; the A8S with 9,5%; the V335I with 6%. We never found mutations R663C, E2666D and I3040V in the control subjects. The presence of E1756K, R3004Q, A8S and V335I in control subjects, suggests they are common polymorphisms. Mutations E2666D, located in the exon 48 and codifying for AAA-3 domain, and I3040V, located in the exon 55 downstream to AAA-4 domain, are extremely conserved during the evolution, indicating the crucial role of these aminoacids in the function of core. Also the mutation R663C, located in WD5-repeat, which is critical for propeller-structure assembly, is conserved in the superior species. By electronic microscopy of ejaculated spermatozoa's tails from patients carrying the mutations R663C (1 patient), I3040V (in 2 unrelated patients) and E2666D (1 patient), we discovered an altered axonemal structure with severe disorganization of microtubules, abnormal dynein outer arm and central pair. The obtained results are very prominent cause of these patients are involved in assisted reproductive programs with probable chance of disease's transmission to the offspring.

2319/F

Preimplantation genetic screening (PGS) for aneuploidy in couples undergoing donor egg *in vitro* fertilization cycles. *A. Benner[1], R. Pen[1], P. Kearns[1], P. Browne[2], W.G. Kearns[1].* 1) Shady Grove Center for Preimplantation Genetics, Rockville, MD; 2) Shady Grove Fertility Reproductive Science Center, Rockville, MD.

Purpose: Preimplantation genetic screening (PGS) is used in an effort to decrease spontaneous miscarriage, prevent the birth of aneuploid offspring and to increase the delivery rate by removing genetically abnormal embryos from the pool of embryos to be transferred. Most currently available information regarding aneuploidy rates among human preimplantation embryos comes from PGS of embryos from patients with impaired fertility. PGS for aneuploidy is not a common recommendation for the donor egg patient group, but we and others have demonstrated preliminary evidence that aneuploidy rates in this patient group exceeds 40% of the embryos analyzed. Therefore, we determined aneuploidy rates in 61 couples undergoing donor egg IVF cycles.

Methods: Sixty-one couples underwent donor egg IVF-PGS due to poor outcomes from prior fertility therapy. Laser-assisted embryo biopsy was performed on day-3 and PGS was done on 797 cleaving embryos from 61 initiated cycles. The mean donor age was 26.5 years (range of 21 to 31). Multi-color fluorescence *in situ* hybridization (FISH) was used to determine aneuploidy for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. Hybridization, stringency washes and fluorescent microscopy was performed according to routine laboratory protocols. Clinical outcomes of these cycles were determined.

Results: All 61 women had an embryo transfer. Five percent (40/797) of the embryos were not diagnosed due to poor blastomere quality. Fifty-one percent (386/757) of the analyzed embryos were abnormal for at least one of the 10 chromosomes tested. The clinical pregnancy rate was 77% (47/61) per patient and per embryo transfer. There were no miscarriages, misdiagnosis, or mosaic embryos.

Conclusions: This study from donor egg cycles provides insight into the presence of aneuploidy in a low risk population. Pregnancy rates were similar in these patients to those undergoing donor egg IVF without PGS.

2320/F

Preimplantation Genetic Diagnosis (PGD)/Screening (PGS) in carriers of structural chromosome abnormalities: The Genzyme Genetics experience. *A. Hajianpour, L. Dong, B. Huang, B. Herbert, D. Burkhardt, S.Y. Kou, Q.Q. Huang, R. Habibian.* Dept Cytogenetics, Genzyme Genetics, Monrovia, CA.

PGD/PGS is now an established procedure for the detection of single gene disorders by PCR, and chromosome aneuploidy /rearrangements by FISH, in cleavage stage blastomeres. Reciprocal translocations are the most common form of chromosome abnormalities observed (1 in 500 live births), followed by Robertsonian translocations and inversions (1 in 1000 each). There has been an increase in demand for PGD/PGS by patients who carry chromosome rearrangements in order to increase their chances of normal pregnancies. Genzyme Genetics is one of the leading laboratories performing PGD/PGS by FISH. A retrospective data analysis performed on 51 couples carrying chromosome rearrangements, using blastomeres fixed on slides, are presented here. When applicable, we used three differentially labeled probes: two subtelomere probes appropriate to the chromosome arms involved in the rearrangement, combined with a centromere probe (or any other probe mapping proximal to the breakpoints). When differentially labeled centromere or proximal probes were not available, we performed two sequential hybridizations with subtelomere probes specific to each arm of the chromosome involved. Using these strategies it is possible to identify all 16 segregation products of reciprocal translocations, all six possible outcomes of Robertsonian translocations, and all major recombinant products of inversions, excluding the recombinant products within the inversion loop. We perform pre-PGD chromosome and FISH analysis on all couples with chromosome rearrangements unless they have been tested by our laboratories previously. Using this protocol we have identified two discrepant results. Therefore, it is recommended that laboratories performing PGD/PGS for chromosomal rearrangements also perform cytogenetic and FISH analysis on parental blood. This is to confirm the rearrangements and identify any possible polymorphisms (by FISH) in order to select the most appropriate probes for PGD/PGS analysis.

2321/F

Direct and indirect mutation analysis for preimplantation genetic diagnosis (PGD) of cystic fibrosis (CF). *B. Tazon-Vega, A. Victor, C. Zhang, O. Davis, S. Spandofer, K. Amoroso, Z. Rosenwaks, KP. Xu.* Center for Reproductive Medicine and Infertility, Weill Cornell Medical College, New York, NY.

CF is a common severe autosomal recessive disorder. Misdiagnosis may occur when PGD for CF is performed by mutation detection only due to allele dropout (ADO) from single cells and it is not applicable to couples carrying unknown mutations. Linkage analysis using polymorphic markers can indirectly diagnose the disease while also assessing ADO. Our objective was to provide more reliable and suitable PGD for most CF couples. Linkage analysis for the couple and available relatives is performed prior to PGD with 1 flanking marker and 3 intragenic markers of the *CFTR* gene: *D7S522*, *IVS1CA*, *D7S677*, and *IVS8CA*. This analysis determines the paternal and maternal haplotypes linked to CF. Informative markers are tested by co-amplification (with the mutation if known) from single peripheral blood lymphocytes prior to IVF-PGD treatment. During IVF-PGD, single blastomeres are obtained on day 3 after fertilization, a multiplex PCR reaction of the informative markers and the mutation is carried out followed by individual nested PCR; amplified products are run on a genetic analyzer. This approach has been applied to 3 couples in our center, 2 of which have both members carrying the ΔF508 mutation. The paternal mutation for the third couple was ΔF508 and the maternal unknown. Amplification efficiency for all markers was 90% and ADO was 10% for lymphocytes. Overall 26 embryos were biopsied obtaining a total of 30 blastomeres. Six embryos were diagnosed as non-carriers, 12 carriers, and 7 affected. One embryo had an inconclusive diagnosis due to ADO. Two embryos were transferred for each couple resulting in one ongoing and one biochemical pregnancies. It is worth noting that in the couple with an unknown maternal mutation, of the 11 embryos analyzed 6 would have been considered non-transferable if microsatellite markers had not been used to distinguish the maternal chromosomes. Linkage markers flanking the *CFTR* gene allow for fully informative PGD in most CF couples even if they carry unknown mutations, thus reduce the risk of misdiagnosis.

2322/F

Clinical usefulness of array CGH in screening test or diagnosis of male infertility. *D. Cha[1], J. Kang[2], J. Park[3], K. Lee[1], S. Lee[1].* 1) Dept OB/GYN, Kangnam CHA Hosp, Seoul, Korea; 2) Macrogen, Seoul, Korea; 3) Dept OB/GYN, Bundang CHA Hosp, Korea.

Objective: The main purpose of this study is to examine the usefulness of array CGH to detect the genetic abnormality in patients with severe male factor infertility. Methods: 13 infertile men were diagnosed with cytogenetic assay and Y chromosome deletions. 10 sequence-tagged sites (STS) markers were used. These markers were amplified by performing 5 separate multiplex PCR reactions. Genomic DNAs were extracted for MacArray Karyo4000 array. Probe labeling, hybridization, and analysis CGH were performed according to the manufacturer's instructions. The relative copy number of chromosomal segments was analyzed with real-time quantitative polymerase chain reaction. Results: Eleven out of 13 patients had various abnormal karyotyping and seven patients had Y chromosome microdeletions. All the results of BAC-chip were identical to the conventional cytogenetic results in the numerical aberration such as Klinefelter syndrome. Also, it could identify the Y microdeletion (sY117, sY127, 143, sY134, sY138, sY152, sY147, sY149, sY269, sY157, and sY158). and was confirmed by the real-time quantitative polymerase chain reaction. Conclusion: Chromosomal abnormalities and deletions of Y chromosome can result in chromosomally derived infertility. These findings strongly recommend the necessity of genetic screening using array CGH in unknown origin of infertile patients.

2323/F

Quality assessment of spermatogenesis in treated hypogonadotropic hypogonadic (HH) men. *K. Krabchi, S. Chantot-Bastaraud, I. Berthaut, C. Ravel, P. Bouchard, S. Christin-Maitre, J. Mandelbaum, J.P. Siffroi.* Universite Pierre et Marie Curie- Paris 6, EA 1533, Paris, F75020, France.

Background: Hypogonadotropic hypogonadism is characterized by failure of gonadal function secondary to deficient gonadotropin secretion, resulting from either a pituitary or hypothalamic defect. The therapeutic use of commercially available FSH and LH appeared to be an adequate way to treat male infertility problems of these patients achieving an apparently normal spermatogenesis. To our knowledge, the quality of such induced spermatogenesis has never been investigated. Because routine semen analysis are not suitable for the measure of nuclear integrity of sperm cells in these cases, we have performed an extensive study to evaluate this aspect. Subjects and methods: Twenty six treated HH patients were recruited. Spermatogenesis has been achieved in 19/26 cases (73%). Spermatozoa were retrieved surgically in testicular samples in 3 cases of azoospermia. A semen cryopreservation has been realized in 14 cases and 7 sperm samples were available for the study. They were compared to 7 normospermic controls. The sperm cells were processed by FISH using probes for chromosomes specific loci of chromosome 13, 21, 18, X and Y. In order to quantify sperm DNA fragmentation, a TUNEL assay was performed. Both FISH and DNA fragmentation results were observed using fluorescence microscopy. Results: FISH analysis revealed that 97 to 99% of induced spermatozoa were normally haploid. TUNEL assay results were also in the range (2 to 10%) of physiologically normal sperm. There was no significant difference in the quality of nucleus status observed between the 7 patients and the 7 normal fertile men (controls). Discussion: The high outcome of a complete spermatogenesis was achieved with a remarkable high success rate owing to the gonadotropins treatment in HH men, which allows either spontaneous fathering or efficient ART. We evaluated the nuclear quality of sperm cells after treatment in 7 patients. No enhancement in the rates of aneuploidy or DNA fragmentation could be shown after this induced spermatogenesis suggesting their nuclear integrity.

2324/F

Candidate-gene association study of non-obstructive azoospermia (NOA): ART3 as a genetic susceptibility to NOA. *A. Tajima[1], H. Okada[2], M. Sekine[2], K. Shichiri[3], A. Tanaka[4], K. Tanaka[2], I. Inoue[1].* 1) Dep Molecular Life Sci, Tokai Univ Sch Medicine, Kanagawa, Japan; 2) Dep Obstet & Gynecol, Niigata Univ Graduate Sch Med & Dent Sciences, Niigata, Japan; 3) Dep Obstet & Gynecol, Tachikawa Hospital, Niigata, Japan; 4) St. Mother's Hospital, Fukuoka, Japan.

Male-factor infertility is believed to be responsible for 20-50% of all infertility cases. Male infertility could be caused by genetic factors altering spermatogenesis, but chromosomal abnormalities associated with azoospermia factor (AZF) regions on Yq are the only genetic defects with moderate incidence rates (up to 15%) so far. Thus, genetic causalities of most infertile patients are not elucidated. Here we perform an extensive candidate gene analysis to identify susceptibilities to non-obstructive azoospermia (NOA). Based on the concept that a common variant of a susceptibility gene could result in altered expression of the gene in testis, we first determined NOA candidate genes representing differences in testicular expression between NOA patients, using Agilent Human 1A(v2) Oligo microarrays. Next, 191 SNPs of 42 candidate genes were evaluated for allelic association with NOA in 442 NOA patients and 475 proven fertile controls. After two-rounds of screening, SNPs of *ART3* (ADP-ribosyltransferase 3) were associated with NOA, and the most significant association was observed with ART3-SNP25 locating intron 11 of *ART3* ($P = 0.0025$). Haplotype-based association analysis revealed that the most frequent haplotype in controls were under-represented in NOA patients with statistical significance ($P = 0.000073$), indicating a protective impact of the haplotype. These results would help promoting the full understanding of genetic causalities of NOA.

2325/F

Development and establishment of cell cultures from placental tissues for the study of confined placental mosaicism. *J.A.M.A. Tan[1], K.K. Ho[2], P.C. Tan[1].* 1) Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; 2) Department of Obstetrics & gynaecology, Faculty of Medicine, University of Malaya, Kulal Lumpur, Malaysia.

Cell cultures orginating from placental tissues are necessary for the study of confined placental mosaicism, defined as the presence of chromosomal abnormalities in placental tissues. A critical step in research in confined placental mosaicism, which has been associated with poor pregnancy outcomes, is the successful culture of primary trophoblast cells. Establishment of primary trophoblast cell cultures was carried out using the direct tissue explant methods and tissue dispersion with enzmye digestion methods. In addition, Percoll density gradient centrifugation was evaluated for purification of homogenous cell populations.

Tissue samples were extracted from human placentas from full term pregnancies with signed consent from subjects involved. Adherence of cells from explanted tissues was observed after two weeks of culture. Attachment of explanted tissues was accompanied by rapid production of newly dividing cells as observed by outgrowth of cells from the edges of the explanted tissues. The tissue dispersion method involved trypsin-dispersed cell suspensions followed by Percol purified cytotrophoblast cells for the initiation of *in vitro* primary cell cultures. Out of the 80 placentas collected, metaphases were successfully harvested and analysed from 12 samples (12%), and out of theses 12 placentas with successful proliferating cultures, 24 karyotypes were obtained and analysed.

2326/F

Genotype-phenotype correlation for *KLHL10* mutations in oligozoospermic men. *A.N. Yatsenko[1], A. Roy[1], R. Chen[1], L. Ma[1], L.J. Murthy[1], S. Veeraragavan[1], W. Yan[2], D.J. Lamb[1], M.M. Matzuk[1].* 1) Baylor Col Medicine, Houston, TX; 2) University of Nevada, School of Medicine, Reno, Nv.

Nearly 25% of infertile males are diagnosed as idiopathic, suggesting the contribution of the genetic factors. The most common semen pathology among infertile men is oligozoospermia. However, cause for the semen defect, except for ~10% of oligozoospermic patients with identified chromosomal aberrations, remains unknown. Recently we identified *KLHL10* mutations that are responsible for oligozoospermia in ~ 2% of more than 600 patients with the semen defect ($0.5\text{-}32\times10^6$ spermatozoa/ml). KLHL10 is known to interact with CUL3 and forms ubiquitin E3 ligase complex. To understand an effect of *KLHL10* mutations we performed genotype-phenotype correlation for identified gene alterations in oligozoospermic men. Preliminary data indicate that most severe effect on sperm count has splicing defect. Interestingly, among missense *KLHL10* mutations, most severe ones are those that are located in predicted functional regions, namely kelch repeat 1 and BACK domains. Preliminary functional evidence indicates that two most frequent missense mutations A313T and Q216P impair natural homodimerization affinity of KLHL10 protein and likely damage its function. All latter identified *KLHL10* alterations in oligozoospermic men mapped to kelch repeats 1, 2, 3 and BACK domains as well. Conversely, most DNA mutations located to predicted linker protein segments were found in normozoospermic patients, suggesting their milder or neutral effect on protein function. Our results imply that severity of oligozoospermia depends on mutation type and position effect on important functional domains of the protein. Since, severe oligozoospermia was coupled with severe teratozoospermia in 4 of 7 patients with *KLHL10* mutations in our initial study; we are currently studying the gene contribution to oligoteratozoospermia and teratozoospermia. These study was supported in part by the NIH Specialized Cooperative Centers Program in Reproductive Research (U54 HD07495) and NIH Infertility Center (P01HD36289) to MMM and DJL, and by NIH grant HD050281 to WY.

2327/F

FIGLA mutations cause premature ovarian failure in a subset of Chinese women with POF. *H. Zhao[1,2], Z-J. Chen[1], Y. Qin[1,2], S. Wang[1], J.L. Simpson[2], A. Rajkovic[2].* 1) Center for Reproductive Medicine, Shandong Provincial Hospital of Shandong University, Jinan,SD,-China; 2) Departments of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA.

OBJECTIVE: Premature Ovarian Failure (POF) is a common cause of hypergonadotropic ovarian failure and infertility, affecting 1-2% of women. POF is a genetically heterogenous disease, with few known causative genes. We utilized a candidate gene approach to study if FIGLA, a transcriptional regulator preferentially expressed in the ovary, is mutated in Chinese women with POF. MATERIALS AND METHODS: 100 Chinese POF women, as well as 304 control age-matched women, were recruited for this study. The coding regions of FIGLA gene were amplified using polymerase chain reaction (PCR) with 4 pairs of specific primers. Sequencing was performed after PCR amplification on ABI Prism Sequencer 3130XL (Applied Biosystems). To further test whether the missense or deleted (c.16C>A and c.423-425delAAC) alleles affect FIGLA's ability to dimerize with itself or E12, we employed a yeast two-hybrid strategy to study protein-protein interactions. RESULTS: Three novel variants were identified in four POF individuals: c.16C>A (p.A4E), c.20-41del (p.P6fsX77) and c.423-425delAAC (p.140delN). The c.20-41delta causes a frameshift mutation with haploinsufficiency. Functional analyses by the yeast two-hybrid assay demonstrated that p.140delN mutation disrupted FIGLA binding to the E12 HLH domain. The c.20-41 delta and c.423-425delAAC mutations were not present in the 304 control women. CONCLUSION: Our findings show that a subset of Chinese women with sporadic premature ovarian failure harbor mutations in FIGLA. Haploinsufficiency in transcription factors is known to cause many human Mendelian disorders, and c.20-41delta frameshift results essentially in haploinsufficiency by terminating FIGLA open reading frame immediately after the first five amino acids. Moreover, we show that the c.423-425delAAC disrupts FIGLA's interaction with E12. High throughput sequencing of genes involved in the FIGLA pathway will be useful to detect other genes that play critical roles in premature ovarian aging.

2328/W

Is Ménière's disease associated with polymorphisms in KCNE1 or KCNE3 in the United States? *C.A. Campbell[1, 2], C.C. Della Santina[3], N.B. Smith[3], J.P. Carey[3], L.B. Minor[3], R.J.H. Smith[1,2].* 1) Dept of Otolaryngology, University of Iowa, Iowa City, IA; 2) Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA; 3) Dept of Otolaryngology, Dept of Biomedical Engineering, and Dept of Neuroscience, The Johns Hopkins University, Baltimore, MD.

Ménière's disease (MD) is a complex disorder of unknown etiology characterized by the symptoms of vertigo, sensorineural hearing loss and tinnitus. Incidence in Caucasians is 1-2 per 10,000 and in the Japanese, 35-160 per 1,000,000 (Morrison 1995). Although candidate genes studies focused on COCH (coagulation factor C homology), ATQ1 (antiquitin) and AQP2 (aquaporin 2) have been unsuccessful in identifying disease-causing allele variants of these genes, Doi and colleagues have reported that two single nucleotide polymorphisms (SNPs) in KCNE1 and KCNE3 are associated with MD in Japanese patients (Doi et al. 2005). These two genes encode potassium channels that are expressed in the stria vascularis and endolymphatic sac, respectively. Their role in ion transport and their expression pattern suggest that they may be important in inner ear homeostasis. To establish whether a similar association exists in the Caucasian MD population, we sequenced the coding regions and exon-intron boundaries of both genes in ~150 persons with MD and compared results to 168 ethnically matched CEPH controls, and a second control group of 150 Caucasians. Neither of the two reported SNPs were significantly associated with MD when compared to the CEPH control population. Population stratification was evaluated for the two control populations using an LCT promoter SNP (rs4988235), and 22 STRP markers spaced throughout the genome. Comparison of the population stratification results will be presented. In addition, the results from the second Caucasian control population candidate gene association study will be presented.

2329/W

***ZNF750*, a novel C2H2 zinc finger protein associated with seborrheic dermatitis and psoriasis, modulates expression of cytokines and proliferation genes in keratinocytes.** *R. Birnbaum, R. Ofir, V. Chalifa-Caspi, O.S Birk.* The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology and Faculty of Health Sciences, Ben-Gurion University of the Negev, and Genetics Institute, Soroka Medical Center, Beer-Sheva, Israel.

Seborrheic dermatitis and Psoriasis are common dermatoses with overlapping features. An Israeli Jewish Moroccan family presented with autosomal dominant seborrhea-like dermatosis with psoriasiform elements: enhanced keratinocyte proliferation, parakeratosis, follicular plugging, *Pityrosporum ovale* overgrowth, and CD4 lymphocyte infiltrate. We showed that the disease gene is *ZNF750*, encoding a C2H2 zinc finger-like protein. *ZNF750* is normally expressed in keratinocytes (not in fibroblasts) and scarcely in CD4 lymphocytes. We deomonstrate that ZNF750 modulates the expression of specific cytokines by keratinocytes, and we elucidate molecular mechanisms of enhanced keratinocyte proliferation in this disease. Our findings open new insights to the molecular mechanisms of Seborrheic dermatitis and Psoriasis.

2330/W

Identification of functional pathways of the immune and hematological systems important to bone health using transcriptional profiling in a nonhuman primate. *L.M. Havill[1], J.M. Proffitt[1], J.C. Charlesworth[1], M.P. Johnson[1], J.E. Curran[1], E.K. Moses[1], J. Blangero[1], C. Brugnara[2], O.S. Platt[2], M.C. Mahaney[1].* 1) Genetics, SFBR, San Antonio, TX; 2) Laboratory Medicine, Harvard University School of Medicine, Cambridge, MA.

Recent research suggests that components of the immune and hematological systems contribute to variation in bone health. Studies of bone mineral density (BMD), a measure of bone fracture resistance, unequivocally show that this trait, and hence, osteoporosis risk, has a strong genetic basis. Many of the genes associated with BMD are also pivotal to immune function and hematopoiesis. We used genome-wide transcriptional profiles to identify functional networks of genes likely to be important to normal variation in BMD in the baboon, an established primate model for human bone maintenance and turnover. We used the Illumina Human Sentrix-6 BeadChip micro-array to interrogate RNA from stored lymphocytes of 495 baboons (Papio hamadryas). Transcript levels were regressed against BMD assessed by DXA at the ultradistal radius (trabecular bone) and the radius shaft (cortical bone). We identified ~500 transcripts showing nominally significant correlations to BMD as genes of interest. We used Ingenuity Pathways Analysis version 5.0 (Ingenuity® Systems, www.ingenuity.com) to overlay these genes/gene products onto a global molecular network developed from literature-reported connectivity recorded in the Ingenuity Pathways Knowledge Base, allowing for the characterization of immune and hematological system-related functional networks represented amongst the genes of interest. Several functional pathways were common to the results for both sites, though the genes implicating these pathways were not entirely redundant between sites. Immune system functions in common to both traits involve cell growth, generation and morphology. Those for the hematological system involve cell binding, generation, migration and morphology. Our results support a role of immune and hematological system-related genes in normal variation in an osteoporosis risk factor, and may provide insight into the mechanisms underlying their role in bone health.

2331/W

A spectrum of molecular variation in a cohort of Italian patients affected by Paget's Disease of Bone. *I. Marino[1], F. Gianfrancesco[1], T. Esposito[1], D. Rendina[2], G. De Filippo[3], R. Nuti[4], D. Merlotti[4], A. Ciccodicola[1], L. Gennari[4], P. Strazzullo[2], G. Mossetti[2].* 1) Institute of Genetics and Biophysics, Italian National Research Council, Naples, Italy; 2) Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy; 3) Unit of Pediatric Endocrinology, Gaetano Rummo Hospital, Benevento, Italy; 4) Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Italy.

Paget disease of bone (PDB) is a chronic disease of the skeleton that affects up to 2-3% of the population aged > 50 years. The disorder is characterized by focal areas of increased osteoclastic bone resorption and a coupled, but disorganized, increase in osteoblastic bone formation. The PDB geographic distribution is not uniform, with a higher prevalence of the disease in North America, Australia, and New Zealand. In Europe, the PDB prevalence shows a north-south gradient, with highest prevalence in England and a lower prevalence in Italy and Greece. Mutations in the ubiquitin protein-binding domain (UBA), of the sequestosome 1 (SQSTM1) gene, which is a scaffold protein in the NF-κB signalling pathway were identified as a common cause of PDB. To examine the prevalence of mutations of SQSTM1 in Italian families, and to assess potential genotype-phenotype associations, we performed mutational analysis in 150 sporadic and familial Italian Paget's cases, recruited in the Campania Region. An increased PDB clinical severity was observed in the PDB cohort from Campania in comparison with patients from other Italian regions. Moreover, neoplastic degeneration of pagetic bones (osteosarcoma and giant cell tumor) was exclusively observed in Campania patients with polyostotic PDB. In 15% of these patients with PDB disease, heterozygous mutations in the SQSTM1 gene were identified. These were the previously described P392L mutation, and four new mutations. All mutations were located in the ubiquitin-associated domain of the gene, representing a mutational hot spot area. Our findings confirm the hypothesis of an involvement of the SQSTM1 gene in the pathogenesis of Italian Paget's cases.

2332/W

HLA-DRB1 is associated with disease susceptibility and severity of rheumatoid arthritis in Japanese. *S. Tsukahara, K. Ikari, S. Momohara, T. Tomatsu, M. Hara, H. Yamanaka, N. Kamatani.* Inst Rheumatology, Tokyo Women's Medical Univ, Tokyo, Japan.

The disease susceptibility to rheumatoid arthritis (RA) has been estimated to have a genetic component of up to 60%, and one-third of the genetic component has been estimated to depend on the human leukocyte antigen (HLA) locus. Several prospective studies suggest that particular HLA-DRB1 alleles encoding a conserved sequence of amino acids called shared epitope (SE) are associated with severe radiographic damage or functional impairment of RA. A recent meta-analysis shows the value of SE for predicting radiographic damage varies among ethnicity. In the present study, we examine whether SE is associated with disease susceptibility and severity of RA in Japanese.

The diagnosis of RA was established using the classification criteria of ACR. Sharp/van der Heijde (SvdH) method was used to assess radiographic joint damage in patients with 5-year disease duration. DNA samples of patients were obtained from IORRA study and population-based control samples were from the Pharma SNP consortium. Sequencing-Based Typing of HLA-DRB1 was performed on 147 cases and 470 controls using the Atria AlleleSEQR HLA-Sequencing-Based Typing Kit. Assign-SBT software was used to determine HLA-DRB1 alleles, and subjects were categorized as having 0, 1 or 2 copies of the SE, defined by the following alleles: 0101, *0401, *0404, *0405, *0410, *1001 or *1406. Association between RA susceptibility and HLA-DRB1 SE were examined by Fisher's exact test. Differences in SvdH scores among copies of the SE were analyzed by linear regression analysis. All statistical analyses were carried out using the R software package.

HLA-DRB1 SE was strongly associated with RA ($P = 5.1 \times 10^{-7}$). Mean SvdH score was 45.0, 50.1 and 77.9 for homozygous negative, heterozygous, homozygous positive individuals for SE allele, respectively. SE had a significant effect on radiographic damage in Japanese RA patients ($P = 0.01$). We conclude that HLA-DRB1 SE is associated with RA susceptibility and severity in Japanese.

2333/W

Clinical and pathological characteristics of Lewy Body disorders in patients with Glucocerebrosidase mutations. *L.N. Clark[1,2,10], R. Wolf Gilbert[3], B. Dorado[1,2], L. Kartsaklis[5], B.M. Ross[1,2], E.D. Louis[1,3,5], L.J. Cote[3,5], H. Andrews[4,5,6], C. Waters[3], B. Ford[3], S. Frucht[3], R. Ottman[3,5,7,8], J.P. Vonsattel[1,2,9], S. Fahn[3], L.S. Honig[3,5], K. Marder[1,3,4,5].* 1) Taub Institute for Research on Alzheimer's Disease and the Aging Brain; 2) Department of Pathology; 3) Department of Neurology; 4) Department of Psychiatry; 5) G.H. Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York; 6) Department of Statistics; 7) Department of Epidemiology, Mailman School of Public Health, Columbia University, New York; 8) The Epidemiology of Brain Disorders Department, New York State Psychiatric Institute, New York; 9) The New York Brain Bank; 10) The Center for Human Genetics, College of Physcians and Surgeons, Columbia University, New York.

Background: Mutations in the Glucocerebrosidase (GBA) gene have been associated with pathologically proven Lewy body disorders (LBD). Methods: We sequenced all GBA exons in 61 samples enriched for LBD from the NYBB. Results: 24.6 % (15/61) were mutation carriers. Among non-carriers, 72.4% of cases who had cortical LB also had definite or probable AD (CERAD), whereas among carriers, 21.4% had both cortical LB and definite or probable AD. (p=<0.001) .In a logistic regression model, GBA mutation status was associated with cortical Lewy bodies (OR 19, 95% CI 2-175; p=0.009) after adjustment for age, gender, and definite or probable AD. AD pathology was an independent predictor of cortical Lewy body pathology in this sample (OR 14, 95% CI 3-67; p=0.001). In separate Cox Regression models, adjusting for age of onset of Parkinsonism and gender, no association was found between 1) the age of onset of dementia, 2) the time of progression from parkinsonism to dementia, or 3) the rate of developing dementia prior to parkinsonism with mutation status. Conclusions: The presence of GBA mutations may be associated with both cortical Lewy bodies and dementia, independent of AD pathology.

2334/W

Partial deletion mouse models for Williams-Beuren syndrome. *U. Francke[1], H-H. Li[1], M. Roy[2], U. Kuscuoglu[1], B. Halm[1], K. Carlsmith Harrison[1], J.H. Bayle[3], A. Splendore[1], F. Ding[1], E. Wright[1], C.M. Spencer[4], C.J. Goergen[2], J. Li[1], L. Tsavaler[1], C.A. Taylor[2], R.M. Myers[1], R. Paylor[4], K. Deisseroth[2, 5].* 1) Dept Genetics; 2) Dept Bioengineering; 3) Dept Pathology, Stanford Univ; 4) Dept Mol Hum Genet, Baylor College of Medicine, Houston TX; 5) Dept Psychiatry Stanford Univ, Stanford CA.

Williams-Beuren syndrome (WBS) is caused by recurrent heterozygous deletions of a ~1.5 Mb region at 7q11.23. Features include distinct craniofacial appearance, mental disability with visuo-spatial construction defects and preservation of speech, vascular stenosis, hypotonia, hyperacusis, social disinhibition and anxiety. The conserved syntenic region in mouse is at 5G1-G2. Most single-gene knock-out mouse models for one of the 25 genes in the deletion have no phenotype in heterozygotes. To functionally dissect the WBS deletion region and to identify genes that are truly haploinsufficient, we created two lines of mice that are deleted either from *Gtf2i* to *Limk1* (Proximal - Pd) or from *Limk1* to *Fkbp6* (Distal - Dd) by using *Cre-Lox*P technology. Double heterozygotes (D/P mice) model the complete WBS deletion. All deletion mice are viable, fertile and have normal lifespan. Gene expression microarray and qRT-PCR studies of brain RNA revealed a reduction of transcripts mapped to the deleted segments. Pd and D/P mice have growth delay, Dd and D/P mice have smaller brains, abnormal cerebella and hernias. Micro CT of skulls revealed shortening in D/P females more than in males. Adults with elastin gene deletion (Dd and D/P) have a less compliant aorta, reducing anterior wall motion due to disorganized and thinner elastin sheets. Elastin transcripts are reduced in thorax at P7. D/P, and Pd mice to some extent, have a deficit in motor coordination and performance, partly due to hypotonia, as determined by rotarod and hanging wire tests. A comprehensive behavioral test battery revealed several phenotypic differences among the mutant lines in assays of exploration, anxiety-related responses, and measures of social interactions, which indicate that these *Wbs* mouse lines can be used to model aspects of the human disorder and identify contributing genes.

2335/W

Genetic studies of familial autoimmune myasthenia gravis. *G. Landoure[1], M. Knight[1], H. Stanescu[2,3], A. Taye[1], R. Kleta[2,3], K.H. Fischbeck[1].* 1) NINDS, NIH, Bethesda, MD; 2) NHGRI, NIH, Bethesda, MD; 3) UCL, London, UK.

About 4% of patients with myasthenia gravis have a positive family history. We studied an Italian-American kindred with parental consanguinity and 5 out of 10 siblings affected by autoimmune myasthenia. The age of onset was 50 to 79 years. A genome-wide scan with 2000 microsatellite markers was performed in 7 family members. The consanguinity indicates autosomal recessive inheritance in this family. Based on this, we performed homozygosity mapping and found linkage to a region of shared homozygosity on chromosome 13 with a LOD score of 3.28. After taking unaffecteds into account the LOD score dropped to 1.57. Haplotype reconstruction showed homozygosity in the affecteds and also one unaffected individual. The region is 7.4 Mb and contains 57 genes, 37 of which are protein coding genes, with 33 expressed in muscle, nervous system, or immune system. A parametric analysis also showed linkage to a region of chromosome 2 with a LOD score of 2.18. Haplotype reconstruction indicated that the affected individuals are compound heterozygotes. This region is 6.6 Mb and contains 69 genes. 57 are protein-coding genes, with 48 expressed in appropriate tissues. Microarray expression analysis was done and showed one upregulated gene in the region of interest on chromosome 13 and two in the region on chromosome 2. These genes were sequenced and no change was found. Seventeen genes were sequenced in the chromosome 2 region, including AchR subunits gamma and delta, and no change was found. The region on chromosome 13 was considered, with the possibility of incomplete penetrance or late onset of the disease in the unaffected individual. Nearly all the genes in this region have been sequenced, and homozygous single nucleotide variants were found in two. One of these changes is located in a 3'UTR, in a region that is not well conserved but contains a miRNA binding site. We have sequenced over 60 controls, and none had the variant. The second change is in a coding region but does not alter the corresponding amino acid. We are now sequencing additional controls and doing quantitative RT-PCR to determine whether these variants affect splicing or gene expression.

2336/W

Association study of SNPs in the PHF11 gene in Italian families with allergic asthma. *C. Bombieri, P. Zorzi, G. Malerba, L. Xumerle, P.F. Pignatti.* Sec Biology & Genetics, Dpt. Mother and Child, and Biology-Genetics, University of Verona, Verona, Italy.

In an our previous genome wide scan for asthma in 123 Italian families, phenotyped for clinical asthma and rhinitis, skin prick test positivity to common aeroallergens, total serum IgE levels (IgE), bronchial hyperresponsiveness to methacholine, linkage on chromosome 13q14 has been detected for elevated IgE. Association of the PHF11 gene with IgE and atopic dermatitis (AD) was found in two recent studies (Nat Genet 34:181;2003; Genes Immun 6:264;2005). We have now performed a linkage and association study of the PHF11 gene polymorphisms in a subset of 24 families (144 subjects) which have shown positive linkage for IgE. The following 7 SNPs, located inside the gene and reported to be associated with IgE and AD in the above mentioned studies, were selected and analysed: 185306b7_2 (intron1); rs2031532 (ex2); rs2247119 (intron3); rs2274276 (intron4); 185752b4_2 (intron5); 185752b5_2 (intron9); rs1046295 (3'UTR). SNPs were genotyped by minisequencing (SNaPShot Multiplex kit, Applera, on ABIPRISM 310 sequencer using Genescan software) or by enzymatic restriction. Linkage analysis was perfomed using MERLIN software and association study was performed by Transmission Disequilibrium Test (TDT) using the unphased software: //www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased. A correction for multiple test of the obtained data was applied. Statistical analysis results confirmed our previous findings of linkage of this chromosomal region to IgE in allergic asthma, but did not show significant association of the PHF11 gene SNPs with any of the studied phenotype. In our population, association might be due to other polymorphisms of the PHF11 gene or to other genes located in this chromosomal region.

2337/W

***VEGF* polymorphisms associate with Kawasaki Disease: replication of a susceptibility locus for pediatric coronary vasculitis.** *W. Breunis[1], S. Davila[2], V. Wright[3], M. Hibberd[2], M. Levin[3], J. Burns and the US KD Genetic consortium[4], D. Burgner[5], T. Kuijpers[1].* 1) Emma Children's Hospital, Netherlands; 2) Genome Institute of Singapore, Sinagapore; 3) Imperial College London, England; 4) UCSD School of Medicine, USA; 5) School of Paediatrics and Child Health, Australia.

Background: Kawasaki Disease (KD) is an acute systemic vasculitis that occurs in young children. Based on clinical and epidemiologic parameters an infectious cause is assumed, however the etiology still remains unknown. A genetic influence is suggested by differences in annual incidence between different ethnicities. **Objective**: In a previous study we have shown that the *VEGF* haplotype CGCC (based on rs699947, rs2010963, rs25648 and rs3025039) was significantly associated with the development of KD (hap score 3.8; p = 0.0002) in a Dutch Caucasian KD cohort of 170 patients and 300 controls. To test this association we conducted a large family-based association study. **Methods**: 14 SNPs in the *VEGF* gene, selected as tagging SNPs to cover common genetic variants, were analyzed as a part of a larger Illumina GoldenGate assay investigating 1,903 members of 583 KD families, including 498 trios, from Australia, UK and US. Genotyping of the families was performed with a Beadstation 500G Genotyping system and genotypes were analyzed with Beadstudio software from Illumina. Allelic association was tested using PBAT. **Results**: Of the 14 analyzed SNPs in the *VEGF* gene 3 SNPs were associated with susceptibility for KD (rs833068, rs3025033 and rs3025039). The SNP C>T at position 236 bp 3' of STP (rs3025039) was seen with a frequency of 14% in the parents which is similar to the frequency in our Dutch control population in our previous study. Asymmetric transmission was observed from heterozygous parents to their affected offspring (p = 0.005, transmitted : untransmitted ratio 141:98). **Conclusion**: Our results confirm our previously observed association of KD susceptibility and polymorphisms in the *VEGF* gene in an independent cohort of KD patients. As VEGF is a multifunctional cytokine the exact role of VEGF remains unclear and further functional studies are warranted.

2338/W

C2 and BF genes in Age-Related Macular Degeneration and joint action with CFH and LOC387715 genes. *M.B. Gorin[1,2,5], Y.P. Conley[3,5], J. Jakobsdottir[4], R.E. Ferrell[5], D.E. Weeks[5,4].* 1) Dept Ophthalmology, Jules Stein Eye Inst - UCLA, Los Angeles, CA; 2) UPMC Eye Center, Department of Ophthalmology, School of Medicine, University of Pittsburgh, PA; 3) Department of Health Promotion and Development, School of Nursing, University of Pittsburgh, PA; 4) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA; 5) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA.

The Y402H variant in the complement factor H (CFH) gene on 1q32 and the S69A variant in the LOC397715 (LOC) gene on 10q26 have been consistently shown to be strongly associated with age-related macular degeneration (ARM). The C2 and BF genes, closely linked on 6p21, have recently been shown to harbor ARM-associated variants. We typed 4 SNPs in those genes (rs9332739 and rs547154 in C2; rs4151667 and rs2072633 in BF) in case-control and family-based data (white subjects only). The data include a total of 601 ARM families (including 494 affected sib pairs, 5 affected half-sib pairs, 60 affected cousin pairs, and 38 affected avuncular pairs) and 149 unrelated controls and 172 unrelated cases. All SNPs were in Hardy-Weinberg equilibrium in both cases and controls. We used Fisher's exact test to test for differences in genotype distributions between cases and controls, and the CCREL test to compare ARM families vs. controls. The C2 SNP rs547154 was associated with ARM in the case-control data (p-value 10^-4) and family-based data (p-value 0.00001). We used logistic regression models to investigate the joint action of C2 and CFH and LOC, using the controls, cases, and one randomly selected case from each family. First, we tested the joint action of all pairs of genes and found that the most parsimonious models (model with lowest AIC) of the action of the locus pairs CFH and LOC, CFH and C2, and LOC and C2, were, in all cases, additive models without interaction between the two loci. The most parsimonious three locus model was the additive model of all 3 genes. The addition of C2 to a model of CFH and LOC significantly improved the model (p-value of likelihood ratio test 0.005).

2339/W

Evidence for interaction between *DCDC2* and *KIAA0319* in dyslexia. *P. Hoffmann[1], K.U. Ludwig[1], D. Roeske[1,2], J. Schumacher[3], G. Schulte-Körne[4], I.R. König[5], A. Ziegler[5], B. Müller-Myhsok[2], M.M. Nöthen[1].* 1) Dept Genomics, Life & Brain Ctr, Bonn, Germany; 2) MPI Psychiatry, Munich, Germany; 3) Inst Hum Genet, Univ Bonn, Bonn, Germany; 4) Dept Child & Adolesc Psychiatry, Univ Munich, Munich, Germany; 5) IMBS, Univ Lübeck, Lübeck, Germany.

Independent linkage studies for dyslexia have pointed towards a susceptibility locus on chromosome 6p21-p22 (DYX2) (1). This region harbours two candidate genes in close proximity to each other, namely *DCDC2* and *KIAA0319* (1). Unfortunately, no single study to date has sufficiently covered both genes, which would be necessary to understand the relative contribution of both genes and to identify possible interactions between them. Harold et al.(2) recently reported a combined analysis of both genes in two UK samples, supporting their previously observed findings for *KIAA0319* and showing evidence for an interaction between the two genes. We have previously reported strong association of variants in the *DCDC2* gene with dyslexia in German families, but did not obtain any evidence for a contribution of the *KIAA0319* gene (3). In the present study we expanded the marker set from our previous study by six markers in order to obtain a more comprehensive picture of the contribution of *KIAA0319*. None of these markers showed significant association with dyslexia, neither in the total sample, consisting of 244 German families with a severely affected child, nor when stratifying for the subdimensions or severity. When testing for interaction between markers in *KIAA0319* and our previously identified risk haplotype in *DCDC2* (3), we obtained no evidence for interaction for dyslexia itself, but identified a nominally significant association for the subdimension "word reading", which was the core phenotype in the study of Harold et al. This may be seen as supportive evidence for an interaction between *KIAA0319* and *DCDC2*. However, an effect of *KIAA0319* alone, as reported for the UK samples, could not be demonstrated in our sample of German origin.

(1) Schumacher J et al. *J Med Genet* 2007; **44**, (2) Harold D et al. *Mol Psychiatry* 2006; **11**, (3) Schumacher J et al. *Am J Hum Genet* 2006; **78**.

2340/W

Genetic association of the CHRNA6 and CHRNB3 genes with tobacco dependence in a nationally representative sample. *N. Hoft[1], I. Schlaepfer[1], R. Corley[1], S. Young[1], B.C. Haberstick[1], D. Huizinga[2], S. Menard[2], M. Ehringer[1]*. 1) Inst Behavioral Genetics, University of Colorado, Boulder, CO; 2) Inst Behavioral Science, University of Colorado, Boulder, CO.

The family of neuronal nicotinic acetylcholine receptors show regulation of activity by both endogenous acetylcholine and exogenous nicotine, making sequence variations in these receptors likely candidates for association with tobacco phenotypes. Our group has previously identified a significant association between SNPs in the genomic region containing the CHRNA6 genes and dependence for tobacco use in a young adult Colorado-based sample (Zeiger et al, submitted). Similarly in that same region SNPs in CHRNB3 have been found to be associated with tobacco dependence in regular smokers (Beirut et al 2007). In this study, we were able to replicate both these findings in the National Youth Survey Family Study wave 10, a nationally representative sample of households. Eight single nucleotide polymorphisms (SNPs) in the CHRNA6 and CHRNB3 genomic region were genotyped in 1002 subjects, approximately half of whom are members of sibling pairs. Association was assessed using a family-based approach as implemented in the statistical package PBAT (FBAT-PC, principal components). Individual SNPs were tested for association with a composite phenotype of frequency and quantity of tobacco use and dependence, and followed by testing of individual phenotypes to validate results from the composite. Variation in CHRNA6 was found to be associated with a composite dependence-frequency-quantity phenotype (p = 0.02) as well as with "dependence in regular smokers" (p=0.025). Additionally, multiple SNPs adjacent to rs6474413, the SNP identified in Beirut et al (2007), were shown to be associated with "dependence in regular smokers" (p=0.004). Together these results further implicate the region upstream of CHRNB3 in susceptibility/resistance to nicotine dependence.

2341/W

Replication and fine-mapping of association of FTO SNPs with obesity. *H. Lyon[1,2], G. Lettre[1,2], E. Speliotes and[1,2], J.N. Hirchhorn for the Diabetes Genetics Initiative (DG[1,2], J. Butler[1,2], Z. Gajdos[1,2], L. Peltonen-Palotie[2,3], M. KuokKanen[2,3], V. Salomaa[3], R. Cooper[4], X.F. Zhu[5], G. Dedoussis[6], C. Papoutsakis[6], N. Vidra[6], K. Ardlie[2], K. DeLellis[7], B. Henderson[7], L. Kolonel[8], M. Palmert[5]*. 1) Div Genetics, Boston Children's Hosp & Harvard Med Sch, Boston, MA; 2) Broad Inst, Cambridge, MA; 3) National Public Health Inst, Helsinki, Finland; 4) Loyola U Med Cen, Maywood, IL; 5) Case Western Reserve U, Cleveland, Ohio; 6) Harokopio U, Athens, Greece; 7) USC, Los Angeles, CA; 8) U of Hawaii, Honolulu, HI.

Background: Two groups (Frayling 2007, Dina 2007) recently reported an association of SNPs in the *FTO* (fat mass obesity associated) gene with body mass index (BMI) and fat mass in adults and children. We sought to replicate this association in population-based and case-control cohorts, to examine the affect on obesity in children, and to extend this finding into African-American cohorts. Results: We genotyped rs9939609 and 15 SNPs selected on the basis of LD patterns in the HapMap CEU and YRI samples. The association with rs9939609 replicated in 2 adult cohorts, a population based cohort from Finland (FINRISK97, N=6488, p=1x10-6) and a case control cohort from US and Poland (N=2873, p=0.053), as well as in a pediatric cohort (GENDAI, N=790, p=0.04). The association with BMI was less evident in a Scandinavian case-control study of diabetes(DGI, N=3048, p values in cases, controls, and combined =0.055, 0.538 and 0.082 respectively). In a case-control study for age at menarche (Multiethnic Cohort, N=1801), the association appeared stronger in women of Caucasian and Hispanic ancestries with early menarche (p=0.001) rather than late menarche (p=0.81). We have not yet found convincing evidence of association between *FTO* SNPs and BMI in African-Americans (N=835 related people, p=0.382 and N=893 unrelated, p=0.99) or stronger associations with nearby *FTO* SNPs. Conclusion: The intronic variants in *FTO* seem to affect obesity in adults and children of European ancestry, and this effect may be stronger in women with early menarche. Fine-mapping is underway to define the causal risk alleles in African-Americans.

2342/W

Confirmation of IL12B and IL23R associations with psoriasis. *R.P. Nair[1], M. Weichenthal[3], P.E. Stuart[1], S. Jenisch[3], H.W. Lim[2], A. Ruether[3], S. Schreiber[3], E. Christophers[3], J.J. Voorhees[1], J.T. Elder[1]*. 1) Univ Michigan, Ann Arbor, MI; 2) Henry Ford Hospital, Detroit, MI; 3) Univ Kiel, Kiel, Germany.

Psoriasis is a common inflammatory and hyperproliferative skin disease with a multifactorial genetic basis. At least 9 linked loci have been reported, and several have been replicated. Attempting to identify loci that may have been missed by linkage analyses, Cargill et al (AJHG 80:273) performed a genome-wide association analysis using gene-centric markers, identifying two associated genes, IL12B and IL23R. They reported association with the major allele (A-G) of the IL12B haplotype rs3212227 (3' UT) - rs6887695 (60 kb 5'). For IL23R, a common allele (C-G) of the haplotype rs7530511 (L310P) - rs11209026 (Q381R) was disease-associated. We examined these four SNPs for association with psoriasis in two groups of North American and German Caucasians: (1) 1,178 psoriasis cases and 2,001 controls and (2) 462 pedigrees of varying sizes. Genotyping of the SNPs was performed by primer extension (SnapShot, ABI). Case-control data were analyzed by the Cochran-Armitage test for linear trend of association and family data were analyzed by the PDT. Results for case-control data were combined across geographic cohorts using the generalized Mantel-Haenszel procedure. Both IL12B markers showed highly significant association with psoriasis in the case-control set (rs3212227 OR=3.05 homoz, 1.96 het, p=1.0 x 10^{-9}; rs6887695 OR=2.34 homoz, 1.56 het, p=1.7 x 10^{-11}) and the family cohort (rs3212227 p=7.4 x 10^{-3}; rs6887695 p=3.7 x 10^{-4}). The IL23R markers tested also showed significant association for the cases and controls (rs7530511 OR=1.33 homoz, 1.06 het, p = 0.014; rs11209026 OR=1.95 homoz, 1.25 het, p = 7.4 x 10^{-4}), but not for the families (rs7530511 p=0.30; rs11209026 p=0.19). The trend in families was in the direction of association for the known risk alleles. Our results confirm associations between IL12B and IL23R and psoriasis in Caucasians. They also provide a likely genetic basis for the strong clinical association between psoriasis and Crohn's disease, and help to explain the efficacy of biologicals targeting p40, the product of the IL12B gene, in both disorders.

2343/W

Association of *CDKAL1* variants with early phase insulin secretion in Hong Kong Chinese. *M.C.Y. Ng, C.H.T. Tam, V.K.L. Lam, W.Y. So, R.C.W. Ma, J.C.N. Chan.* Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong.

Recently, several genome-wide association studies in diverse Caucasian populations have found association between variants at cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) and type 2 diabetes. The association was further replicated in 1500 type 2 diabetic patients and 1000 controls from our Chinese population in a previous study. It is speculated that CDKAL1 may inhibit CDK5/p35 complex in pancreatic β cells and alter insulin response to glucotoxicity. This study aimed to investigate for the association of *CDKAL1* variants and insulin response in Hong Kong Chinese.

We studied 616 healthy subjects without diabetes for 3 tagging SNPs (rs7752906, rs7756992 and rs9356744, $r^2 < 0.8$) in the associated linkage disequilibrium block reported in the previous studies. We assessed the SNP association with insulin resistance (HOMA_IR) and insulinogenic index during OGTT [(insulin 30 min - 0 min) / (glucose 30 min - 0 min)] using linear regression with adjustment for age and gender.

We did not observe association of any *CDKAL1* SNPs with HOMA_IR. However, significant associations to insulinogenic index were observed for two correlated SNPs (r^2 = 0.77), rs7752906 and rs9356744 (P = 0.009-0.014). The risk allele A of rs7752906 showed the strongest association with reduced insulinogenic index under an additive model [geometric mean (95%CI) = 76.8 (63.9-92.4) for AA carriers; 84.7 (78.6-91.2) for AG carriers and 99.4 (92.3-107.0) for GG carriers].

Our results support others' finding that variants at *CDKAL1* influence early phase insulin secretion, which may affect susceptibility to type 2 diabetes.

2344/W

Novel Sequence Variants in the HLX Gene in Patients with Isolated Congenital Diaphragmatic Hernia. *A. Slavotinek[1], A. Moshrefi[1], A. Mendell[2], GM. Shaw[3], L. Pennacchio[4], MD. Bates[2]*. 1) Dept Pediatrics, U585P, Univ California, San Francisco, San Francisco, CA; 2) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) California Birth Defects Monitoring Program, Berkeley, CA; 4) U.S. Department of Energy Joint Genome Institute, Walnut Creek, CA.

Congenital diaphragmatic hernia (CDH) is a common, life threatening birth defect. The genes that cause isolated CDH are largely unknown and only one mutation and two sequence variants of unknown significance have been reported in the FOG2 gene in isolated CDH. The Hlx gene is a divergent member of the homeobox transcription factor family with homology to the Drosophila homeobox gene H2.0. Hlx is highly expressed in intestinal mesenchyme and is detectable in the murine diaphragm at the time of diaphragm closure. Homozygous null mice for Hlx have had extremely small livers, reduced intestinal length and herniation of the diaphragm. In addition, HLX is located at 1q41-1q42 in humans, a chromosome region known to harbor a gene required for normal diaphragm development. We therefore re-sequenced this gene in 122 CDH patients. We identified four novel sequence alterations (3.2%) - c.C35T, predicting p.S12F, c.C53T predicting p.S18L and c.G517T predicting p.D173Y in three patients with isolated, right-sided CDH and c.C1161T, predicting p.A235V, in a patient with left sided-CDH, an atrial septal defect and a patent ductus arteriosus. These sequence alterations affect highly conserved amino acids outside the DNA-binding homeodomain. The alterations were absent in more than 186 ethnically matched control chromosomes. Parental samples were unavailable for all but the mother of the last patient, who was normal. Functional studies showed that only p.S18L resulted in a protein of reduced activity in regulating a smooth muscle actin promoter. The mouse model of CDH in the Hlx null mouse involved two null alleles, whereas these patients had single sequence variants. We have detected four novel, non-synonymous sequence variants in HLX in CDH patients. Our data suggests a minor role for HLX sequence variants in the pathogenesis of isolated CDH in humans, and further functional evaluations are in progress.

2345/W

Polymorphisms in the interleukin-12β and interleukin-23R genes are associated with psoriasis of early onset in a UK cohort. *Rh.LI. Smith[1,2], R.B. Warren[1,2], S. Eyre[1], P. Ho[1], X. Ke[1], H.S. Young[2], C.E.M. Griffiths[2], J. Worthington[1]*. 1) ARC-EU, The University of Manchester, UK; 2) Dermatological Sciences, The University of Manchester, UK.

Psoriasis is a chronic inflammatory skin disease that affects approximately 2% of the population worldwide. A recent genome-wide association scan (GWAS) focussing on gene-centric single nucleotide polymorphisms (SNPs) identified four non-Human Leucocyte Antigen (HLA) SNPs associated with psoriasis in a North American population (Cargill, *et al.* 2007), one of which (rs3212227) had previously been associated with psoriasis in a study of Japanese patients. These polymorphisms were found within the interleukin (*IL*)-*12*β and *IL-23R* genes located on chromosomes 5 and 1 respectively. The purpose of this study was to investigate these associations in a UK cohort of Type I psoriasis patients (onset of disease ≤40 yrs of age). Each marker was investigated independently in a case-control setting. The four SNPs, 2 in *IL-12*β and 2 in *IL-23R*, were genotyped in 597 UK Type I psoriasis patients (53.6% male; mean age of onset 19.8 years) using the Sequenom iPLEX genotyping platform. Genotype frequencies were compared against healthy volunteers (data made available by the Welcome Trust Case Control Consortium, ~2700; and 1958 Birth Cohort, ~4700). All four SNPs: rs3212227, rs6887695, rs11209026 and rs7530511 were significantly associated with Type I psoriasis (p = 0.003, 0.001, 0.001 and <0.001 respectively). In *IL-12*β, SNP rs3212227 conferred risk by carriage of two copies of the major allele with an odds ratio (OR) = 1.38 (95% CI 1.14 - 1.68, p = 0.0004); and rs6887695 conferred risk on the basis of a dominant model of inheritance for the major allele OR = 1.72 (95% CI 1.18 - 2.56, p = 0.0016). The *IL-23R* polymorphism rs11209026 also conferred risk by a dominant model of inheritance for the major allele OR = 1.67 (95% CI 1.20 - 2.37, p = 0.0008) with rs7530511 conferring risk by carriage of two copies of the major allele OR = 1.98 (95% CI 1.51 - 2.63, p < 0.0001). These results confirm the association of both *IL-12*β and *IL-23R* in a UK population of patients with early-onset psoriasis.

2346/W

Polymrophisms In The NPPA Gene Associate With Asthma. *J. Wang[1], S. Mohapatra[2], H. Feng[3], J. Marks[1], L. Chepenik[1], M. Castro[4], C.G. Irvin[4], J.A. Johnson[3], J.E. Sylveste[1], J.J. Lima[1].* 1) Pharmacogenetics Center, Nemours Children's Clinic, Jacksonville, FL; 2) University of South Florida, Tampa, FL; 3) Pharmacy Practice, University of Florida, Gainesville, FL; 4) Wake Forest University, Winston-Salem, NC.

Asthma is a common chronic complex disease characterized by inflammation, constriction of the airways and bronchial hyperresponsiveness to external stimuli. Susceptibility loci for asthma have been mapped to regions in many chromosomes including Chromosome 1p36 where the *NPPA* (natriuretic peptide precursor A) gene is located. *NPPA* gene encodes for atrial natriuretic peptide (ANP). ANP is produced mainly in the atria and ventricles and distributed in most tissues of the body, playing an important role in vasodilation, bronchorelaxation, pulmonary permeability, and in augmenting allergic inflammation and asthma. We hypothesized that *NPPA* polymorphisms are associated with asthma. Participants with well-characterized asthma (Cases, 297 Whites and 114 Blacks) were selected from clinical trials sponsored by the American Lung Association (NEJM 2001;345:1529; Am J Respir Crit Care Med 2007;175:235). Healthy subjects (Controls, 114 Whites and 73 Blacks) were recruited from a recent study (Metabolism 2007;56L757). Three *NPPA* SNPs were analyzed by a LightTyper instrument using a case-control approach: A/G (rs5063) in Exon 1 resulting in Met32 to Val substitution; C/T (rs5065) in Exon 3 resulting in a change of Arg152 to Ter and A/G (rs5067) in the 3'UT region. All 3 SNPs were in HWE for both ethnic groups, except rs5063 in Blacks. Minor allele frequencies for rs5063 A, rs5065 C and rs5067 A were significantly higher in Blacks compared to Whites: 0.074, 0.369, and 0.381 vs. 0.045, 0.160, and 0.100, respectively ($p \leq 0.002$). In both ethnic groups, significant associations were found between asthma and rs5065 ($p \leq 0.003$) and rs5067 ($p \leq 0.009$), with a trend for rs5063 ($p \leq 0.02$). The frequencies of the minor allele carriers for rs5063, rs5065 and rs5067 in Controls vs. Cases combined from the 2 ethnic groups were 15.8% vs. 7.1%, 52.3% vs. 31.6%, 40.5% vs. 23.3% respectively. We conclude that *NPPA* is an asthma susceptibility gene.

2347/W

Significant epistasis among susceptibility genes confers increased asthma risk in farmers. *M. Zucchelli[1], A. Dixon[3], E. Melen[2], M. Kronqvist[4], M. Moffatt[3], W.O. Cookson[3], M. van Hage[4].* 1) Centre for Biotechnology at Novum, Karolinska Inst, Stockholm, Sweden; 2) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3) National Heart and Lung Institute, Molecular Genetics Division, Imperial College, London, UK; 4) Department of Allergy and Clinical Immunology, Institute of Medicine, Karolinska Institutet, Stockholm, Sweden.

Studies of the molecular genetics of asthma and IgE-mediated allergy have pointed out the complexities of these diseases. Multiple chromosomal regions of genetic linkage have been observed and a number of candidate genes for allergy and asthma have been described. Gene-gene interactions, the effect of one locus being altered by effects at another locus/loci, have previously been reported between several candidate genes, especially those involved in the inflammatory response such as the TH2 cytokines. We have genotyped 56 SNPs in 12 common asthma and allergy susceptibility genes in 461 farmers of European descent. Individuals were tested for asthma, allergic disease, atopy and sensitisation to a number of common inhalant allergens. At the SNP level we could replicate, although with modest effect size, that the recently cloned asthma susceptibility gene PHF11, is also associated with asthma and allergy in this specific population. When exploring epistatic effects, we found significant interactions between TNF-? (TNF), FCER1B (MS4A2) and TLR9 with regard to asthma and atopy. Other biologically interesting effects were observed between other genotyped genes but the p-values were not significant after correction for multiple testing. Our results confirm that gene-gene interaction is a common phenomenon in complex diseases such as asthma and allergy and that epistatic analyses should be evaluated when conducting genetics studies of these diseases.

2348/W

GENETIC/NUTRIENT DETERMINANTS OF CONGENITAL HEART DEFECTS IN THE INUIT OF NUNAVUT. *L. Arbour[1], G. Osborne[2], R. Rupps[1], M. Forth[2], M. Nowdluk[2], L. Field[1], R. Rozen[3].* 1) Dept of Medical Genetics, University of British Columbia, Vanouver, BC, Canada; 2) Dept of Health and Social Services, Iqaluit, Nunavut; 3) Dept of Human Genetics and Pediatrics, McGill University, Montreal,Quebec.

There is suggestion that not only neural tube defects (NTDs) but other anomalies, such as heart defects, might be reduced with folic acid, either with multivitamin use or grain fortification. Folate has been long considered a nutrient of concern in Northern communities where the availability and preference for folate rich foods is lower than in the south. Although NTDs are not more frequent in Nunavut, septal heart defects were documented to be increased by nearly 4 times (ICD-9 745) prefortification. Methods: To explore determinants of the increased rate of septal heart defects Inuit mothers of children with and without heart defects were invited to participate in a case-control study evaluating nutrient intake, pregnancy exposures, RBC folate, serum cobalamin, homocysteine, and six functional polymorphisms for genes important in folate metabolism and uptake (MTHFR A222V and E429A, MTRR I22M, RFC-1 H27R, BHMT R239Q, MTHFD1 R653Q). Results: 61 children with isolated heart defects and their mothers (n=60) with 58 community matched controls participated. There were no differences in RBC folate (953 Vs 957 nmol/L p=.94), serum cobalamin (380 Vs 354 pmol/L p= 0.35), and homocysteine (8.8 Vs. 8.9 μmol/L p=0.93) between mothers of cases and controls. There was no difference in alcohol (≈20%) and cigarette use (≈80%) in pregnancy. No women were taking multivites at conception nor at the time of this study. However, RFC-1 H27R was more common in cases (OR 3.2 CI 1.1-9.2 p=.03) and in mothers of cases (3.9 CI 1.43-10.9 p=.01) than controls. Although the allele frequency was low for MTHFR A222V, this variant was also increased in cases (p=.04) alone. Conclusion: Previously implicated in infants with heart defects, RFC-1, a gene important in cellular transport of folate may contribute to the multifactorial etiology of septal heart defects in this population. Further study is underway to determine if heart defects have decreased since folic acid fortification was commenced.

2349/W

Angelman syndrome mouse model with a large chromosomal deletion from Ube3a to Gabrb3. *Y-H. Jiang[1], Y. Pan[1], L. Landa[1], C. Spencer[1], M. Brilliant[2], A.L. Beaudet[1].* 1) Department of Molecular and Human Genetics, Houston, TX; 2) Department of Pediatrics, University of Arizona Health Science Center. Tucson, AZ 85724.

Angelman syndrome (AS) is a neurobehavioral disorder associated with severe mental retardation, absence of language development, epilepsy, happy disposition, and movement disorders. The molecular defects underlying AS are heterogeneous, including large chromosomal deletions of 15q11-q13 of exclusively maternal origin (70%), paternal uniparental disomy (UPD) of chromosome 15, imprinting mutations, and mutations in the E6-AP ubiquitin ligase gene (*UBE3A*). Previously, we have characterized an AS mouse model by inactivation of the *Ube3a* gene in mice. Using chromosomal engineering strategy by the cre-loxP and Hprt technique, we have generated mutant mice with a deletion from *Ube3a* to *Gabrb3* which inactivates both of these genes as well as *Atp10a* as is the case for the majority of human AS patients (70%) with a large chromosomal deletion. Homozygous mutant mice with this deletion died in the perinatal period largely due to the cleft palate resulting from null mutation of *Gabrb3* as previously reported. Mice with a maternal deletion are viable and have no apparent developmental defect. There is no significant difference for the expression *Atp10a* gene is sub-regions of brain between the maternal and paternal deletion which suggest that the *Atp10a* gene is biallelicly expressed in these regions. The behavioral analysis revealed significant impairments in motor function, spatial and fear conditioning memory, open-field, and light dark testing in maternal deletion mice. The hippocampal long-term potentiation is impaired in maternal deletion mice. In addition, we have recorded ultrasonic calls emitted from pups with a maternal deletion. This analysis revealed a significant difference of ultrasonic calls between pups with a maternal deletion and wild type. Thus, mice with a deletion from *Ube3a* to *Gabrb3* provide another valuable mouse model for exploring the potential of treatment strategies or for making comparisons to that of *Ube3a* maternal deficiency mice to dissect the molecular pathogenesis of AS.

2350/W

Genome-wide expression profiling of urinary bladder identifies candidate genes for the bladder exstrophy-epispadias complex (BEEC). *L. Qi[1], K. Chen[2], D. Hur[3], Y. Lakshmanan[4], L. Kotch[5], G. Ashrafi[3], F. Martinez-Murillo[5], A. Blackford[6], J. Kowalski[6], J. Gearhart[4], S. Boyadjiev[7,4].* 1) Rowe Program in Human Genetics, UC Davis, Davis, CA; 2) Dept. of Statistics, UC Davis, Davis, CA; 3) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 4) Division of Pediatric Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University, Baltimore, MD; 5) Dept. of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD; 6) Dept. of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; 7) Section of Genetics, Dept. of Pediatrics, UC Davis, Sacramento, CA.

BEEC represents a spectrum of rare congenital anomalies ranging from isolated epispadias and classic bladder exstrophy, to cloacal exstrophy. While the causes of BEEC are unknown, there is evidence that genetic factors are involved in its etiology. In order to identify candidate genes of BEEC, we performed a genome-wide expression analysis of human urinary bladder at two timepoints: embryonic mesenchyme (EM) surrounding the urogenital sinus at 8 - 16 weeks of gestation, normal postnatal bladder (NB) and exstrophic postnatal bladder (EB). Gene expression profiles were obtained for 3 independent EM samples, 3 independent EB and 3 gender and ethnicity matched NB samples using Affymetrix GeneChip Human U133 Plus 2.0 arrays. In addition, the expression profile of mouse bladder at gestational day (GD) 13 was also determined using the GeneChip Mouse Genome 430 2.0 arrays. We identified 170 genes with at least 2-fold expression difference between NB and EB samples, which are also consistently expressed in the 3 EM samples. Moreover, about 90% of these 170 human genes were also detected in the mouse GD 13 sample, indicating most of the genes that involve in embryonic bladder development are expressed at the same developmentally relevant period in both human and mouse. Using pathway analysis, we identified 5 potential networks composed of at least 10 candidate genes. Further study of these candidate genes may lead to better understanding of the genetic etiology of BEEC.

2351/W

Haptoglobin genotyping and cardiovascular risk in subjects with diabetes mellitus. *A. Millson[1], B.D. Horne[2], J.L. Anderson[2], J.F. Carlquist[2], W.L. Roberts[1,3], E. Lyon[1,3].* 1) ARUP Inst for Clin & Exp Path, ARUP Laboratories, Salt Lake City, UT; 2) Intermountain Health Care, Salt Lake City,UT; 3) Pathology Dept, University of Utah, Salt Lake City, UT.

Haptoglobin (Hp) is a serum protein with many functions. The best known is as an antioxidant, binding hemoglobin released during red cell hemolysis, thus reducing kidney damage. Hp is composed of 4 polypeptide chains, 2 alpha and 2 beta. The alpha chain has two common alleles, Hp1 and Hp2, the Hp2 allele resulting from a duplication of Hp1. The beta chain is identical in all Hp types. The biochemical properties of the haptoglobin molecule vary depending on which alleles are present (Hp1-1, Hp1-2 or Hp2-2). Haptoglobin genotype has been shown to be an independent risk factor in individuals with diabetes mellitus for coronary artery disease (CAD). We evaluated a series of 3,137 subjects enrolled in the Intermountain Heart Collaborative Study Registry to assess possible association of haptoglobin genotype and CAD. About 70% of the study group had severe CAD and 60% showed abnormal glucose metabolism. 705 out of 3,137 subjects were diabetic. All subjects had >3 years of clinical follow-up. Haptoglobin genotyping was performed on the LightCycler™ using two polymerase chain reactions, one for the Hp1and one for the Hp2 allele, followed by fluorescent monitoring using hybridization probes. One of the study objectives was to assess risk of atherosclerotic complications in the diabetic subjects. Our primary endpoint was angiographic CAD. Our secondary endpoints were death due to myocardial infarction (MI), all-cause death and MI. The genotype frequencies were similar between the diabetics and non-diabetics. We found our primary endpoint of angiographic CAD to be significant, p=0.013 with the p-trend = 0.0003, in only the diabetic subjects with the Hp1-1 genotype. Our findings are similar to the Framingham Offspring cohort study (2004) yet contradict other studies which found diabetics subjects with the Hp2-2 genotype at increased risk for CAD.

2352/W

Limited evidence of association to type 2 diabetes in African Americans with WGA diabetes SNPs. *D.W. Bowden, J.P. Lewis, N.D. Palmer, M.M. Sale, B.I. Freedman.* Wake Forest University School of Medicine, Winston-Salem, NC.

Recently, several genome-wide association (WGA) studies have reported identification of multiple type 2 diabetes mellitus (T2DM) susceptibility genes in various Caucasian populations. However, little or no investigation of these loci has been reported in African Americans (AA). Striking differences between these populations suggest they may not share identical genetic risk factors. Previously we have shown that common genetic variants in *TCF7L2* contribute to T2DM in AAs (Sale et al., submitted). Our objective was to examine the influence of genes recently identified in WGA studies in a large AA case-control population. We genotyped 17 SNPs in 11 T2DM loci previously associated in Caucasians including *HHEX*, *SLC30A8*, *CDKAL1*, *PKN2*, *IGF2BP2*, *FLJ39370*, *EXT2*/*ALX4*, *FTO*, and LOC387761 in a sample of 1048 T2DM AA cases enriched for diabetic nephropathy and 1128 AA controls. In contrast to prior reports of association, our analysis of SNP data provided little evidence of association with T2DM. However, a SNP in intron 5 of *CDKAL1* (rs10946398) was marginally associated with T2DM in this population (2df P=0.0394, OR 0.88). In addition, rs7480010 located in LOC387761 also reached statistical significance (2df P=0.0036, OR 0.77) but was inconsistent with Hardy-Weinberg proportions (P=0.0035). All other SNPs investigated were not associated with T2DM with 2df P-values ranging from 0.06-0.97. Interestingly, 4 of the SNPs are nonpolymorphic in the Yoruba population of the HAPMAP project but were polymorphic in AAs. This may represent true allele frequency differences within different African-derived populations or may be a consequence of admixture. Overall, despite the highly significant evidence of association of these genes in Caucasian samples, this study suggests these variants do not contribute in a major way to diabetes susceptibility in the AA populations. These results also suggest genes contributing to T2DM in African Americans are, at least in part, different from those in Caucasians.

2353/W

Angiotensin converting enzyme gene polymorphism and diabetic nephropathy in Filipino type 2 diabetes mellitus patients. *E.M.C. Cutiongco[1], E. Paz-Pacheco[2], G.V. Jasul[2], M.C.A. Cruz[2].* 1) Institute of Human Genetics, National Institutes of Health Philippines, Manila, Philippines; 2) Department of Medicine, University of the Philippines - Philippine General Hospital, Philippines.

Objective: To determine the frequencies of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism among Filipino type 2 diabetic patients and normal controls. Methods: We performed a preliminary analysis of the ACE gene polymorphism in diabetic patients with nephropathy, diabetic patients without nephropathy, and normal controls. Patients with established renal disease other than diabetic nephropathy were excluded from the study. The diabetic patients were evaluated by the following parameters: duration of diabetes, presence of comorbid conditions, body mass index (BMI), systolic blood pressure (BP), diastolic BP, glycosylated hemoglobin (A1c), and presence of nephropathy. We extracted DNA from peripheral blood and determined the type of polymorphism (II homozygote, DD homozygote or ID heterozygote) via polymerase chain reaction, restriction enzyme digestion, and gel electrophoresis techniques. We analyzed the data using independent T-tests and chi square tests to compare the clinical characteristics of the two groups of diabetic patients, and logistic regression analysis to determine odds ratio for development of nephropathy. Results: Among the patients with diabetic nephropathy (n=21), the ID polymorphism was more frequent (52.4%) compared to the homozygous II and DD polymorphisms. In those without diabetic nephropathy (n=21), the II genotype was more common (61.9%). The ID polymorphism was the more frequent genotype in the normal controls (n=24) (58.3%). The odds of developing diabetic nephropathy were increased by 4.8 times in those with ID polymorphism, and 2.9 times in those with DD polymorphism. Conclusion: We found that the D allele (ID and DD genotypes) was more common in patients with diabetic nephropathy, similar to the observation in South Indian patients. Genetic studies on larger diabetic populations are needed to establish the hypothesized role of the D allele in susceptibility to diabetic nephropathy.

2354/W

Association of the distal region of the Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) gene with Type 2 Diabetes enriched for nephropathy in an African American Population. *K.L. Keene[1], J.C. Mychaleckyj[2,3], S.G. Smith[1], T.S. Leak[1], C.D. Langefeld[4], B.I. Freedman[5], S.S. Rich[2,3,6], D.W. Bowden[1,5,7], M.M. Sale[1,2,5,6,8].* 1) Molec Med/Ctr Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Center for Public Health Genomics; 3) Department of Public Health Sciences,Univ of VA, Charlottesville, VA; 4) Division of Public Health Sciences; 5) Department of Internal Medicine; 6) Department of Medicine, University of Virginia, Charlottesville, VA; 7) Department of Biochemistry; 8) Department of Biochemistry and Molecular Genetics.

Variants in the Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) gene have shown positive associations with diabetes and several diabetes-related phenotypes including insulin resistance, metabolic syndrome, and type 1 diabetic nephropathy. Additionally, evidence for linkage for type 2 diabetes mellitus (T2DM) in African Americans (AA) was observed at 6q24-27, with the proximal edge of the peak encompassing the ENPP1 gene. To comprehensively evaluate variants in ENPP1 for association with T2DM-ESRD, forty-nine SNPs located in the coding and flanking regions of ENPP1 were genotyped in 577 AA individuals with T2DM-ESRD and 596 AA without a diagnosis for T2DM. Haplotypic association and genotypic association for the dominant, additive, and recessive models were tested by calculating a c2 statistic and corresponding P value using the program SNPGWA. Nine SNPs showed nominal evidence for association (P< 0.05) with T2DM-ESRD in one or more genotypic model. The most significant associations were observed with rs7754586 (P= 0.003 dominant model, P= 0.0005 additive, and P=0.007 recessive), located in the 3' UTR, and an intron 24 SNP (rs1974201: P=0.004 dominant, P=0.0005 additive, and P=0.005 recessive). This study was the first to comprehensively evaluate variants of the ENPP1 gene for association in an AA population with T2DM and ESRD and suggests that variants in the distal region of the ENPP1 gene may contribute to T2DM-ESRD susceptibility in AA.

2355/W

Variants in the ELMO1 gene are associated with diabetes and nephropathy in African Americans. *T.S. Leak[1], P.S. Perlegas[2], S.G. Smith[1], P.J. Hicks[2], L. Lu[3], C.D. Langefeld[3], K.L. Keene[1], M.M. Sale[1,4,5,6,7,8], B.I. Freedman[4], D.W. Bowden[1,2,3,4].* 1) Molecular Gen/Human Genetics; 2) Department of Biochemistry and Molecular Genetics; 3) Public Health Sciences; 4) Internal Medicine, Wake Forest Univ Sch Med, Winston-Salem, NC; 5) Center for Public Health Genomics; 6) Department of Public Health Sciences; 7) Department of Medicine; 8) Department of Biochemistry and Molecular Genetics, Univ of VA, Charlottesville, VA.

The engulfment and cell motility 1 (ELMO1) gene on chromosome 7p14.2-14.1 has been associated with type 2 diabetes mellitus (T2DM)-associated nephropathy in a Japanese cohort. Three hundred-one HapMap and 10 reportedly associated SNPs were genotyped in 577 African American (AA) T2DM patients with end-stage renal disease (ESRD), 596 AA controls without a current diabetes diagnosis or kidney disease, with an average density of 1 SNP every 2kb. Of the 311 SNPs tested, 98 (31.5%) showed suggestive evidence of association (P ≤ 0.05) in one or more tests of association: allelic, overall genotypic, dominant, additive, or recessive genotypic models, allelic, 2- and 3-SNP haplotypic analyses. Haplotype analysis revealed 8 and 11 overlapping 3 and 2-SNP haplotypes, respectively, containing a total of 12 and 22 independent SNPs spanning introns 1 and 13 that were associated with susceptibility to T2DM-ESRD (P ≤ 0.05). The associated SNPs were genotyped in independent populations of 564 AA controls, 328 AA with T2DM lacking nephropathy, 326 AA with non-diabetic forms of ESRD and 558 additional AA T2DM-ESRD subjects. Significant associations were detected between SNPs spanning introns 1 and 13 of the ELMO1 gene in T2DM without nephropathy (P range <0.0001 - 0.049), non-diabetic ESRD (P = <0.0001 - 0.049) and T2DM-ESRD (P = 0.001 - 0.049). In the combined analysis of 1135 T2DM-ESRD cases and 1160 controls also showed evidence of association (P = <0.0001 - P=0.049). We have observed evidence that ELMO1 is associated with T2DM and both diabetes and non-diabetes associated kidney disease. The consistent evidence of association suggest pleiotropic effects for this gene, possibly through metabolic syndrome- a phenotype common to each patient group.

2356/W

CDKAL1 and diabetes in Mexican Americans. *J.H. Lieman[1,2], R.J. Leach[2,3], M. Escamilla[2,3], H.H.H. Goring[4], J. Blangero[4], R. Duggirala[4], M.P. Stern[2,3], D.M. Lehman[2,3].* 1) The University of Texas Pan American, Edinburg, TX; 2) South Texas Medical Genetics Group University of Texas Health Science Center San Antonio, Edinburg, TX; 3) The University of Texas Health Science Center, San Antonio, TX; 4) Southwest Foundation for Biomedical Research, San Antonio, TX.

It is now widely established that hereditary factors influence risk for development of type 2 diabetes (T2D), yet few gene variants have been confidently identified through consistent replication. Recently, several genome-wide association studies, conducted primarily in Caucasian populations, have identified and replicated T2D-associated variants in and near novel candidate genes. These data are consistent with the notion that multiple genetic factors affect T2D risk, with each conferring incremental risk. Since the genetic risk factors may vary between ethnic groups, we sought to determine whether these same variants contribute to T2D risk in a Mexican American population, the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) which consists of large pedigrees (n=692 genotyped). The variants tested were rs7756992 and rs10946389 (CDKAL1), rs10811661 (near CDKN2A/CDKN2B), rs4402960 (IGF2BP2), rs1111875 (near HHEX), rs94759 (near CEP55), and rs8050136 (near FTO). All SNPs conformed to HWE expectations. Each SNP was tested for association with the traits diabetes and diabetes age-of-onset using a measured genotype approach, as implemented in SOLAR. We observed nominal association with diabetes for the 2 highly correlated SNPs located in CDKAL1 (rs7756992 p=0.02 RR=1.20 for GG genotype; rs10946398 p=0.01 RR=1.24 for GG genotype). No other associations were detected. Further, accounting for bmi did not affect any of the diabetes association results (average bmi = 31.5). These results provide supportive evidence for a potential role for alterations in the CDKAL1 gene in T2D pathogenesis with comparable risk among Mexican Americans as that reported in Caucasian populations. The risk for the homozygous carriers of the minor alleles at the SNPs in CDKAL1 was much stronger than that for the heterozygotes, consistent with results reported in Steinthorsdottir et al (Nature Genetics, 2007).

2357/W

Sequence variants of insulin-secreting pathway genes contributing to type 2 diabetes risk in the Yakut population of Eastern Siberia. *Z. Odgerel[1], H.S. Lee[1], F.A. Platonov[2], N. Sambuughin[1], P.M Ignatiev[2], L.L. Alekseeva[2], V.L. Osakovskiy[2], T.M. Sivtseva[2], V.G. Krivoshapkin[2], L.G. Goldfarb[1].* 1) NINDS, NIH, Bethesda, MD; 2) Institute of Health, Yakutsk, Russian Federation.

Yakut (Sakha) population originated from a nomadic Central Asian tribe that migrated about 900 years ago to the Siberian plains, the coldest area in the Northern hemisphere with average January temperatures of -41°C and a world record of -72.2°C. Basic metabolic rate in Yakut people is elevated, the level of blood glucose increased, and the amount of circulating insulin decreased as a result of adaptation to chronic and severe cold stress. A recent change in lifestyles and food composition from traditional to predominantly carbohydrate diets led to a 10-fold increase in the prevalence of type 2 diabetes, with an alarming tendency of further growth. We investigated whether adjustment to colder environment led to adaptive selection of functional polymorphisms in genes involved in the insulin-signaling pathway. The frequency of alleles previously shown to be associated with T2D was determined in the Yakut T2D patients and compared to the baseline population in the format of a case-control association analysis. The study population consisted of 178 subjects diagnosed with T2D and 125 individuals in which diabetes was excluded. Fifteen sequence variants in nine genes were selected on the basis of their association with T2D replicated in at least two previously studied populations. After computing odds ratio based on presence/absence of risk allele (allelic positivity test), we identified three variants showing significant association with T2D. Two were identified in the ABCC8 gene: a C>T change in the third nucleotide of codon 562 leaving His as the encoded amino acid (P=0.018) and an A>G substitution at codon 1273 with Arg residue remaining unchanged (P=0.041). The C variant at the minus 1031 position of the regulatory region in TNFalpha gene also shows association (P=0.006). The prevalence of risk alleles ABCC 562T and TNFalpha -1031C in non-diabetic Yakuts were 51% and 30% vs. 30 to 32% and 16 to 18%, respectively, in Southern Asian populations, from which Yakuts originated a millennium ago.

2358/W

The Type 2 Diabetes Gene CDKAL1 is Expressed in Beta Cells and Modulated by Glucose Concentration. *V. Steinthorsdottir[1], I. Reynisdottir[1], G. Thorleifsson[1], S. Ghosh[1], R. Benediktsson[2], G. Sigurdsson[2], A. Kong[1], M. Gurney[1], J.R. Gulcher[1], U. Thorsteinsdottir[1], K. Stefansson[1].* 1) deCode Genetics, Reykjavik, Iceland; 2) Landspitali University Hospital, Reykjavik, Iceland.

The CDKAL1 gene, identified through genome-wide association study, was found to be associated to increased risk of T2D in nearly a recessive manner, with genotype odds ratio for the homozygous carrier 1.45 and 1.55 for individuals of European and Asian ancestry, respectively. The function of the CDKAL1 gene product is unknown but it is similar to another protein, CDK5RAP1, an inhibitor of the CDK5/p35 complex in neuronal tissue. This complex is also expressed in pancreatic beta cells and, in the presence of its active form, insulin expression is decreased under glucotoxic conditions. This led to the hypothesis that CDKAL1 might be an inhibitor of the CDK5/p35 complex in pancreatic beta cells. Furthermore, we have shown that the risk variant of CDKAL1 is associated with reduced insulin secretion and this effect is mostly seen for the homozygote where a 24% reduction in insulin response is observed compared to the heterozygous carriers or non-carriers. This is in line with the nearly recessive mode of inheritance observed for this variant with respect to disease risk. The aim of this study was to gain further insight into the role of CDKAL1 using the rat pancreatic beta cell line INS-1. The rat pancreatic beta cell line INS-1 was cultured in the presence of variable glucose concentration, ranging from 2.5-30 mM, to evaluate whether CDKAL1 expression is regulated by glucose concentration. We demonstrated that the expression of CDKAL1 in rat INS-1 cells varied according to glucose concentration in the culture medium with reduced expression detected under glucotoxic conditions compared to normal glucose concentration. This indicates that CDKAL1 expression in pancreatic beta cells is sensitive to glucose concentration. It is possible that this response to glucose may be affected in individuals carrying the variant of CDKAL1 that is associated to T2D. This could explain the reduced insulin secretion observed by these individuals in response to an oral glucose challenge.

2359/W

Association of HTRA1 polymorphism and bilateral neovascular age-related macular degeneration. *H. Chen[1,2,3], Z. Yang[1,2], D. Gibbs[1,2], C. Olson[1,2], J. Harmon[1,2], Z. Tong[1,2], S. Tang[3], K. Zhang[1,2].* 1) Ophthalmology, University of Utah, Salt Lake City, UT; 2) Human Molecular Biology and Genetics, University of Utah, Salt Lake City, UT; 3) Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.

PURPOSE: Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in elderly people. Recently, a single nucleotide polymorphism (SNP), rs11200638, in the promoter of the HTRA1 gene was found to be associated with wet and dry form of AMD. The purpose of this study is to investigate the association of rs11200638 SNP with bilateral and unilateral wet AMD. METHODS: AMD patients and age matched controls were enrolled and genotyped for the rs11200638 polymorphism. AMD patients were classified as bilateral wet AMD, unilateral wet AMD, bilateral dry AMD and unilateral dry AMD. Wet AMD eyes were also classified according to choroidal neovascularization subtype including classic, occult and mixed choroidal neovascularization. Allele frequencies and genotype frequencies were compared among different phenotype groups by a chi square test. Odds ratios (ORs) and 95% confidential intervals were calculated to estimate risk. RESULTS: The A allele and AA genotype at the SNP rs11200638 of HTRA1, were significantly more prevalent in bilateral wet AMD patients than unilateral wet AMD patients (p=0.04 and 0.03 respectively, chi square test). The homozygote OR of binocular wet AMD(12.41; 95% CI, 5.70-27.03) was 1.95 fold greater than that of monocular wet AMD (6.36; 95% CI, 2.98-13.55). The same trend was seen in dry AMD. There is no significant difference of allele or genotype frequencies among the subtypes of choroidal neovascularization. CONCLUSIONS: The SNP rs11200638 in the promoter of HTRA1 was associated with bilaterality of AMD. The risk allele A conferred higher risk to binocular AMD than monocular AMD. This is consistent with the role of HTRA1 in the pathogenesis. Further study on HTRA1 will provide insight into pathogenesis of AMD.

2360/W

SNP Analysis of the PHF11 gene in Italian families with Allergic Asthma. *P. Zorzi, C. Bombieri, G. Malerba, L. Xumerle, E. Trabetti, P.F. Pignatti.* Department of Mother and Child, Biology and Genetics, University of Verona, Italy.

Asthma is a chronic inflammatory disorder of the airways characterized by reversible obstruction, and bronchial hyper-reactivity. Asthma has an important genetic component but no clear pattern of inheritance. In a previous genome scan for asthma, conducted on 123 Italian families, characterized for clinical asthma, rhinitis, elevated total serum IgE, positive Skin Prick Test, and bronchial hyper-responsiveness (BHR) to methacholine, it has been observed the presence of linkage between region 13q14 and elevated total serum IgE. Two studies, recently reported in literature, Zhang et al., 2003, and Jang et al., 2005, identified an association of the PHF11 gene with elevated IgE and atopic dermatitis, respectively. An association study was performed by Transmission Disequilibrium Test (TDT) on 23 Italian families (144 individuals) presenting positive linkage to elevated total serum IgE, using 7 SNPs on the PHF11 gene, reported in literature associated with elevated IgE and atopic dermatitis. 5 SNPs (rs2031532 G/A, rs1046295 G/A, 185752b5_2 C/T, 185306b7_1 A/C, and 185752b4_2 A/G) have been analyzed in multiplex by ddNTP Primer Extension technique, using the SNaPshot Multiplex kit (Applied Biosystems) on the ABI PRISM 310 Genetic Analyzer with the Genescan software. Two SNPs (rs2247119 C/T, and rs2274276 C/G) have been analyzed through PCR and enzymatic restriction. The observed frequencies for the minor allele were rs2031532 A 12%, rs1046295 A 25%, 185752b5_2 T 37%, 185306b7_1 C 38%, e 185752b4_2 G 27%, rs2247119 T 10%, and rs2274276 G 29%. Linkage analysis through the Merlin program showed presence of linkage for atopy (p=0.001) and IgE (p=0.002). TDT did not show any significant association between the analyzed SNPs and IgE in allergic asthma. No significant preferential transmission of the haplotype was observed for any of the analyzed phenotypes. The analysis in our population suggests that association could be linked to the presence of other polymorphisms in the PHF11 gene or neighbouring genes. It may be appropriate to further extend the analyzed region to other neighbouring region, which demonstrated positive linkage to asthma and related phenotypes.

2361/W

B9 - A Potential Basal Body Localization Domain. *J.F. Robinson, N. Katsanis, PhD.* McKusik-Nathans IGM, Johns Hopkins School of Medicine, Baltimore, MD.

Primary cilia are cellular appendages originally thought vestigial organelles; however recent work has demonstrated their involvement in a myriad of sensory functions. Defects in primary ciliary signaling are also implicated in human disorders termed collectively ciliopathies, which include Polycystic Kidney Disease, Bardet-Biedl (BBS), Meckel-Gruber (MKS), and Joubert Syndrome. Although there is a vast and growing number of proteins that have been shown to localize to the cilium and/or its anchor, a modified centriole termed the basal body, the mechanism of targeting ciliary proteins remains completely elusive. One protein, MKS1, has been implicated recently in several ciliopathies, including MKS and BBS, has been shown to localize to the basal body, where it has been shown to be necessary for ciliogenesis. The C. Reinhardtii orthologue of MSK1 has been suggested to encode a core structural component of the centriole termed the B9 domain. Through searches of the human genome we have identified a total of three predicted proteins, MKS1, LOC80776 and EPPB9, encoding polypeptides with B9 domains. Interestingly, each of these three proteins has been predicted to serve as ciliary function as it is present in the integrated ciliary proteome (www.ciliaproteome.org), suggesting a cilia-specific role for the poorly characterized B9 domain. In this project we combine localization studies with mutational studies to further characterize the B9 domain and its importance in ciliary localization.

2362/W

The GLI1 gene as a risk factor for ulcerative colitis.*GLI1.* *R.W. Bentley[1], R.L. Roberts[1], R.B. Gearry[2,3], T.R. Merriman[4], M.A. Kennedy[1], M.L. Barclay[2,3].* 1) Department of Pathology; 2) Department of Medicine; 3) Department of Gastroenterology, Christchurch School of Medicine, Christchurch, New Zealand; 4) Department of Biochemistry, University of Otago, Dunedin, New Zealand.

Introduction. Crohn's disease (CD)and ulcerative colitis (UC)are the major forms of inflammatory bowel disease (IBD) and recent work has shown a high prevalence of IBD in New Zealand. For CD, mutations in the *IL23R, ATG16L1* and *NOD2* genes have been reported to predict the age of onset, severity and location of disease but no strong genetic marker for UC has yet been found. Preliminary analysis of four SNPs(rs2228224, rs2228225, rs2228226 and rs3817474) of the *GLI1* gene in a Scottish cohort indicated that two of the haplotypes generated had a highly significant association with UC susceptibility. *GLI1* encodes a transcription factor that plays an important role in the formation and maintenance of a healthy gut and defective *GLI1* function in people with IBD suggests that it may be a risk factor for UC.

Aim. To determine whether polymorphic variation in the *GLI1* gene at the four sites described is implicated in susceptibility to IBD in Canterbury, New Zealand.

Methods. SNPs were assayed using allele-specific PCR in controls (n=479), CD (n=525) and UC (n=479). Direct sequencing was used to validate the assay. Haplotypes were estimated and tested for association.

Results. Three haplotypes described the majority (>99%) of the variation in the *GLI1* gene at the SNPs investigated. Comparative analysis of genotype data demonstrated a significant association of the *GLI1* gene with UC (p<0.05)but not CD. In addition, a significant association was observed between the major haplotype and susceptibility to UC (p<0.05, OR(95% CI)1.227, 1.019-1.479).

Conclusion. Polymorphic variation at the SNP sites investigated suggests a role for the *GLI1* gene in ulcerative colitis, but not Crohns's disease, in a population of IBD patients from Canterbury, New Zealand.

2363/W

Detecting Loci That Confer Susceptibility to Dust Mite-Induced Asthma Using a Combined *In Vivo* and *In Silico* Approach. *S.N.P. Kelada[1], D.M. Brass[2], S. Maruoka[2], D.A. Schwartz[2,3], F.S. Collins[1].* 1) Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD; 2) Laboratory of Environmental Lung Diseases, National Heart Lung and Blood Institute, Research Triangle Park, NC; 3) National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Asthma is a disease of major public health concern. The etiology of asthma is multifactorial in nature, and involves interactions between genes and environment. Allergen exposure is a well known inciting factor for asthma among atopic individuals. In particular, house dust mite (HDM) exposure has consistently been linked to the development of asthma and exacerbations of symptoms. We aim to identify loci that confer susceptibility to HDM-induced asthma by examining the effects of HDM exposure *in vivo* across thirty inbred strains of mice whose genetic variation has previously been well characterized by a public mouse HapMap effort.

Mice are sensitized by two intra-peritoneal injections (on days 0 and 7) of purified natural dust mite allergen (nDer p 1), followed by oro-tracheal administration of the allergen on day 14. Forty-eight hours after airway challenge, cytokine levels and inflammatory cell influx into the lungs are measured, as well as pulmonary function by means of the Flexivent technique. Changes in gene expression in airway epithelial cells and T cells from lymph nodes are also examined. Strain-dependent responses (phenotypes) can then be mapped to loci *in silico* using a newly developed genome-wide association method that accounts for the population structure of the inbred strains of mice and employs a set of approximately 150,000 publicly accessible haplotype tagging SNPs. Both cis- and trans- expression determinants can be mapped using this method. Results from these experiments will be used to guide candidate gene selection in a case-control study of asthma susceptibility in humans.

2364/W

Human PON1 During Development: Effects of Age and Genotype. *K. Huen[1], K. Harley[1], A. Bradman[1], C. Furlong[2], B. Eskenazi[1], N. Holland[1].* 1) Sch Public Health,Univ of California, Berkeley, CA; 2) University of Washington, Seattle, WA.

Paraoxonase 1 (PON1) is a high density lipoprotein (HDL) - associated enzyme, which can detoxify organophosphate (OP) pesticides. Additionally, it can prevent LDL oxidation, a marker of oxidative injury resulting from oxidative stress. Studies in both animals and humans report significantly lower serum and liver PON1 activity at birth in comparison to adults. Therefore, young infants may be more susceptible to OP pesticide exposures and oxidative stress. PON1 provides a distinctive example of a gene containing a common missense SNP (Q192R) that dramatically affects functional activity. PON1 activity is easily quantified by measuring rates of hydrolysis of various substrates. Previously, we showed that haplotype analysis of PON1 polymorphisms did not provide an advantage to analysis of individual genotypes when examining the effects of PON1 genotypes on PON1 functional activity. Here, we genotyped several PON1 polymorphisms in the coding and promoter regions and measured PON1 enzyme activity and PON1 levels in n=172 children at birth, 24 months, and 60 months of age. Hierarchical linear models were used to examine how PON1 levels and activity change over time from birth through 60 months of age and to determine if PON1 polymorphisms affect this age-related change. Both age and PON1 Q192R genotype were associated with PON1 levels and PON1 activity although no significant interaction between the two variables was identified. Contrary to previous reports that children reach a plateau comparable with adults at 24 months, average PON1 levels measured by arylesterase activity increased from birth through 39 months ($p<0.005$) while PON1 activity towards the substrate paraoxon, continued to increase from birth through 47 months ($p<0.005$). Further, children with the PON1 192 RR genotype have slightly lower PON1 levels than children with fewer R alleles ($p=0.003$) after adjusting for age. In contrast, mean PON1 activity was highest in children with the PON1 192 RR genotype after adjusting for age ($p<0.005$). Thus, early childhood represents a critical period of vulnerability to OP exposures and oxidative stress which is further influenced by PON1 genotypes.

2365/W

Worldwide incidences of type 1 diabetes are correlated with the frequencies in allele T of dbSNP rs2476601 of the PTPN22 gene. *YJ. Lee[1,3], CY. Huang[1], WH. Ting[1], CK. Chen[1], ZC. Wang[1], CL. Lin[1], HF. Liu[1], FS. Lo[2].* 1) Dept Pediatrics & Medical Res, Mackay Memorial Hosp, Tamshui, Taipei, Taiwan; 2) Division of Endocrinology, Department of Medicine, Chang Gung Children's Hospital, Taoyuan, Taiwan; 3) Department of Pediatrics, Taipei Medical University, Taipei, Taiwan.

The global variation in the incidence of type 1 diabetes (T1D) is thought to relate to the distribution of genetic or environmental factors. Although non-Asp-57 alleles of HLA-DQB1 gene are associated with the differences in the incidence of T1D, the association can not completely explain the variations of the incidence. Other genetic and/or environmental factors must play a role. dbSNP rs2476601 in the PTPN22 gene was found to be associated with T1D. The minor allele (T) confers risk. This SNP is absent in Asians who have a low incidence of T1D. Thus, we investigate the association between incidences of T1D and frequencies of allele T. **Subjects:** The subject were 305 hospital personnel. Their genomic DNA was genotyped for this SNP. **Literature search:** A literature search was done using both PubMed and OVID as well as a search in dbSNP of NCBI, HapMap and JSNP. All available reports on rs2476601 were evaluated. The incidences of T1D in various ethnic groups were compiled. **Results:** All 305 subjects were C/C. This SNP was absent in all subjects tested. The frequencies of allele T are 9.8% in Caucasians, 4.7% in Hispanic Americans, 4.4% in Colombians, 2.1% in Sardinians, 2.1% in African Americans, 1.3% in Indians, 0.04% in Africans, and 0.03% in Asians. Among Caucasians, Finns have the highest frequency of 15.4% and Spanish have the lowest one of 7.0%. Linear regression analysis showed the frequencies of allele T (T, %) were significantly correlated with the worldwide incidences of T1D (I, number/100,000). $I = 1.282 \times T + 5.301$ ($R = 0.548$, $p = 0.015$) when Sardinians were included and $I = 1.661 \times T + 0.952$ ($R = 0.798$, $p = 0.0001$) when Sardinians were excluded from analysis. **Conclusion:** Population variation in the frequencies of allele T of rs2476601 of the PTPN22 gene may explain much of the worldwide differences in the incidence of type 1 diabetes.

2366/W

Vitamin D Receptor Gene Genotypes are not Associated with Low Bone Density in RA Mexican Mestizo Women. *L. Sandoval-Ramírez[1,4], M.P. Casillas-Avila[1,4], L. González-López[3], M.A. López-Olivo[3], M.F. Alcaraz-López[3], E.A. Aguilar-Chávez[3], J.M. Oliva-Ortiz[1,4], I.P. Dávalos[1,4], E.R. Ochoa-Martínez[5], A. Celis[6], J.I. Gámez-Nava[2,4], M. Salazar-Páramo[2].* 1) División de Genética, Centro de Investigación Biomédica de Occidente, CMNO, IMSS; 2) Hospital de Especialidades, CMNO, IMSS; 3) Hospital General Regional No. 110, IMSS; 4) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara; 5) CUCBA, Universidad de Guadalajara; 6) Departamento de Salud Pública, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México.

Introduction. Patients with rheumatoid arthritis (RA) have risk factors to osteoporosis, but not all become sick even intake the same drugs and doses. The variance in BMD (Bone Mineral Density) has been attributed to genetic factors. Studies have been found association between polymorphisms of the vitamin D receptor (VDR) gene and BMD and they account for 75% primary genetic factor for BMD. Purpose. To determine whether polymorphisms of VDR gene are associated with osteoporosis in Mexican RA women patients. Methods. Genotyping of VDR polymorphisms were performed by PCR-RFLP analysis from 129 Mestizo Mexican women patients with RA (mean age 54 years; age range 75-40 years) and 36 healthy women controls (mean age 54 years; age range 87-46 years). Three VDR gene polymorphisms (ApaI, FokI, BsmI and TaqI) were investigated. BMD of vertebral spine and hip were made to everyone to determined osteoporosis presence (OMS T < -2.5 SD). Chi square and OR was made to estimate risk of osteoporosis. Results. The polymorphisms frequency were in osteoporotic RA group: ApaI (AA= 51.6%; Aa= 29%; aa= 19.4%); BsmI (BB= 6.2%; Bb= 68.8%; bb= 25%): FokI (FF= 35.3%; Ff= 52.9%; ff= 11.8%); TaqI (TT= 46.9%; Tt= 40.6%; tt= 12.5%); and nonosteoporotic RA group: ApaI (AA= 42.7%; Aa= 37.8%; aa= 19.5%); BsmI (BB= 14.3%; Bb= 51.6%; bb= 34.1%); FokI (FF= 28.4%; Ff= 49.5%; ff= 22.1%); TaqI (TT= 45.8%; Tt= 43.4%; tt= 10.8%). There was no significant difference ($p> 0.05$) between groups. Conclusion. Our results suggest that VDR polymorphisms do not play a major role in low bone mineral density predisposition in Mexican RA women patients.

2367/W

HTRA1 variant increases risk to neovascular age-related macular degeneration in Chinese population. *Z. Yang[1, 2], F. Lu[2], J. Hu[2], Q. Zhao[3], Y. Lin[2], Y. Yang[2], X. Liu[2], Y. Fan[2], B. Chen[3], S. Liao[2], C. Lei[3], D. Cameron[1], K. Zhang[1].* 1) Ophthalmology Research, Univ Utah, Salt Lake City, UT; 2) Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, Sichuan, China; 3) Xing Hua Hospital, Shanghai Jiao Tong University, Shanghai, China.

Purpose: Age-related macular degeneration (AMD) is a leading cause of irreversible visual impairment in the world. The two forms of advanced AMD, geographic atrophy (GA) and choroidal neovascularization (wet AMD), represent two types of degenerative processes in the macula that lead to loss of central vision. Drusen are characterized by deposits in macula without visual loss and are considered a precursor of advanced AMD. Recently rs11200638 in the promoter of HTRA1 has been shown to increases the risk for wet AMD in Caucaian and Hong Kong Chinese population. We investigated association between rs11200638 and wet AMD and drusen in a Mainland Chinese cohort. Methods: We genotyped rs11200638 for 128 Chinese patients (64 wet AMD and 64 drusen) and 106 normal controls. We performed chi square analysis for an additive allelic model. Results: rs11200638 was significantly associated with wet AMD (P=1.9X10-9). However, it is not associated with drusen in Chinese population . Conclusions: rs11200638 in HTRA1 is significantly associated with wet AMD in Mainland Chinese. These findings confirmed the role of HTRA1 in wet AMD. Understanding the underlying molecular mechanism will provide an important insight in pathogenesis of AMD. **Purpose**.

2368/W

Family-based genome-wide mapping of expression trait loci from peripheral blood CD4+ lymphocytes as a powerful means of identifying functional variation. *A. Murphy[1], V. Carey[1], R. Lazarus[1], B. Klanderman[1], J. Sylvia[1], J. Ziniti[1], C. Allaire[1], E. Silverman[1], C. Lange[2], S. Weiss[1], B. Raby[1].* 1) Channing Laboratory, Brigham & Women's Hospital, Harvard Medical School, Boston MA; 2) Harvard School of Public Health, Boston MA.

Regulatory genetic variation contributes substantially to phenotypic diversity, yet few approaches are available for identification of such variation. One proposed solution is expression quantitative trait loci (eQTL) mapping, with preliminary studies demonstrating the potential of this approach in both animal models and human cell lines (i.e. Schadt 2004). Herein we demonstrate both the feasibility and power of eQTL mapping in human populations using RNA derived from freshly harvested peripheral blood CD4+ lymphocytes from 96 young-adults participating in a genetic study of asthma. We generated VSN-normalized gene expression profiles using Illumina HumanRef8 arrays that survey 20,589 RefSeq-curated mRNA transcripts. Genome-wide genotype data (534,290 autosomal SNP, Illumina Infinium 550K array) were available for these subjects and their parents. Family-based association testing was performed (additive model) using PBAT. We screened for cis-acting variants (within 200kb of transcripts), resulting in 1.64 million tests. We adjusted for multiple comparisons using the conditional power screening approach (Van Steen 2005). Significant eQTL associations in 33 genes were observed for 74 of 100 SNP with highest power ($p=10^{-4}$-10^{-12}). This large number of significant associations was observed despite the relatively small sample size, due in large part to the strong genetic effects conferred by these loci (SNP-specific h^2=0.31-0.65). We also note that several of these significant associations have been previously described (Cheung 2005, Qu 2007), suggesting reproducibility. Finally, the power screening method outperformed Bonferroni correction; the latter identified only 30 significant ($p<10^{-8}$) associations. These results highlight the potential of family-based integrative genomic approaches for the identification of functional expression-related polymorphisms. Funding: U01 HL065899, P01 HL083069, R01 HL086601, K08 HL74193.

2369/W

No association between OPA1 polymorphisms and primary open-angle glaucoma (POAG) in three different populations. *Y. Liu[1], D. Munro[1], X. Qin[1], S. Schmidt[1], J. Wiggs[3], MA. Hauser[1,2], RR. Allingham[1,2].* 1) Center for Human Genetics, Duke Univ Medical Center, Durham, NC; 2) Department of Ophthalmology, Duke University Eye Center, Duke University Medical Center, Durham, NC; 3) Harvard Medical School, Boston, MA.

Mutations in the optic atrophy 1 (OPA1) gene have been associated with optic atrophy type 1, which is a dominantly inherited optic neuropathy resulting in progressive loss of visual acuity. SNPs rs10451941 and rs166850 of OPA1 have been associated with normal tension glaucoma (NTG) in the Caucasian and Japanese populations, as well as high tension glaucoma (HTG) in the Japanese population. No such association was found with NTG in the Korean or the African-Caribbean population of Barbados in West Indies, or with HTG in Caucasian population. We investigated the association between these SNPs and POAG with elevated intraocular pressure in the Caucasian (279 cases, 227 controls), African American (193 cases, 97 controls), and Ghanaian (West African) (170 cases, 138 controls) populations. We found no significant differences in OPA1 allele or genotype frequencies between POAG cases and controls at the rs10451941 and rs166850 SNPs in either dataset. In conclusion we report no association between two previously implicated OPA1 polymorphisms and a POAG phenotype that includes elevated IOP. This represents the first association analysis of OPA1 in high tension glaucoma in the African American and Ghanaian populations. OPA1 association with POAG may be limited to patients with normal tension glaucoma in these populations.

2370/W

***LPIN2* variations in psoriasis.** *H. El-Shanti[1], P.J. Ferguson[1], C. Madison[1], S. Leal[2], L.Y. Tan[1], T. Helms[1].* 1) University of Iowa, Iowa City, IA; 2) Baylor College of Medicine, Houston, TX.

STATEMENT OF PURPOSE: One approach to the identification of genes involved in a complex disorder is to examine the involvement of a candidate gene - identified due to its physiologic role or its causal role in a monogenic disorder of a similar phenotype - by genotyping for polymorphisms within the gene and performing association studies. Majeed syndrome is an autosomal recessive disorder characterized by chronic recurrent multifocal osteomyelitis, congenital dyserythropoietic anemia and an inflammatory dermatosis. Some of the carriers have psoriasis. We showed that homozygous mutations in *LPIN2* are responsible for Majeed syndrome. Furthermore, *LPIN2* is located within a psoriasis susceptibility locus. We hypothesize that *LPIN2* is the gene that predisposes to psoriasis at this locus. METHODS: We performed a case-control association study of 78 individuals with psoriasis and 44 controls. Genotyping was performed on 8 tag SNPs within *LPIN2* utilizing Taqman assays. Subsequently, the exons and splice sites of *LPIN2* were sequenced to identify variations in coding regions and splice sites. Further analysis utilizing techniques to detect large deletions or duplications within the genomic structure of *LPIN2* are ongoing. RESULTS: Preliminary assessment of the case-control association study did not reveal significant results; likely due to small numbers in our cohort. Sequencing revealed 3 coding variants that were present in affected individuals and not present in controls. The three variants are A331S, P348L and L504F. Conservation across species suggest that A331S and L504F may be significant. The variant A331S was not found in a large cohort of controls (CEPH-Human Diversity Panel). CONCLUSION: Although we have not demonstrated an association of *LPIN2* and psoriasis in our case-control association study, we have detected coding variations in at least one individual that is probably significant. Given Kryukov et al.'s recent data suggesting that most rare missense alleles are deleterious in humans, these variants need to be studied in further detail and in a larger cohort to determine their significance in the etiology of psoriasis.

2371/W

Lack of association between genotypes or haplotypes of ADRB2 and Juvenile Idiopathic Arthritis. *G. Pont-Kingdon[1], K. Sumner[1], B. Clifford[3], A. Whiting[3], E. Lyon[1,2], J. Bohnsack[3], S. Prahalad[3].* 1) Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Pathology Dept, University of Utah, Salt Lake City, UT; 3) Pediatrics Dept, University of Utah, Salt Lake City, UT.

The β2 adrenergic receptor (β2-AR) is present in numerous cell types and ADRB2 polymorphism(s) has been associated with asthma severity, response to beta agonist drugs, and rheumatoid arthritis (RA). The β2-AR has also been shown to contribute to the initiation and progression of joint damage in animals with experimental arthritis.. Our objective was to investigate ADRB2 variants for association with juvenile idiopathic arthritis (JIA) or major JIA subtypes. SNPs at position 46 and 79 result in substitution of glycine to arginine at position 16 (G16R), and of glutamine to glutamic acid at position 27 (E27Q). Both of these have been shown to have functional consequences. ADRB2 haplotypes were established in a cohort of 348 children with JIA and 448 autoimmunity free controls matched for ethnicity by direct molecular haplotyping using melting-curve analysis of a fluorescently labeled loci-spanning probe (LSProbe) that analyzed both SNPs simultaneously. Both ADRB2 SNPs were in Hardy-Weinberg equilibrium among controls. The minor allele frequencies in the controls at positions 16 and 27 were 36.7 % and 41.1% respectively. No association was found between JIA and the genotypes of the 2 ADRB2 SNPs as well as ADRB2 haplotypes. Specifically the haplotype that demonstrated a strong association with RA (R16-Q27) was not associated with JIA (36.7 % among cases, 38.3% among controls). Furthermore none of the variants demonstrated association after stratification by JIA subtypes, including the rheumatoid factor positive polyarticular JIA, although the number of patients with this subtype (~9%)was underpowered to replicate results in adults with RA. Our results indicate that ADRB2 variants are not associated with JIA or major JIA subtypes. These observations suggest that although they share several clinical and pathological features, JIA and RA have unique genetic associations.

2372/W

Genome-wide Linkage Study of Periodontitis. *R.L. Hanson[1], W.A. Shultis[1], H.C. Looker[1], M. Shlossman[2,3], R.J. Genco[2], R.G. Nelson[1], W.C. Knowler[1].* 1) Diabetes Epidemiology Clin Res, NIDDK, Phoenix, AZ; 2) Dept of Oral Biology, SUNY-Buffalo, Buffalo, NY; 3) Arizona School of Dentistry and Oral Health, Mesa, AZ.

Periodontal disease may have important genetic determinants, but there have been few genome-wide studies to identify susceptibility loci. We conducted genome-wide linkage analyses of periodontitis in 758 Pima Indians from 243 sibships who had participated in a genome-wide linkage study of type 2 diabetes.

Periodontitis severity was assessed for each tooth from the percentage of alveolar bone loss on a panoramic radiograph. The sum of the severity scores for all teeth was adjusted for age, sex and, among those with diabetes, for duration of diabetes, and these residuals were normalized to create an overall periodontitis severity score for genetic analyses. Genotypes from 516 autosomal microsatellite markers were used for linkage studies. Variance components methods were used to assess heritability and for linkage analyses.

The periodontitis severity score was significantly heritable among the 407 nondiabetic individuals (h^2=0.28, 95% CI 0.03-0.54) and among the 351 diabetic individuals (h^2=0.61 95% CI 0.36-0.84). Since bivariate analyses suggested substantial, but incomplete, overlap between genetic determinants in diabetic and nondiabetic individuals (genetic correlation=0.63, 95% CI 0.12-0.91), linkage analyses were conducted in each group separately and in the combined group. Among diabetic individuals, there was suggestive evidence for linkage on chromosome 17 (LOD=2.17) at 46 cM, and among all individuals there was suggestive linkage on chromosome 5 (LOD=2.49) at 102 cM. The highest LOD score among nondiabetic individuals was 1.64 on chromosome 16 at 38 cM.

These analyses suggest that a genetic locus that influences susceptibility to periodontitis in diabetic individuals is located on chromosome 17 and one that influences susceptibility in both diabetic and nondiabetic individuals is located on chromosome 5.

2373/W

Indian Genome Variation Initiative for Genotype to Phenotype correlations in complex diseases. *S.K. Brahmachari[1], Indian Genome Variation Consortium[1,2,3,4,5,6,7].* 1) Functional Genomics Unit, Institute of Genomics and Integrative Biology, Delhi, India; 2) Centre for Cellular and Molecular Biology, Uppal Road , Hyderabad, India; 3) Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata, India; 4) Central Drug Research Institute, Chattar Manzil Palace, Post Box No. 173 , Lucknow, India; 5) Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, India; 6) Institute of Microbial Technology, Sector 39-A, Chandigarh, India; 7) Indian Statistical Institute, 203 Barrackpore Trunk Road, Kolkata, India.

Genetically isolated populations have assumed importance in dissecting complex diseases and mapping underlying genes with the availability of large number of polymorphic genetic markers. However, there has been limited success in SNP associations in complex disease possibly due to a nonlinear correlation of genome variations to phenotype. Genome information of an individual is stable while phenotype is a consequence of dynamic interactions. In the Indian Genome Variation Consortium we have undertaken a detailed genome variation analysis to decipher the various components of a set of complex disorders and drug response genes. Informative SNPs (5-8/gene) from nearly a thousand pathway based candidate genes have been genotyped on Indian populations of diverse linguistic, geographical and ethnic origins. In the 1st phase, SNPs from 72 disease candidates/drug responsive genes and two disease associated genomic regions (6Mb) were analyzed in 2,014 individuals from 55 contrasting endogamous populations. This study revealed (1) large genetically related clusters that correlate with linguistic and ethnic histories (2) genes influenced by natural selection (3) population stratification issues (4) extent of portability of HAPMAP data and (5) Indian populations represent entire world population. Based on Phase 1 inferences we identified 23 reference populations on which variation analysis of over a thousand genes, genome wide neutral markers and CNV regions has been completed. This data would serve as a rich resource and guide for disease gene mapping, pooling populations and cohort validation studies.

2374/W

PDE4D and ALOX5AP are not major risk factors for stroke in the Portuguese population. *T. Krug[1], H. Manso[1,2], B.V. Fonseca[1], L. Gouveia[3], S. Violante[1], R. Taipa[3], I. Albergaria[2], G. Gaspar[2], M. Correia[4], M.V. Baptista[5], A. Pinto[6], R. Silva[6], F. Gonçalves[7], G. Lopes[4], J.P. Gabriel[8], I. Matos[9], J.M. Ferro[3], A. Vicente[1,2], S.A. Oliveira[1].* 1) Inst Gulbenkian de Ciência, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal; 3) H. de Santa Maria, Portugal; 4) H. Geral de Santo António, Portugal; 5) H. Garcia de Orta, Portugal; 6) H. Fernando Fonseca, Portugal; 7) H. Univ. de Coimbra; 8) H. de São Pedro, Portugal; 9) H. Distrital de Mirandela, Portugal.

Stroke is the third cause of death in developed countries and is even more disabling than lethal. The most common form of stroke is a complex disorder resulting from the interplay of environmental and genetic factors, but its genetic underpinnings remain elusive. Recent whole-genome linkage screens followed by fine-mapping association studies have strongly implicated phosphodiesterase 4D (PDE4D) and arachidonate 5-lipoxygenase-activating protein (ALOX5AP) as susceptibility genes for stroke in the Icelandic population. PDE4D degrades second messenger cAMP, a key signal transduction molecule in different cell types, including inflammatory, vascular endothelial and smooth muscle cells. ALOX5AP is required for the synthesis of the leukotrienes secreted by various types of inflammatory cells clustering at the injured sites in blood vessels. The risk conferred by these genes in other populations is unclear since replication studies have reported conflicting results. Our aim was to test the association of these genes with stroke in a Portuguese sample of 533 patients (82% with ischemic stroke) and 507 unrelated controls. We genotyped 52 SNPs in the 5'end of PDE4D and 21 SNPs in ALOX5AP and 10kb flanking region on each side (SNPs are either tagging SNPs from HapMap or SNPs previously found associated), and performed single-marker and haplotype association tests. We found only weak evidence for association (0.02<p-value<0.05) of 3 SNPs and 1 haplotype in PDE4D, and 2 SNPs in ALOX5AP. This study suggests that variants in PDE4D and ALOX5AP are not major risk factors for stroke in the Portuguese population.

2375/W

Detection of novel mutations in mitochondrial tRNA genes by mitochondrial resequencing microarray analysis in two families. *J.H. KYHM, A. MILUNSKY, M. ITO.* Center for Human Genetics, Boston University School of Medicine, Boston, MA.

Mitochondrial disorders are believed to be present in 1 per 10000 live births. These disorders are genetically complicated due to maternal inheritance of mitochondrial DNA as well as biparental inheritance of nuclear DNA. It is necessary to screen the whole mitochondrial genome for unknown mutations to confirm the diagnosis precisely. To identify mitochondrial mutations, we used the Affymetrix mitochondrial resequencing microarray to sequence the entire 16.5 kb mitochondrial genome including all 37 genes. We found two novel mutations in mitochondrial DNA. The first case, we found a patient with pseudo-obstruction to be heteroplasmic for C15925G, a well conserved nucleotide in the Ac-Stem region of the tRNA threonine gene. Her daughter, who was also found to be heteroplasmic for the same mutation manifested fatigue and exercise intolerance. The second case, we detected a near homoplasmy for the A10018G, a well conserved nucleotide in the Ac-Stem region of the tRNA glycine gene. This patient had migraine and her child died of lactic acidosis and multiple organ failure with a clinical diagnosis of multiple complex deficiencies. These mutations in tRNA genes may result in the disruption of the Ac-Stem structure, which cause the instability of tRNA tertiary structure. Mitochondrial resequencing microarray analysis is a powerful, fast, reliable and sensitive method to detect mutations.

2376/W

The detection of C677T gene polymorphism of methylenetetrahydrofolate reductase with real time PCR combined with probe melting curve and the study of its association with liver cirrhosis. *G.Z. Liu[1], Y.R Li[2].* 1) Suizhou Central Hospital,Sui Zhou,Hubei P.R.China, Sui Zhou,Hubei, hubei, China; 2) Union Hospital, Tongji Medical College, Huazhong University of Science and Technology,Wuhan.

Objective To set up a real time fluorescent PCR to detect the C677T gene polymorphism of methylenetetrahydrofolate reductase(MTHFR) and to investigate the correlation between the C677T gene polymorphism of MTHFR and liver cirrhosis. Method The real time detection of the C677T gene polymorphism of MTHFR was performed in 60 healthy volunteers and 64 patients with liver cirrhosis and 42 patients with liver disease excluding liver cirrhosis with double probe hybrid combined with probe melting curve, then compare it with traditional PCR-RFLP. Results There are three genotypes(C/C, C/T and T/T) in the C677 position of MTHFR. For the patients with liver cirrhosis, the rate of C/C, C/T and T/T is 18.8%, 50% and 31.3% respectively. It is 45.2%, 40.5% and 14.3% respectively in patients with liver disease excluding liver cirrhosis as well as 53.3%, 33.4% and 13.3% in healthy volunteers. There is significant difference of genotype frequency of MTHFR between the group of liver cirrhosis and liver disease excluding liver cirrhosis as well as healthy volunteers(χ2=16.7553, P<0.01; χ2= 9.4664, P<0.01).T allele frequency is 56.3% in the patients with liver cirrhosis and the frequency is higher that in the patients with liver disease excluding liver cirrhosis and healthy volunteers(χ2=13.14, P<0.01; χ2=0.34, P>0.05), furthermore the T allele frequency correlates with the occurrence of liver cirrhosis significantly(OR=2.73, 95% credit region: 1.43~5.19). The results came from real time PCR consists with that of traditional PCR-RFLP completely. Conclusion Real time fluorescent PCR combined with probe melting curve is an effective and a rapid method to detect the C677T gene polymorphism of MTHFR. T allele frequency in C677 position in the patients with liver cirrhosis is higher than that in the patients with liver disease excluding liver cirrhosis and in the healthy volunteers. Furthermore the T allele frequency correlates with the occurrence of liver cirrhosis closely.

2377/W

NRAMP1 polymorphisms and susceptibility to tuberculosis in Turkish adult population. *A.Y Ekmekci[1], F. Ozkinay[1], H. Onay[1], F. Bacakoglu[1], A. Sayiner[1], S.Z Guclu[2], S. Pehlivan[3], O. Cogulu[1], A. Aykut[1], C. Gunduz[1], C. Ozkinay[1].* 1) Ege University Medical Faculty, Izmir, Turkey; 2) Health Ministry Suat Seren Chest Diseases and Thoracic Surgery Education and Research Hospital, Izmir, Turkey; 3) Gaziantep University Faculty of Medicine, Gaziantep, Turkey.

The natural resistance-associated macrophage 1 (NRAMP1) gene has been thought to be a strong candidate gene for human tuberculosis (TB) susceptibility. The results of the study that investigating the association between TB and NRAMP1 gene are controversial. We aimed to investigate the relationships between INT4, D543N and 3'UTR polymorphisms of the NRAMP1 gene and TB susceptibility in Turkish adult population. This case-control study included 319 TB patients (mean age: 42.6 ± 16.4) and 273 age matched healthy controls. Three polymorphisms (INT4, D543N and 3'UTR) of the NRAMP1 gene were genotyped by using polymerase chain reaction of genomic DNA with specific primers followed by restriction fragment length polymorphism method. Digested samples were analyzed by electrophoresis on 4% agorose gel. No statistically significant association was observed between susceptibility to TB and three investigated polymorphisms of the NRAMP1 gene in the population studied. In conclusion the polymorphisms, INT4, D543N and 3'UTR, of the NRAMP1 gene were not associated with TB susceptibility or resistance in Turkish adult population. To clarify the entire role of NRAMP1 gene in TB susceptibility, further studies including clinical analysis of patients and haplotype analysis with the other polymorphisms of this gene are needed.

2378/W

Functional consequence of a common and a novel *CYP17A1* promoter polymorphism for association studies. *V.M. Hayes.* Cancer Genetics Group, Garvan Institute of Medical Research, Sydney, NSW, Australia.

Cytochrome P450c17α is a key enzyme in sex hormone production and is encoded by the *CYP17A1* gene. A single nucleotide polymorphism (SNP) located 34-bases upstream of translation initiation, rs743572T>C, has been studied extensively for its potential role, although often controversial, in conferring risk to a large number of hormone-related diseases and conditions. The majority of these studies are based on the assumption that this polymorphism directly influences gene transcription. In prostate cancer, numerous studies have reported associations with increased/decreased risk, or lack of association. In our large European-Australian cases-control study (n=1,563) we report a lack of association between this marker and prostate cancer risk (1) in agreement with a meta-analysis (n=5,159) for European-based populations (2). The latter study did however suggest a potential influence of this marker in African-based populations. We have identified a novel SNP at the -34 position, resulting in a T to A nucleotide change, in two African-based populations using denaturing gradient gel electrophoresis. We demonstrate how commonly used genotyping methods, restriction fragment length polymorphism analysis and TaqMan allelic discrimination, result in genotype misclassification. Although the common C- and the novel A-allele variants create putative SP-1 and AP-4 transcription factor binding sites, respectively, we show no biological effect of these polymorphic alleles on transcription factor binding or gene expression compared to the wild-type allele. The implication of our findings on association studies of the *CYP17A1* -34T>C SNP are apparent, not only with respect to genotype misclassification in Africans, but also the lack of evidence supporting its role as a functional (direct association) polymorphic marker.

(1) Severi G, et al. Brit J Urol, in press.
(2) Ntais C, et al. Cancer Epidemiol Biomarkers Prev 2003;12:120-6.

2379/W

Associations of HNF4α Variants with Type 2 Diabetes in Hong Kong Chinese. *J. Ho[1], S. Germer[2], M. Ng[1], R. Ma[1], W. So[1], M. Martin[2], J. Chan[1].* 1) Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong; 2) Human Genetics Department, Roche Molecular Systems, Alameda, CA 94501, USA.

Hepatic nuclear factor 4α (HNF4α) is a transcription factor that regulates both lipid and glucose metabolism. Mutations in this gene are partly responsible for maturity-onset diabetes of the young (MODY). In addition, populations of European and Asian ancestries demonstrated association of common polymorphisms at the P2 promoter region of *HNF4α* with type 2 diabetes (T2D). In this study, we aimed to investigate the association of common variants at *HNF4α* with T2D in Hong Kong Chinese.

We selected 19 tag SNPs (single nucleotide polymorphism) from HapMap CHB data that span the exons, introns and two promoter regions of *HNF4α*. Using the allelic specific *Tm shift* assay, we genotyped 487 young-onset T2D diabetic patients and 294 healthy controls from Hong Kong.

We found that three common SNPs (rs4812828, rs1884614 and rs2144908) were significantly associated with T2D in our Chinese samples (P <0.05). These associated SNPs are located within a single linkage disequilibrium block ($D' \geq 0.97$, $r^2 \geq 0.69$) at the P2 promoter region that is specific to pancreatic β cells. Rs4812828 showed the strongest allelic association with the C allele conferring increased risk for T2D (odds ratio [OR] = 1.3, 95% CI 1.09-1.60, P = 0.006), in addition to T allele of rs1884614 (OR = 1.3, 95% CI 1.05-1.60, P = 0.014) and A allele of rs2144908 (OR = 1.29, 95% CI 1.05-1.60, P = 0.017). Haplotype analysis did not reveal stronger associations than single marker associations.

In summary, we found that common polymorphisms at P2 promoter region of *HNF4α* were associated with type 2 diabetes in Hong Kong Chinese. The risk alleles at rs1884614 and rs2144908 in our study replicated findings from other populations including Danish, Finnish, Ashkenazi Jewish and Japanese.

2380/W

Association of gene SCL11A1 with Rheumatoid Arthritis in a Mexican population. *E.R. Ochoa-Martínez[1,5], M.P. Casillas-Avila[4], L. González-López[3], E.A. Aguilar-Chávez[3], I.J. Gámez-Nava[1], V.M. Anguiano-Alvarez[4], I.P. Dávalos-Rodríguez[1], M. Salazar-Páramo[2], L. Sandoval-Ramírez[1].* 1) División de Genética, Centro de Investigación Biomédica de Occidente, CMNO, IMSS, Guadalajara Jalisco Mexico; 2) Hospital de Especialidades, CMNO, IMSS; 3) Hospital General Regional No 110 IMSS; 4) Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud; 5) Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico.

The SLC11A1 gene codifies for macrophage to a natural resistant protein 1, it has been considered as a candidate gene for autoimmune diseases susceptibility. This suggests that SLC11A1 is a candidate gene for autoimmune diseases genetic susceptibility, like Rheumatoid Arthritis (RA) and infections that involve macrophage response. Objective: To determine if there is an association between 823CT, D543N, 1729+55del4 polymorphisms and Rheumatoid Arthritis susceptibility. The polymorphisms were analyzed in 109 healthy individuals as control and 148 RA patients diagnosed according to the ARA (American Rheumatology Association), this patients are from IMSS Guadalajara, Jalisco. Genotypes analyses were made by PCR-RFLP technique, using primers and enzymes described by Liu et al (1995). The enzymatic digestion products, were solved with electrophoresis of poliacrilamide gels (29:1) final concentration at 6%, and were dyed with silver nitrate (AgNO3). The allelic frequencies of 823CT indicate a statistically significant difference (p= 2.9x10-4 OR= 0.45 (IC 95% 0.29 - 0.71)). Comparing genotype frequency between patients and control was significant to the CC variant (p= 1.8x10-4 OR 0.37) CT (p= 1.8x10-3 OR 2.33), but not significant for TT (p= 0.14 OR 2.32) in 823CT. The D543N and 1729+55del4 polymorphisms can not be associated as a susceptibility factor to Rheumatoid Arthritis, because genotypes and allelic frequencies were similar for both groups. Statistical analysis of 823CT polymorphism between controls and patients suggest that this polymorphism is associated with susceptibility to Rheumatoid Arthritis, specifying that the C allele confers protection to RA in Mexican population.

2381/W

Interferon-γ gene and interferon-γ receptor-1 gene polymorphisms in tuberculosis children from Turkey. *H. Onay[1], A.Y. Ekmekci[1], B. Durmaz[1], E. Sayin[1], H. Cosar[1], N. Bayram[1], D. Can[2], H. Akin[1], C. Ozkinay[1].* 1) Ege University Faculty of Medicine, Izmir, Turkey; 2) Dr. Behcet Uz Children's Hospital, Izmir, Turkey.

It has been reported that macrophage activation by interferon-γ (IFN-γ) is important in mycobacterium tuberculosis infection. In this study, the relationships of the +874 T/A polymorpism in the first intron of IFN-γ (IFNG) gene and intronic (CA)n polymorphic microsatellite marker of the interferon γ receptor 1 (IFNGR1) gene to tuberculosis susceptibility were investigated in children. Forty four children (mean age: 7.02 ± 4.56) with tuberculosis (TB) and 75 age matched controls were included in the study. The IFNG gene was genotyped for the polymorphism +874 T/A found in the first intron by using amplification refractory mutation allele-specific polymerase chain reaction method. For the intronic (CA)n polymorphism of the IFNGR1 gene, genomic DNAs were amplified using specific FAM-labelled primers and the polymerase chain reaction products were genotyped by ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). There were no significant differences between the allele frequencies and genotype frequencies of patient and control groups for the polymorphism +874 T/A in the IFN γ gene. We identified 13 (CA)n alleles for the intronic (CA)n microsatellite of IFNGR1 in both TB children and controls. Only one allele was different in both groups. A significant difference was found for the allelic markers (170 and 180) between the TB children group and control group. The allele 170 was significantly associated with the susceptibility to TB (p=0,0212), whereas the allele 180 was significantly associated with the protection to TB in children (p=0.0199). In conclusion no significant association was observed between the +874 T/A polymorphism found in the first exon of IFN-γ gene and TB susceptibility in Turkish children. Allelic variants of the (CA)n polymorphism in the sixth intron of IFNGR1 gene may be associated with susceptibility to TB or protection against TB.

2382/W

NRAMP1 polymorphisms and susceptibility to tuberculosis in Turkish children. *F. Ozkinay[1], A.Y Ekmekci[1], H. Onay[1], H. Cosar[1], O. Cogulu[1], A.R Bakiler[2], E. Turker[1], C. Gunduz[1], C. Ozkinay[1].* 1) Ege University Faculty of Medicine, Izmir, Turkey; 2) Adnan Menderes University Faculty of Medicine, Aydin, Turkey.

Tuberculosis (TB) is an important public health problem worldwide causing significant morbidity and mortality all over the world. The researches have shown that genetic factors as well as environmental factors contribute to the development of this devastating disease. The natural resistance-associated macrophage protein 1 (NRAMP1) is one of the most extensively studied gene in the susceptibility to tuberculosis. The results of the NRAMP1 studies in the association with tuberculosis are inconclusive and the role of this gene in the pathogenesis of tuberculosis in humans is debated. We aimed to investigate the association between 3 polymorphisms (D543N and 3' UTR and 5'(CA)n) of the NRAMP1 gene and TB susceptibility in Turkish children. This study included 43 children with TB (mean age 7.02 ± 4.56) and 70 age matched controls. Polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis were used to type the polymorphisms D543N and 3' UTR. Genotyping for the polymorphism 5'(CA)n was performed by using ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA) after PCR amplification of genomic DNA with the FAM-labelled specific primers. The genotype and allele frequencies of the 3'UTR polymorphism were significantly different in patient group and control group (p=0.0354 and p= 0.0373 respectively). Only allele 200 among the alleles of the microsatellite 5'(CA)n marker was significantly higher in patient group (p=0.0374). No significant differences were found between the genotype and allele frequencies of patient and control groups for the polymorphism D543N. As a conclusion NRAMP 1 gene may play a role in the susceptibility or resistance to TB in children.

2383/W

Identification of functional SNPs in TIM3 promoter region. *J. Zhang, J. He, A. Sandford, P. Paré.* Respiratory Medicine Division, St. Paul's Hospital iCAPTURE Center, Vancouver, B.C., Canada.

Background: T-cell immunoglobulin mucin-3 (TIM3) is a TH1-specific type 1 membrane protein that regulates TH1 proliferation and the development of tolerance. TIM3 protein and its genetic variants have been suggested to play a role in regulating allergic diseases. One association study reported that 3 single nucleotide polymorphisms (SNPs) in TIM3 were significantly related to atopy and eczema using white and Hispanic family samples. Similar results were obtained using Korean samples. Objective: The aim of this study is to determine the promoter region of TIM3 and the influence of genetic variation in that region on transcriptional regulation of TIM3. Methods: We performed 5' rapid amplification of cDNA ends (RACE) and reverse transcription-polymerase chain reaction (RT-PCR). We screened for polymorphisms in the promoter region. Deletion analysis was used to localize the promoter region of TIM3. Results: We found that there are two promoter regions in TIM3. One is from -214 bp to +58 bp and another is from -1.6 kb to -914 bp relative to the transcription start site. None of the SNPs or the haplotype affected the transcriptional activity. Conclusion: Our findings indicate that SNPs and haplotypes in TIM3 promoter region do not have a functional effect but it is possible that other SNPs in the gene could account for the association with asthma.

2384/W

Replication of association to ATG16L1 and IL23R in a Norwegian population repesentative cohort with inflammatory bowel disease (IBD), but no association with primary sclerosing cholangitis (PSC). *A. Franke[1], M.B. Kirsten[2], T.H. Karlsen[2], T. Balschun[1], A. Bergquist[4], C. Solberg[3], E. Schrumpf[2], M.H. Vatn[2], S. Schreiber[1].* 1) IKMB, Christian-Albrechts University, Germany; 2) Medical Department, Rikshospitalet-Radiumhospitalet Medical Center, Norway; 3) Ullevaal University Hospital, Norway; 4) Department of Gastroenterology and Hepatology, Karolinska University Hospital, Sweden.

Crohn disease (CD) and ulcerative colitis (UC) are the 2 major forms of IBD. Recently, variants in the IL23R and ATG16L1 gene were identified to be associated with IBD and CD, respectively. We previously showed that NOD2 is only weakly associated with CD in Norway. We now replicated ATG16L1 and IL23R in a 10 year incidence cohort from Southern Norway (IBSEN I study). As Norway has very high incidences of PSC, a disease associated with IBD, a large PSC patient panel was tested in a 2nd experiment for ATG16L1 and IL23R. 368 controls, 145 CD, 327 UC, and 365 PSC patients were investigated for the ATG16L1 variant T300A and the IL23R variant Arg381Gln. T300A was significantly associated with CD (p= 0.008; OR=1.89, 95% CI [1.10-3.23]), but not with UC. The Il23R variant Arg381Gln was replicated in both the CD and the UC panel (IBD: p=0.005; OR=0.49, 95% CI [0.29-0.82]). However, no significant association between the two tested variants and PSC was detected. Subphenotype analyses for CD revealed an association between ATG16L1 predisposition and smoking status, as well as with disease localization. We replicated associations between IBD and the susceptibility genes ATG16L1 and IL23R in the Norwegian population. Since other reported IBD genes previously failed to replicate or gave only weak signals, ATG16L1 and IL23R are the first *bona fide* susceptibility genes in the Norwegian population. However, both variants do not play a role in the genetic predisposition to PSC. Associations of ATG16L1 with smoking status and ileal localization were identified in subanalyses and need to be confirmed in future studies.

2385/W

Autism associated alleles affect the transcriptional regulation of *ENGRAILED 2*. *J. Millonig[1, 2, 3], R. Benayed[1, 2], P. Matteson[1, 2], J. Choi[1, 2], N. Gharani[3], L. Brzustowicz[3].* 1) Center for Advanced Biotechnology and Medicine, UMDNJ-RWJMS, Piscataway, NJ; 2) Department of Neuroscience and Cell Biology, UMDNJ-RWJMS, Piscataway, NJ; 3) Department of Genetics, Rutgers University, Piscataway, NJ.

We have previously demonstrated that two intronic SNPs (*rs1861972* and *rs1861973*) in the homeobox transcription factor, *ENGRAILED 2*(*EN2*), are consistently associated with Autism Spectrum Disorder (ASD) in 3 separate datasets (A-C haplotype; *P*=0.000000427; 518 families)(Gharani et al., 2004; Benayed et al., 2005). Population Attributable Risk calculations for the associated haplotype determined that the *EN2* risk allele contributes to ~40% of ASD cases. Hapmap LD data indicate that *rs1861973* is not in strong LD with any SNP within 2Mb of *EN2* (r^2<.45) identifying the associated haplotype as a candidate risk allele. Previous resequencing and LD analysis of *EN2* support this possibility.

Because risk alleles for other common diseases affect the transcriptional regulation of the associated gene, luciferase reporters for the *EN2* intron were generated and transiently transfected into 3 cell types: HEK293T cells, PC12 cells and primary cultures of mouse cerebellar granule neurons. In all three cell types the intron functioned as a transcriptional repressor (P<.0001; two tailed paired Student's T test). To test for a functional difference between the associated A-C and non-associated G-T haplotypes, both versions of the intron were cloned into luciferase reporters that contained either a minimal SV40 promoter or the *EN2*promoter. These constructs were transfected into the same 3 cell types and in all experimental conditions the non-associated G-T intron was a stronger repressor than the associated A-C version (P<.005; two tailed paired Student's T test). EMSAs using granule cell extracts were performed which identified proteins binding specifically to the associated alleles. These data indicate that *EN2* is an ASD susceptibility gene and that the A-C haplotype is a risk allele responsible for *EN2* association with ASD.

2386/W

Significant association of human CR1 polymorphisms and cerebral malaria. *P. Teeranaipong[1], J. Ohashi[1], R. Kimura[1], J. Patarapotikul[2], P. Nuchnoi[2], H. Hananantachai[2], I. Naka[1], C. Putaporntip[3], S. Jongwutiwes[3], S. Looareesuwan[2], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; 3) Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Complement receptor type 1 (CR1 or CD35) is immune-regulatory membrane glycoprotein found on various cell types including erythrocytes and granulocytes. In human malarial infection, CR1 has been shown to be a ligand for rosette formation and the higher expression of CR1 on the erythrocyte in patients with cerebral malaria than in those with mild malaria has been reported in several populations, suggesting an important role of CR1 in the pathophysiology of cerebral malaria. Therefore, we investigated the possible association of *CR1* polymorphisms with cerebral malaria in a Thai population. A total of 473 malaria patients infected with *Plasmodium falciparum* were classified according to WHO criteria into 3 groups: 202 mild malaria, 165 non-cerebral severe malaria and 108 cerebral malaria patients. Seven nonsynonymous coding SNPs, one SNP in intron 27, so called HindIII SNP, four promoter SNPs and five 3'UTR SNPs were analyzed. Of these, a SNP in the *CR1* promoter region (PSNP02C>T) was strongly associated with protection against cerebral malaria (P-value = 0.0009). The frequency of the derived allele (T) was increased in mild malaria patients than in cerebral malaria patients. The PSNP02 was located in the putative transcription binding site of Brn-2, a member of POU domain transcription factor. Thus, PSNP02 may influence the expression level of CR1 and confer the protection against cerebral malaria. Interestingly, the frequency of the derived allele (T) of PSNP02 showed high frequency (97.8%) in the present population as well as in HapMap East Asian populations. This might reflect some strong selective forces that have acted on this promoter SNP during human evolution.

2387/T

Frequency of chromosomal abnormalities in 99 couples prior ICSI and in 82 couples who fail to conceive after one or more ICSI attempts. *N.B. Abdelmoula[1], M. Meddeb[2], L. Mokaddem[2], F. Bouzid[2], A. Sallemi[2,1], A. Amouri[3], T. Rebai[1]*. 1) Histology Laboratory, University of Medicine of Sfax, SFAX, Tunisia; 2) Private Sector, Tunisia; 3) Histology and Cytogenetic Laboratory, Pasteur Institute, Tunis, Tunisia.

We report results of cytogenetic investigations performed in 181 couples prior to ICSI or after unsuccessful attempts, between January 2001 and May 2007. Couples were referred to our laboratory for cytogenetic investigations and genetic counselling. Couples were classified according to the ICSI indications as: Group 1: 99 couples undergoing ICSI for the first time and group 2: 82 couples failing to conceive by ICSI. ICSI was indicated respectively for group 1 and group 2 because OAT(n=42/n=41), azoospermia (n=13/n=5), asthenoteratospermia (n=14/n=13) or other indications with normospermic men (n=30/23). Out of these 362 patients, sixteen had an abnormal karyotype (4,42 per cent patients and 8,84 per cent couples); fourteen men (14/181: 7,73 per cent) and two women (2/181: 1,10 percent). Frequencies of chromosome abnormalities are estimated to 6,06 per cent in group 1 and 12,2 per cent in group 2. If we consider inversions of chromosome 9 and 12 (n=4) and add21p; as minor abnormalities, frequencies became 4,04 per cent in group 1 and 8,53 per cent in group 2. Most chromosomal abnormalities detected were structural rearrangements: 6 reciprocal translocations t(11;22)(q24;q11),t(2;3)(p24;q26),t(11;21)(q13;p11), t(16;22)(q13;q12) and 2 t(1;18)(p21;q12) detected in 2 normospermic men, 2 robertsonian translocations, 5 inversions, one case of add21p and 2 sex mosaicism: 46,XY/47,XXY and 45,X/46,XX/47,XXX. Our results stress the importance of karyotyping both male and female partners before ICSI is started to ovoid couples, unsuccessful attempts. Adequate genetic counselling, followed by preimplantation or prenatal diagnosis, should be offered if a chromosomal abnormality is detected. Thus, much greater overlap between reproductive medicine and genetics is necessary and a close collaboration between professionals working in these two fields is imperative in treating patients with infertility in the best possible way.

2388/T

Co-optimization of Powerplex 16 and restriction analysis for use in the prenatal diagnosis of HSAN4. *C. Oddoux, L. U, K. Hoang, H. Ostrer*. Human Genetics Program, NYU School of Medicine, New York, NY.

Maternal cell contamination (MCC) complicates the analysis of prenatal diagnostic cases. The degree of MCC varies greatly from sample to sample, and with specimen type and culture status and the impact of MCC on the interpretation of an assay's results is assay dependent. Thus, a reliable means of assessing the likelihood of MCC interference for every prenatal performed is required and it must be tailored for the assay in question. Various combinations of polymorphic markers have been used to assess MCC, but there is little agreement on the number of markers or the best ones to use. The Powerplex 16 panel of markers is a forensic panel of 15 highly polymorphic markers provided in commercially available FDA approved kits with validated reference standards. This represents a highly polymorphic quality controlled marker panel that could be applied to MCC applications. Here, we present our experience with the optimization of Powerplex 16 for MCC assessment in a prenatal diagnosis using a restriction assay for the detection of the GLY571ARG mutation in an HSAN4 family with an affected child in whom whole gene sequencing revealed the presence of two copies of the mutation in NTRK1. We prepared for the prenatal testing by evaluating the informativeness of each marker in the panel for distinguishing the two maternal alleles from each other and from the paternal alleles as well as by performing mixing experiments to calibrate the sensitivity of detection of contamination in both the Powerplex 16 and the restriction assays. We found that it was extremely important to assess the informativeness and the sensitivity to contamination in each prenatal case using both maternal and paternal DNA because the sensitivity varies with the specific alleles competing in the assay. In addition we found that the sensitivity of the Powerplex 16 assay and the restriction assay need to be adjusted to be similar to avoid erroneous conclusions. Thus, assays should not necessarily be optimized to the most sensitive mutation detection, and mixed samples should be run concurrently for the Powerplex 16 assay and the mutation detection assay, for each prenatal performed.

2389/T

Prenatal identification of a novel R937P L1CAM missense mutation. *P.L. Wilson[1], H. Ferguson[2], B.L. Blaisdell[2], J. Wilkins[1], S. Li[3], J.J. Mulvihill[3], A. Maddalena[2], A.F. Wagner[1], J.R. Goodman[1]*. 1) Obstetrics & Gynecology, Section of Maternal Fetal Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) GeneDx, Inc, Gaithersburg, MD, USA; 3) Department of Pediatrics, Section of Genetics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

The L1 cell adhesion molecule (L1CAM) is a member of the immunoglobulin superfamily of neuronal cell adhesion molecules and plays a role in CNS development and maturation. It is active in neurite overgrowth, adhesion, fasciculation, migration, myelination, and axon guidance. Mutations in the gene have been associated with phenotypic changes including hydrocephalus, agenesis or hypoplasia of the corpus callosum and corticospinal tracts, mental retardation, spastic paraplegia, and adducted thumbs. A 19-year-old G1P0 Caucasian female was referred at 27-3/7 weeks. Ultrasound evaluation identified a male fetus with hydrocephalus, ventriculomegaly, aqueductal stenosis, and polyhydramnios. An amniocentesis identified a hemizygous mutation of G>C in exon 21 of the L1CAM gene. The patient was later tested and identified to be a carrier of the same mutation. This mutation results in the replacement of the normal Arginine codon (CFC) with a Proline codon (CCC) at position 937 of the resultant protein, R937P. Follow-up ultrasound at 32-1/7 weeks identified the additional findings of bilateral adducted thumbs and short femurs and a right clubbed foot. The fetus was delivered at 38-5/7 weeks with Apgars of 7 and 9, birthweight of 3.4 kg, length of 51.5 cm, and FOC of 43.5 cm. There was marked frontal bossing, contractures of the feet with rocker bottom appearance, and hyperactive reflexes with ankle and knee clonus. The CT showed hydranencephaly as opposed to hydrocephalus, with very abnormal brainstem, posterior fossa, and cerebral hemispheres. Family history identified a maternal half brother born in 1984 who died at the age of 4 years of hydrocephalus, mental retardation, and presumed aqueductal stenosis. Here we present the prenatal and neonatal evaluation of a male infant with a novel L1CAM missense mutation.

2390/T

Mutation screening of FLT-1 in preeclampsia. *S.M. Zeng[1], J. Yankowitz[1], D. Merrill[2], J. Murray[3]*. 1) Department of OB/GYN, Univ of Iowa, Iowa City, IA; 2) Department of OB/GYN, Wake Forest School of Medicine; 3) Department of Pediatrics, Univ of Iowa, Iowa City, IA.

Preeclampsia (PE) is a complex pregnancy-specific disorder, characterized by hypertension and proteinuria in the second or third trimester of pregnancy. PE is a leading cause of maternal and neonatal mortality and morbidity worldwide. The etiology of PE remains unclear, but genetic susceptibility is widely accepted as an etiological factor. A number of candidate genes have been reported. FLT-1 (fms-like tyrosine kinase 1 also known as vascular endothelial growth factor receptor 1 (VEGFR-1)) has been considered a good candidate gene from evidences from molecular biology, clinical and animal models. This gene is composed of 30 exons coding a transmembrane receptor protein. We previously found that 2 SNPs (single nucleotide polymorphisms) in the non-coding region of FLT-1 (intron 17 and upstream) are linked to PE risk. The current study will describe mutations of the coding area of FLT-1 in patients with PE. Diagnosis of PE is according to standard criteria. Each exon and its flanking regions (20-50 base pairs) were amplified, and sequence analysis performed with POLYPHRED and CONSED programs. We sequenced all 30 exons and flanking regions of FLT-1 in 92 Caucasian patients with PE and predicted an effect of mutation on FLT-1 protein structure with PolyPhen program. Sequencing analysis demonstrated 9 mutations in the coding region of FLT-1, including 1 nonsense, 4 missense and 4 silent mutations. One nonsense mutation is caused by alteration of tyrosine codon into stop codon due to C to A transversion at third nucleotide at codon 1213. The 4 missense mutations were I623V in exon 13, K828R in exon 17, E1002A in exon 21 and S1247G in exon 29. Polyphen showed that the alteration of amino acid in all 4 missense mutations had a benign effect on the protein structure of FLT-1. The 4 silent mutations were P365P in exon 8, T568T in exon 13, A897A in exon 19 and P1068P in exon 24. In the flanking regions we found 6 SNPs 3' to exons 3, 14, 19, 23, 28 and 30, and one T insertion 5' to exon 17. Analysis showed that all 4 missense mutations, 1 silent mutation, and one mutation in a flanking region are at sites with high conservation. The association of these mutations and PE risk may warrant further study.

2391/T

A possible Role for the PTPN11 gene in Sex Determination. *M.T. Thomas[1], J.J. Jessen[1], S.K. Keating[2], D.C. Chitayat[1]*. 1) Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Department of Laboratory Medicine and pathobiology, Mount Sinai Hospital.

The SH2 domain-containing protein-tyrosine phosphatase PTPN11(Shp2) has a major role in normal organogenesis and is an essential component of signaling pathways. A germline gain of function mutation is known to be associated with Noonan syndrome as well as in different malignancies. However, to the best of our knowledge, there have been no report regarding the role of PTPN11 gene in sex determination. We report a case of abnormal sex determination in association with a germline mutation in the PTPN11 gene. Case Report: The mother was a 41-year-old G2P0TA1L0 woman of Jamaican descent and her partner was 45 years old and of the same descent. The couple was healthy and non-consanguineous. The pregnancy was complicated with fetal ultrasound finding of polyhydramnios and cystic hygroma detected at 12.3 weeks gestation. CVS was done subsequently and was 46, XX. Repeat fetal ultrasound at 15.7 weeks gestation showed oligohydramnios, hydrops fetalis, a large cystic hygroma, bilateral club feet and left genu recurvatum. The stomach and bladder could not be seen, the left ventricle was smaller than the right and there was a query AVSD. The mother developed a "mirror image" at 17 weeks gestation and delivery was induced. The autopsy showed IUGR, facial dysmorphism, low set ears and ambiguous genitalia with normal testes. No prostatic tissue was identified and a possible rudimentary uterus between the bladder and the rectum. There was bilateral club feet, pulmonary hypoplasia, AVSD and absent thymus. DNA analysis for PTPN11 showed a heterozygous C→A nucleotide change in exon 13 of the PTPN11 gene denoted T7507K. This mutation has not been reported previously. FISH analysis for SRY and 22q11.2, were negative. Parental mutation analysis failed to identify this mutation in them. Our patient had normal female genotype, normal testes, ambiguous genitalia and absent Wolffian duct derivatives in the absence of SRY gene and in association with a germline mutation in the PTPN11 gene. This raises a possible important role for the PTPN11 gene in sex determination.

2392/T

Chromosome copy-number detection through methylation-sensitive DNA amplification and microarray analysis. *S. Brown[1], G. Brown[2], L.Y. Brown[1]*. 1) Dept OB/GYN, Univ Vermont, Burlington, VT; 2) Columbus Regional Hospital, Columbus IN.

Fetal DNA, most likely of trophoblast origin, is present in both the blood and cervical mucus of pregnant women and provides a potential basis for non-invasive fetal diagnostic tests. However, fetal DNA from both sources is generally highly contaminated with maternal DNA, and this contamination is the main technical challenge in trying to accomplish non-invasive detection of fetal chromosome abnormalities. Existing methods for the selective amplification of fetal DNA have generally relied on specific sequence differences between the mother and fetus. As an alternative, we have developed a method for selective amplification of fetal DNA that makes use of observation that trophoblast DNA is globally hypomethylated in comparison with DNA from other sources. In this method, a DNA mixture is first digested with a methylation sensitive restriction enzyme and then amplified by linker-mediated PCR. After an initial amplification, a second isothermal rolling circle amplification is performed. This procedure results in the differential amplification of short, relatively hypomethylated fragments. After amplification, the resulting "representations" are comparatively hybridized to a microarray consisting of oligonucleotides that correspond to restriction fragments generated by the initial digest. Copy number differences are then detected through statistical analysis of array addresses that have been previously shown to exhibit trophoblast-specific amplification. To test the feasibility of this method for detecting aneuploidy, we have prepared mixtures of peripheral blood DNA and first trimester trophoblast DNA from either normal or aneuploid samples. We present data showing that aneuploidy can be detected even when 90% of the starting DNA sample was derived from a euploid source and only 10% was from an aneuploid trophoblast sample. Future work will focus on testing whether our approach can be used for non-invasive prenatal diagnosis.

2393/T

Optimized criteria for using interphase FISH in the prenatal diagnosis of common aneuploidies. *S. LECLERCQ[1], A. LEBBAR[1], V. TSATSARIS[2], D. LETESSIER[1], G. GRANGE[2], JM. DUPONT[1].* 1) Unite de Cytogénétique, GROUPE HOSPITALIER COCHIN SAINT VINCENT DE PAUL, PARIS, France; 2) Service de gynécologie obstétrique,GROUPE HOSPITALIER COCHIN SAINT VINCENT DE PAUL, PARIS, France.

Interphase FISH is commonly used in addition to karyotype for rapid detection of selected aneuploidies with a result usually available in 24 to 48 hours. This test offers an obvious benefit for the management of women presenting a high risk of fetal chromosomal anomaly giving them, in most cases, early reassurance. However, this test is time consuming and labour intensive increasing the cost of the prenatal diagnosis procedure. In order to evaluate medical and economic performances of different strategies selecting patients eligible for interphase FISH, we compare three different protocols used in our last 6 years activity. The three strategies differs in terms of probes selection according to the reason for referral and technical modification of the procedure. In this time frame, 2707 women were referred to our laboratory for prenatal diagnosis either because of maternal age over 38 (48%), abnormal maternal serum screening with a calculated risk over 1/250 (35%) or abnormal ultrasound (17%). A grand total of 4.8% chromosomal anomalies were diagnosed after karyotyping. Theoretically, interphase FISH would detect 79.4% of the unbalanced anomalies. The last protocol adopted, which offers a rapid test to 57% of women undergoing amniocentesis, allows the best aneuploidies detection rate (68% of total aneuploidies,87%of trisomy 21). Aneuploidies not selected for FISH testing were mostly observed in samples referred for maternal age under the protocol cut-off level. Selecting probes according to the patient risk evaluation combined to technical procedure modification allows medico economic improvement of interphase FISH in general practice.Thus, number of eligible patients is increased for a same reagent cost leading to better pregnancy management. However,analysis time increases,limiting the number of tests to perform. Although we improve the use of interphasic FISH in prenatal diagnosis, its generalization will need further technical improvements such as automated spot counting to become reality.

2394/T

Targeted chromosomal microarray analyses (CMA) in 639 newborn patients. *X.Y Lu, M.T Phung, C.A Shaw, K. Pham, T. Sahoo, P. Stankiewicz, A.C Chinault, A.L Beaudet, J.R Lupski, A. Patel, S.W Cheung.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Chromosomal abnormalities are a leading cause of birth defects including multiple congenital anomalies (MCA), dysmorphic features (DF), congenital heart disease, congenital diaphragmatic hernia, and cleft lip/palate. To investigate genomic imbalance as a potential etiology in neonatal patients, we implemented a targeted BAC and oligo array that interrogates over 150 common disease loci, pericentromeric and subtelomeric regions, and the remainder of the genome as backbone coverage. Using this chromosomal microarray analysis (CMA), we analyzed a total of 639 patients aged 1 month or younger between June 2005 and May 2007. Clinically significant abnormalities were detected in 15.5% of the patients (99/639). Ten patients (1.6%) were found to have a common chromosomal aneuploidy, trisomy 21 (n=5), trisomy 13 (n=3), and trisomy 18 (n=2); thirty-two patients (5.0%) had microdeletion or microduplication including the most common ones, involving chromosomes 22q11.2 (n=14), 5p15.3 (n=7), 4p16.3 (n=5) and 15q11-q12 (n=4). Fifty-two patients (8.1%) had interstitial or terminal segmental aneusomies that were distributed among the other relatively rare disease loci covered by the array. The remaining five cases (0.7%) were mosaic for trisomy 9 (n=3), trisomy 22 (n=1) and 45,X/46,X,i(Y)(q10) (n=1). In addition, a total of 67 cases (10.5%) (67/639) exhibited copy-number variations (CNVs) with uncertain clinical significance. After family studies and comparison with the public CNV databases, 19 (2.9%) were reported as benign moderately common variants, 22 (3.4%) were interpreted as rarer familial variants (a phenotypically normal parent has the variant), and the remaining 26 (4.0%) still await parental studies. This study demonstrates that the targeted CMA is a valuable clinic diagnostic tool in newborns, especially for those with neonatal presentations of DF and/or MCA. CMA is valuable for comprehensive neonatal care, enabling precise and reliable diagnosis, prognostic information, and recurrence risk estimates.

2395/T

Fetal parietal foramina: ultrasound and MRI findings. *T. Friedberg[1,3], S. Pentazi[2], S. Blaser[4], K. Murphy[3], D. Chitayat[1,3,5].* 1) The Prenatal Diagnosis and Medical Genetics Program; 2) Department of Diagnostic Imaging; 3) Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON; 4) Department of Diagnostic Imaging; Divisions of Diagnostic Imaging and Neuroradiology; 5) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON.

Enlarged parietal foramina are caused by poor or delayed ossification around the parietal notch causing bilateral openings in the parietal bones on the sides of the posterior sagittal suture. They are generally benign but can be associated with abnormal venous anatomy and seizures. Mutations in the *MSX2* and *ALX4* genes have been reported to cause these defects and can be inherited as an autosomal dominant condition. We present a family with parietal foramina detected through fetal ultrasound findings suggestive of parietal encephalocele. Case report: The couple was healthy, non-consanguinous and of European descent. The pregnancy history was unremarkable. The couple presented at 21 weeks gestation with fetal ultrasound findings of bilateral large choroid plexus cysts extending to the anterior horns and a focal midline deformity raising the possibility of encephalocele. After the ultrasound, the patient reported a family history of a posterior calvarial defect in herself, her daughter, mother, grandfather, and great-grandfather. Physical examination of the mother and daughter showed bilateral calvarial defects measuring 3x3cm at the posterior part of the parietal bones. Fetal MRI verified the finding of bilateral parietal foramina measuring 1.9x2.0cm with protrusion of 6.7mm of CSF filled spaces with no brain substance protruding. The isolated defects were confirmed in the newborn. DNA analysis of the *MSX2* and *ALX4* genes done on the mother identified a novel, but disease-causing mutation in the *ALX4* gene thought to be the cause of the autosomal dominant parietal foramina. To the best of our knowledge, there have been four case reports of parietal foramina detected in the second trimester by fetal ultrasound and one case detected by fetal MRI. Recognition of this condition is important as it can alleviate anxiety associated with suspected findings of encephalocele and to prevent head trauma at the time of delivery.

2396/T

Cleft lip and palate in a fetus with thanatophoric dysplasia (TD) type 1. *S. Ramanathan[1], D. Lewis[2], R.A Morotti[3], H.K Rosenberg[4], L. Mehta[1].* 1) Div. of Medical Genetics, Schneider Children's Hospital at North Shore, Manhasset, NY; 2) Div. of Maternal Fetal Medicine, North Shore Hospital, Manhasset, NY; 3) Dept. of Pathology and; 4) Dept. of Radiology Mt. Sinai Medical Center, New York, NY.

TD is a lethal skeletal dysplasia classified as TD type 1 with micromelia, bowed femurs, with or without cloverleaf skull and TD type 2 with straight femurs and cloverleaf skull. We report on a fetus with TD1 and the unusual finding of unilateral cleft lip and palate (CLP). This was the second pregnancy for a 24 year old woman and her 29 year old partner. Family history was not significant for birth defects. Comprehensive ultrasound done at 20 weeks of pregnancy noted severe micromelia of all the long bones with the femurs and humeri measuring 14w2d, small thoracic circumference and possible cloverleaf skull. Unilateral cleft lip was noted. Following pregnancy termination, post-mortem external examination of the fetus showed narrow thorax, short limbs, relative macrocephaly and unilateral CLP. Post-mortem fetal X-rays confirmed marked shortening and bowing of the long bones with "telephone receiver" femurs, short ribs and diffuse severe platyspondyly. Cloverleaf skull was not confirmed. Testing on amniocytes for common TD mutations in the *FGFR3* (fibroblast growth factor receptor 3) gene showed the Nt742C>T (R248C) mutation. R248C is the most common TD mutation and is associated with TD1. To our knowledge, this is the first report of cleft lip and palate in a fetus with TD1. The fibroblast growth factors (FGFs) have pleiotropic effects in craniofacial development. Mutations in *FGFR3* have been associated with a range of clinical phenotypes, including achondroplasia, Muenke syndrome and LADD syndrome. Orofacial clefting is not a common finding in any of these syndromes. However, clefting is associated with *FGFR2* mutations (Apert and Beare-Stevenson syndromes) and *FGFR1* mutations (Kallmann syndrome 2). While the clefting in this fetus may be coincidental, this report adds to the evidence that impaired FGF signaling contributes to the etiology of cleft lip and palate.

2397/T

Associated malformations in patients with anorectal malformations. *M.P. Roth, B. Dott, Y. Alembik, C. Stoll.* Genetique Medicale, Faculté de Médecine, Strasbourg, France.

Patients with congenital anorectal malformations (ARM) often have other associated congenital defects. The reported incidence and the types of associated malformations vary between different studies. The purpose of this investigation was to assess the prevalences at birth of associated malformations in patients of a geographically defined population with ARM which were collected between 1979 and 2003 in 334,262 consecutive births. Of the 174 patients with ARM during the study period, 49.4% had associated malformations.Patients with associated malformations were further classified into groups with non syndromic multiple congenital anomalies; chromosomal abnormalities; non chromosomal syndromes including Townes-Brocks, Walker-Warburg, Ivemark,Fetal alcohol,Klippel Feil,Pallister-Hall,Facio-auriculo-vertebral spectrum, deletion 22q11.2;sequences,including OEIS,Pierre Robin and sirenomelia;and associations including VATER and MURCS. Malformations of the urogenital system (81.1%) and of the skeletal system (45.5%)were the most common other congenital anomalies occurring with ARM in multiply malformed patients without recognized entities, followed by malformations of the cardiovascular system,the digestive system,and the central nervous system. Weight, length, and head circumference of children with ARM and multiple associated malformations were lower than in controls, as was the weight of the placenta. Prenatal detection by fetal ultrasonographic examination was rarely made in isolated ARM. However, even in multiple associated malformations, prenatal detection by fetal ultrasonographic examination had a low sensitivity, 36%.In conclusion the overall prevalence of malformations, which was close to one in two infants, emphasizes the need for a thorough investigation of patients with ARM. A routine screening for other malformations may be considered in patients with ARM, and genetic counseling seems warranted in most of these complicated cases.

2398/T

Associated malformations in patients with abdominal wall defects. *C. Stoll, Y. Alembik, B. Dott, M.P. Roth.* Genetique Medicale, Faculté de Médecine, Strasbourg, France.

Gastroschisis and omphalocele (exomphalos) are the most common types of congenital abdominal wall defects(AWD).The reported types of associated malformations in AWD vary between different studies,as well as the percentage of associated malformations. The purpose of this investigation was to assess,in a geographically defined population, the prevalences at birth of associated malformations in patients with AWD which were ascertained between 1979 and 2003 in 334,262 consecutive births.Of the 86 patients with omphalocele (total prevalence 2.57 per 10,000),64 had associated malformations which were further classified into groups with chromosomal abnormalities(25 cases),non chromosomal recognized syndromes including Goltz, Beckwith-Wiedeman, Marshall-Smith,Meckel-Gruber,Oto-palato-digital type II, pentalogy of Cantrell, CHARGE, and fetal valproate; sequences,including body stalk anomaly, exstrophy of bladder,and OEIS; and patients with non syndromic multiple congenital anomalies(MCA)(26 cases).Malformations of the musculo-skeletal system,the urogenital system,the cardiovascular system,and the central nervous system were the most common other congenital anomalies occurring in patients with MCA. For gastroschisis,the total prevalence was 1.85 per 10,000.However,there was a significant increase over the study period in the total prevalence.The maternal age-specific prevalence was highest in the 15-19 year age group.Of the 62 patients with gastroschisis,12 had associated malformations including one chromosomal abnormality, 2 limb-body wall complex, one skeletal dysplasia, one amyoplasia congenita, and 7 non syndromic MCA. Prenatal detection identified all the cases with AWD after 1999. In conclusion the overall prevalence of associated malformations, which was close to three in four patients in omphalocele and to one in five patients in gastroschisis, emphasizes the need for a thorough investigation of patients with AWD. A routine screening for other malformations need to be considered in patients with AWD. Genetic counseling seems warranted in most of these complicated cases.

2399/T

The dysmorphologic features of a newborn following an abdominal pregnancy. *C.G. Abarquez[1,2], M.L. Alcausin[1,2], C.D. Padilla[1,2], E.M.C. Cutiongco-de la Paz[1,2].* 1) Institute of Human Genetics, National Institutes of Health, Manila, Philippines; 2) Section of Genetics, Department of Pediatrics Philippine General Hospital, Manila, Philippines.

Abdominal pregnancy is an implantation of an ectopic gestational sac in the peritoneal cavity. It is a rare occurrence and has an incidence of 1 per 11,000 pregnancies but may be more common or less frequent depending on race and country. Abdominal pregnancy is considered advanced if it survives beyond 20 weeks of gestation. It exceptionally reaches term but delivery of a live infant is rare. A high incidence of fetal deformations and mortality has been frequently reported. A deformation sequence occurs when an abnormal mechanical force produces several related deformations. We report a case of a live dysmorphic newborn with an estimated gestation of 40 weeks following an advanced primary abdominal pregnancy. The abdomen, being an abnormal site for fetal development contributed to the pressure deformities noted. The findings of a segmental atelectasis on radiograph, facial asymmetry, torticollis and the clubfeet were secondary to the external abdominal constraint. It is important to detect dysmorphism and malformations because these impact on the overall prognosis and will allow interventions that will prevent, anticipate or treat complications.

2400/T

Racial/ethnic disparity and regional variation in participation in buccal DNA collection - The National Birth Defects Prevention Study. *K. Crider[1], J. Reefhuis[1], A. Woomert[2], S. Rasmussen[1], P. Romitti[3], C. Hobbs[4], M. Royle[5].* 1) Centers for Disease Control and Prevention, NCBDDD, Atlanta, GA; 2) Battelle/CPHRE, Durham, NC; 3) University of Iowa, Iowa City, IA; 4) University of Arkansas, Little Rock, AR; 5) New Jersey Department of Health, Trenton, NJ.

The National Birth Defects Prevention Study is a multicenter, case-control study (begun in 10/97) to examine environmental and genetic risk factors for birth defects. A 1-hour telephone interview was completed by 70% of mothers, 56% of whom returned the buccal DNA cell collection kit mailed to them after the interview. We assessed demographic characteristics associated with return of the kits to investigate possible factors related to participation rates for DNA collection. This analysis was limited to mothers with an estimated delivery date on or before 12/31/03. A total of 15,920 interviewed mothers were eligible to receive a buccal DNA kit (11,805 case mothers, 4,115 control mothers). Of these, 60% were non-Hispanic white (NHW), 11% were non-Hispanic black (NHB), 23% were Hispanic (H), and 6% were other (O) races. Rates for DNA collection were highest among NHW (60%) mothers, followed by H (53%), O (49%), and NHB (39%). The Iowa center had the highest overall participation rate (74%), while the New Jersey center had the lowest (41%). Participation ranged from 76% in Iowa to 45% in New Jersey among NHW mothers; from 52% in California to 27% in New York among NHB mothers; and from 64% in Arkansas to 34% in New Jersey among H mothers. DNA collection rates were slightly higher overall among cases (57%) than among controls (52%). Participants who received a $20 incentive with the kit had higher participation rates than those who did not (56% vs. 41%). Maternal age, income, and education had little effect on overall participation rates. Among women who were willing to participate in an extensive interview, participation in the genetic component varied, limiting both the study power and generalizability. We found substantial variation in buccal collection participation rates by region and by race/ethnicity. Incentives increased participation.

2401/T

African-American and Caucasian preterm and term pregnancies exhibit different patterns of cytokine expression. *S.M. Williams[1,2], D.R. Velez[1,2], T.L. Edwards[2], S.J. Fortunato[3], R. Menon[3].* 1) Division of Cardiovascular Medicine, Vanderbilt University, Nashville, TN, USA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA; 3) The Perinatal Research Center, Nashville, TN, USA.

Pregnancy is regulated by homeostasis of multiple cytokines and chemokines. If this balance is disrupted it can induce a variety of outcomes including preterm birth (PTB). The rate of PTB also differs significantly between African Americans (AA) and Caucasians (C) and significant differences exist between AA and C amniotic fluid (AF) cytokine concentrations of both preterm and term mothers. We hypothesize that differences in patterns of cytokine gene expression contribute to the PTB racial disparity. Therefore, we examined correlations among several cytokine concentrations in AF previously associated with PTB. We examined pairwise correlations of cytokine AF concentrations between: Interleukin (IL)-1β, IL-6, IL-8, IL10, Tumor Necrosis Factor-alpha (TNF-a), soluble TNF Receptors-1 and 2 (sTNFR1 and sTNFR2). Analyses were performed to test for differences in correlation coefficients between status and racial groups. Correlation differences were also evaluated in PTB where microbial invasion of the amniotic cavity (MIAC) was observed. Heterogeneity between correlation patterns was documented between C and AA in PTB and in controls (normal term births) for multiple cytokine correlations. In term births the strongest C/AA differences were between Th1/Th2-related cytokines, while in PTB differences were equally distributed between Th1/Th2-related and Th1/Th1 cytokines. Within AA PTB three correlations differed significantly between PTB with and without MIAC (IL-10/IL-1β, TNF-a/IL-6, and sTNFR2/IL-1β), while no differences were observed between C with and without MIAC. This suggests that while infection may play a prominent role in disruption of AA cytokine homeostasis, infection may not play as prominent a role C cytokine homeostasis. Data indicate that the coordination of cytokine networks differs greatly between C and AA and that network heterogeneity exists between PTB and term individuals within each race.

2402/T

Analysis of the oligonucleotide microarray on placentae of pre-eclamptic pregnancies without labor. *J. Park[1], S. Lee[2], W. Lee[3], C. Ryu[3], K. Lee[2], D. Cha[2].* 1) Ob and Gyn, Bundang CHA hospital, Sungnam, Kyoungki-Do, Korea; 2) Ob and Gyn, Kangnam CHA hospital, Seoul, Korea; 3) Digital Geneomics, Seoul, Korea.

Objective: Preeclampsia is a severe disorder of widespread vascular endothelial malfunction that occurs beyond the 20th week of gestation. But, little is known about its etiology. The aim of this study is to investigate the placental specific mRNAs and the related mechanism associated with the progression of this disease using the genome wide expression profiling. Methods: Placentae from 16 normal pregnancies without labor and 17 pregnancies complicated by preeclampsia without labor were collected. We performed genome-wide expression profiling using CodeLink Human Whole Genome Bioarray (55k). Results: Among the 55,000 genes that were analyzed in the microarray, 467 genes were found to be differentially expressed. Among these candidates, 424 were up-regulated and 43 were down-regulated. The up-regulated genes included Fas, Heat shock protein 1 and TRADD, which are well-known biological markers for apoptosis, as well as plasminogen activator inhibitor type 1. Several biological processes associated with the development of preeclampsia were analyzed by gene ontology classification, including response to oxidative stress (selenoprotein P), apoptosis, immune response, and vascular constriction. Conclusion: mRNA microarray is a high throughput and time-saving method to monitor altered gene expression. The results could provide interesting clues to the etiology of pre-eclampsia and lead to further studies in a more targeted fashion.

2403/T

Rapid detection of Down syndrome & Edward syndrome using FISH in amniocentesis. *K. Lee, S. Lee, D. Cha, J. Park.* Ostetrics and gynecology, CHA general hospital, Kangnam-Gu, Seoul, Korea.

PURPOSE : The purpose of this study was to evaluate the clinical utility of rapid detection of down syndrome and Edward syndrome by Interphase Fluolescence in Situ Hybridization (FISH) analysis. METHODS : A retrospective study in 309 cases of amniotic fluid samples, analysed by interphase FISH with DNA probes specific to chromosome 18 and 21, was performed. All FISH results were compared with conventional cytogenetic karyotypings. RESULTS : The results were considered as informative and they were obtained within 48 hours. A case of Down syndrome and a case of Edward syndrome were diagnosed by FISH and confirmed by subsequent cytogenetic analysis. In 12 cases with normal FISH results, the cytogenetic analysis showed a case of partial trisomy 22, three cases of sex chromosomal aneuploidy, two cases of mosaicism, two cases of microdeletion, and four cases of structural rearrangement. CONCLUSION : FISH is a rapid and effective diagnostic method, which can be used as an adjunctive test to cytogenetic analysis, for prenatal idetification of chromosome aneuploidies. For the more genome-wide screening with variety of probes, the technique of FISH is both expensive and labour-intensive.

2404/T

Prenatal False Positive for Trisomy 21 with Fluorescent in situ Hybridization (FISH). *H. Sroka[1], E. Kolomietz[2, 3], E.J.T. Winsor[2,3], J. Ng-Kcomt[2], E. Cappa[2], D. Chitayat[1,3].* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital,Toronto, Canada; 2) Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada; 3) University of Toronto, Toronto, Canada.

We present a case with clinical information and interphase FISH results consistent with with trisomy 21 and normal male karyotype. A 32-year-old primigravida woman presented with an increased fetal nuchal translucency of 4.1mm at 12.7 weeks gestation. First trimester combined prenatal screening yielded a risk of > 1/8 for Down syndrome and 1/36 for trisomy 13/18. The couple declined CVS and proceeded with amniocentesis at 16 weeks gestation. Rapid FISH analysis on uncultured amniotic fluid cells was performed using AneuVision (Vysis) probes for chromosomes 13, 18, 21, X and Y. Ninety-four percent of nuclei showed 3 signals for chromosome 21 and a normal pattern for the other chromosomes. The couple was informed of the FISH results and arranged for termination of the pregnancy, which was delayed due to the holiday season. Seven days later metaphase chromosome analysis revealed a normal male karyotype and the couple was immediately informed. FISH studies using the same probe mixtures were carried out on metaphase slides and revealed no evidence of a cryptic translocation involving chromosome 21. In retrospect, the most likely explanation for the false positive FISH result was contamination of the probe mixtures during application on the slide. Analysis was performed on two areas of the microscope slide (probes for chromosomes 13 and 21 on one site and 18, X and Y on the other site) and the probes for chromosome 21 and Y are both labelled with SpectrumOrange. Fetal anatomy ultrasound and fetal echocardiography at 19 weeks did not reveal any anomalies. The pregnancy is currently ongoing. The ACMG/ASHG statement (2000) on FISH testing recommends that clinical decision-making should be based on information from 2 of 3 of the following: positive FISH results, confirmatory chromosome analysis, or consistent clinical information. We present this case to warn clinicians and patients that, in rare circumstances, errors occur despite meeting the usual counselling precautions.

2405/T

Autosomal Dominant Small intestinal atresia/stenosis - Case Report. *P. Kim[1], J. Jessen[2], S. Koenen[2], D. Chitayat[1,2].* 1) Hospital for Sick Children, Dept. of Pediatric Surgery (PK), Clinical and Metabolic Genetics (DC); 2) Mount Sinai Hospital,Dept. of Obstetrics and Gynecology (SK), Prenatal Diagnosis and Medical Genetics (JJ,DC) Toronto, Ontario, Canada.

Intestinal atresia (IA)caused by intramural web is an uncommon variant of congenital IA, typically reported neonatally or in childhood and occasionally remaining asymptomatic throughout the entire life span. Type I [mucosal web (Martin and Zerella, 1976)] jejunoileal atresia (JA) is generally thought to be a sporadic event with hereditary types believed to be uncommon although familial cases with autosomal recessive mode of inheritance have been reported. We report a mother with JA and her son with duodenal atresia (DA), both due to web, suggestive of an autosomal dominant mode of inheritance. Case Report: The pregnancy was conceived via IVF following 3 years of infertility. The mother was of Ashkenazi-Jewish Russian descent, the father of Polish descent. The couple was non-consanguineous. The mother was born with intestinal obstruction and on surgery was found to have JA due to a web. The mother's maternal great aunt had a son with reportedly DA and this son has a son with the same condition. The pregnancy was initially uncomlicated, and FTS was done and showed a nuchal translucency of 0.9 mm with an adjusted risk for Down syndrome of 1:724. Mother presented due to rupture of membrane at 30 weeks gestation, fetal ultrasound showed severe oligohydramnios, distended stomach and loop of proximal small bowel, likely duodenum. Emergency cesarean section was performed at 33 weeks gestation. Duodenal obstruction was confirmed postnatally. At the time of surgery, baby was found to have a duodenal web. Duodenojejunostomy was done. The baby is doing well at 6 months follow up. There are 2 theories regarding the etiology of IA. The first proposed a lack of recanalization during the embryological solid cord stage of intestinal development. The second is that IA is caused by an embryological vascular accident resulting in necrosis of the affected segment. To the best of our knowledge this is the first report of a mother and son affected, suggestive of autosomal dominant mode of inheritance.

2406/T

Introduction of QF-PCR as a rapid aneuploidy screen for all women undergoing amniocentesis: A pilot project. *D. Allingham-Hawkins[1], E. Winsor[2], D. Chitayat[2], V. Cirigliano[3], A. Summers[1], K. Chun[1].* 1) Genetics Program, North York General Hosp, Toronto, ON, Canada; 2) Mount Sinai Hosp, Toronto, ON, Canada; 3) General Lab, Barcelona, Spain.

Aneuploidies of chromosomes 13, 18, 21, X and Y are the most common abnormalities detected in prenatal specimens, representing ~70% of all chromosome abnormalities detected and ~77% of unbalanced abnormalities. Given that conventional chromosome analysis typically takes 10-14 days due to the need for cell culture, it is desirable to have a rapid, cost effective method of ruling out these common abnormalities thereby reducing patient anxiety. Quantitative fluorescent PCR (QF-PCR) uses multiple short tandem repeats (STRs) on each of the chromosomes of interest analysed on an automated DNA sequencer to detect numerical changes. To investigate the feasibility of introducing QF-PCR as a routine rapid aneuploidy screen, we are conducting a pilot study comprised of 200 blind validation specimens followed by 1000 prospective amniotic fluid specimens from two large prenatal diagnostic centres in Toronto, Canada. The Aneufast™ (Genomed, UK) kit is being used for all analyses. This kit uses two initial multiplex reactions with 4 STRs on each of chromosmes 13, 18 and 21, two polymorphic X/Y markers and HPRT, SRY and amelogenin for sexing. Reflex reactions with additional markers are available for each chromosome to confirm abnormals or clarify ambiguous results. Among the validation specimens, 194/195 were identified correctly while 5 (failure rate = 2.5%) failed to amplify. The one incorrect result was a XX/XO mosaic interpreted as a normal female. The first 644 study specimens have shown a positive predictive value of 100% (27/27 predicted to be abnormal were true positives) and a negative predictive value of 99.8% (607/608 predicted to be negative for aneuploidy were true negatives). One XX/XXX specimen was interpreted as normal. The failure rate was 1.4%. The other major outcome measure being studied is the turnaround time in hours from receipt of the specimen and issuing of the report. The results for all 1000 specimens will be presented and next steps in delivery of this service discussed.

2407/T

Globin Gene Mutations in Turkey. *M.A. Curuk.* Biochemistry, C.U. Medical Faculty, Adana, Balcali, Turkey.

Hemoglobinopathies are the most common genetic abnormalities causing health problems in the world. Beta thalassemia (β-thal)and sickle cell anemia (SCA)constitute the majority of hemoglobin (Hb) disorders in Turkey. β-thal is seen throughout the country but SCA is prevalent in the çukurova Region, Southern Part Turkey. The overall frequency of β-thal is 2 per cent. The highest frequencies are observed in Antalya and Mugla (10 and 4.8 per cent). The incidence of β-thal trait is 3.7 per cent and of HbAS 10 per cent in the çukurova region (Adana, Hatay and Mersin). The sickle cell gene is very common in çukurova and prevalent among Eti-Turks living in this region. The frequency of sickle cell trait (HbAS) ranges from 0.5 to 44.2 per cent. In addition to HbS [β6;Glu→Val] more than 42 abnormal Hb variants have been reported in Turkey. Furthermore, about 40 different β-thal mutations were characterized in Turkish population. The most common β-thal mutation is IVS1-110 (G→A) and almost ten of the mutations constitute about 90 per cent all of the cases. Since, there is no cure for β-thalassemia and sickle cell disease at present, the type of the mutation must first be identified for prenatal and preimplantation diagnosis. The incidence of alpha thalassemia (α-thal) is about 1-2per cent in Turkey. HbH disease is a severe form of α-thalassemi but is compatible with life. Combinations of α-thal-1 (--/αα)and α-thal-2 (-α/αα) determinants cause HbH (β4) disease. A patient who inherited a single α-globin gene (--/-α) has HbH disease with a chronic hemolytic anemia. HbH disease in the Turkish population is most commonly produced by the deletions of three structural α-globin gene (--/-α) and rarely by the combination of α-thal-1 and nondeletional mutations (--/αα)affecting the α-globin genes (α2 or α1). Three α-thal-1 (-17.4 kb, -20.5 kb and -26.5 kb), two α-thal-2 (-3.7 kb and -4.2 kb) and four nondeletional mutations PA1, PA2 [(PA1: AATAAA→AATAAG or PA2: AATAAA→AATGAA) -5nt, and codon 59 (GGC→GAC)] have been reported in Turkey. The molecular basis of HbH disease is quite heterogeneous in Turkey. The presences of 12 different genotypes in 32 patients with HbH have been presented in this study.

2408/T

Assessment of Liquid Microbead Arrays for the Newborn Screening of Spinal Muscular Atrophy. *R.E. Pyatt, D.C. Mihal, T.W. Prior.* Dept Pathology, Ohio State Univ, Columbus, OH.

Spinal muscular atrophy (SMA) is common neurodegenerative disorder with an incidence of 1 in 6,000 births. Ongoing clinical trials are evaluating therapeutic agents, and recent reports have suggested that motor denervation occurs within weeks of birth especially for the most severely affected. The success of these agents depends on identifying individuals as early as possible in order to begin treatment before irreversible neuronal loss. Identification during the newborn period can only be accomplished by direct DNA testing since SMA has no biochemical marker. DNA analysis has been described as the next innovation in newborn screening, but currently in the United States it is used primarily for reflex testing. The object of this study was to validate liquid microbead arrays for the identification of affected individuals by direct DNA analysis. Assays were created to detect the homozygous deletions in SMN1 exon 7 found in approximately 95% of affected individuals using two different microbead chemistries on the Luminex 200: MultiCode-PLx and Tag-It. A series of 367 blood spots including 164 affected, 46 known carrier, and 157 unaffected individuals were then analyzed with each assay. The MultiCode-PLx assay required 4.2 hours and provided correct identification of all 164 affected samples demonstrating 100% sensitivity. Correct exclusion was also made for all 46 carrier and 157 unaffected samples demonstrating 100% specificity. Conversely, the Tag-It assay required 6.8 hours and demonstrated 100% sensitivity and 99.5% specificity. A single false positive sample was observed with the Tag-It chemistry which upon repeat analysis presented values for SMN1 exon 7 which were intermediary between unaffected and affected samples. Neither chemistry displayed sensitivity to increasing copy numbers of the SMN2 pseudogene. Both chemistries showed high levels of sensitivity and specificity for the detection of SMA affected patients. Furthermore ample DNA was extracted from all bloodspots for analysis and SMN2 levels did not interfere with either assay. Liquid bead arrays represent a robust methodology for DNA analysis in newborn screening laboratories.

2409/T

Evaluation of Different Array-CGH Procedures for Preimplantation Diagnosis. *Y. Yang, M.J. Simovich, W. Jin, M.L. cooper, K.L. Pham, S.W. Cheung.* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Screening for chromosome abnormalities in preimplatation diagnosis (PGD) is currently performed by Fluorescent in situ hybridization (FISH), which can detect aneuploidy for chromosomes 13, 15, 16, 18, 21, 22, X and Y. This detection efficiency can be improved by utilizing the novel array-CGH technology, which scans the whole genome for large deletions and duplications. However, performing single cell array-CGH for PGD has a number of challenges. First, the amplified DNA from single cells should have a good coverage of the entire genome. Second, the amplified products from samples and references should be as similar as possible in order to reduce noisy hybridization signals caused by bias genome representations during amplification. Third, the turn-around time for the test should be within 48 hours.

We are evaluating different protocols in order to optimize conditions for single cell array-CGH in PGD. Single cells were obtained from discarded embryos on day 6. These embryos had undergone PGD for aneuploidy screening by FISH on day 3 at 6-8 cell stage and chromosomal abnormalities were identified. The whole genome amplification was performed by both ligation-mediated PCR and multiplex displacement amplification (MDA) using Phi29 DNA polymerase. Different reference samples including normal single cells as well as different concentrations of purified DNA were used. Array-CGH was performed using Baylor CMA array version 5, which is consisted of 853 BAC clones. The hybridization results will be compared and the optimized procedures for array-CGH in PGD will be proposed.

2410/T

Noninvasive Prenatal Testing for RhD Status by Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) Mass Spectrometry: A Pilot Study. *M.J. Basehore[1], J. Wiszniewska[1], B. Dragon[2], J. Tynan[2], J.A. Lee[1], T. Legler[3], D. van den Boom[2], M. Ehrich[2], C.M. Eng[1].* 1) Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX; 2) Sequenom Inc., San Diego, CA; 3) University Medicine Goettingen, Germany, SAFE Network Partner.

Prenatal genetic analysis of fetal status is currently performed using techniques that require invasive procedures to obtain fetal-derived samples. Chorionic villous sampling and amniocentesis are associated with fetal loss rates ranging from 0.5-1%. The discovery that cell-free fetal DNA circulates in maternal plasma has lead to the consideration of its use as a source of fetal material, thereby providing a noninvasive approach to determining prenatal genetic status. Cell-free fetal DNA is detectable around four weeks of gestation and comprises approximately 6% of total free plasma DNA by the third trimester of pregnancy. However, the limited amounts of fetal DNA in maternal plasma and the need to differentiate fetal genotype from the maternal background present challenges that have hindered the implementation of noninvasive prenatal genetic testing in clinical diagnostics. In this study, peripheral blood samples were obtained from 130 RhD-negative, pregnant women at an average gestational age of 26 weeks. Cell-free fetal DNA was extracted from maternal plasma and fetal samples were tested for RhD status, as well as the presence or absence of SRY, by MALDI-TOF mass spectrometry. These results showed complete concordance with fetal RhD genotype determined by real-time PCR and gender determined postnatally. Additionally, 30 of the 130 samples were also tested in a clinical laboratory on a research basis and results were concordant with previous studies for RhD genotype by real-time PCR and MALDI-TOF, gender, and postnatal serological phenotype. These preliminary studies indicate that MALDI-TOF mass spectrometry is a potential method for the noninvasive prenatal assessment of RhD status. Further studies are needed to delineate the stability and optimal extraction conditions for cell-free fetal DNA before this method can be routinely implemented in a clinical diagnostic setting.

2411/T

Reliable capture of fetal cells from maternal blood and detection of trisomy using a novel cell capture and enrichment device. *F.Z. Bischoff[1,3], R. Wapner[2], J. Williams[4], P. Cotter[3], J.L. Simpson[5].* 1) Baylor College of Medicine, Houston, TX; 2) Columbia Univ, New York, NY; 3) Biocept, Inc., San Diego, CA; 4) Cedars-Sinai Medical Center, Los Angeles, CA; 5) Florida International Univ College of Medicine, Miami, FL.

Recovery and analysis of fetal cells from maternal blood could yield non-invasive definitive prenatal diagnosis. Though many reports support successful detection of common fetal aneuploidies, methods for isolation are inconsistent. The problem may be surmounted by MEMS (microelectromechanical system), whose principle is to capture cells based on attachment chemistry and microfluidics. Attachment is facilitated through antibodies linked to a hydrogel used to coat the post-filled device (75μ x 12mm x 30mm).**OBJECTIVE** Determine feasibility of a MEMS based approach to capture and analyze fetal nRBCs by FISH.**METHODS** Under IRB approval, maternal blood (30ml) was obtained from three women suspected of having either trisomy 21 (10.5 and 11.1 wks) or trisomy 18 (12.0 wks); investigators were blinded and unaware of a potential abnormality. Concurrently a sequential series of 15 first trimester maternal samples was studied from patients having CVS (9-12.6 wks). Following ficoll separation, recovered mononuclear cells were passed through the MEMS device coated with glycophorin-A antibodies. Antibody fluorescent staining to epsilon hemoglobin is used to confirm fetal cell origin. FISH (21-specific; 18-, X-,Y- centromeric probes; Vysis Inc.) was performed within the device (FirstCEE) using standard fluorescent microscopy.**RESULTS** Fetal trisomic cells in robust number (7 to 10 cells) were correctly identified in each of the 3 trisomic cases. In the 10 controls from a pregnancy with a male fetus, 8 were informative (3 to 6 fetal cells per 10ml of blood). No false-positive male cells were detected in 5 female cases.**CONCLUSIONS** Our results demonstrate reliable detection of both euploid and aneuploid fetal cells using MEMS technology. Clinical validation studies are currently underway to define sensitivity and specificity. This novel technology can offer most patients a definitive first trimester diagnostic test that is non-invasive.

2412/T

Systematic search for placental epigenetic markers on chromosome 21: towards noninvasive prenatal diagnosis of fetal trisomy 21. *S.S.C. Chim[1], S. Jin[2], T.Y.H. Lee[2], F.M.F. Lun[2], W.S. Lee[2], L.Y.S. Chan[2], Y. Jin[2], N. Yang[2], Y.K. Tong[2], T.Y. Leung[1], T.K. Lau[1], C. Ding[3,4], R.W.K. Chiu[2,3], Y.M.D. Lo[2,3].* 1) Dept of Obstetrics & Gynaecology; 2) Dept of Chemical Pathology; 3) Li Ka Shing Institute of Health Sciences; 4) Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong SAR, China.

The presence of fetal DNA in maternal plasma has offered a source of fetal genetic materials for noninvasive prenatal diagnosis. However, co-existing background maternal DNA complicates the analysis of such fetal DNA for aneuploidy detection. Recently, differential methylation patterns between the placenta and maternal blood cells have been shown for *SERPINB5* on chromosome (Chr) 18. Fetal trisomy 18 was further shown to be detectable noninvasively based on the allelic ratio of hypomethylated *SERPINB5* in maternal plasma. To develop a similar method for the noninvasive detection of trisomy 21, we systematically searched 114 CpG islands (CGIs), representing 76.5% of all 149 CGIs on Chr21, for differential DNA methylation patterns in the placenta and maternal blood cells. Most of the CGIs not studied contained repetitive DNA. Extent of CpG methylation in 5 placentas and 5 maternal blood cells were determined by methylation-sensitive single nucleotide primer extension and/or bisulfite sequencing. The methylation index (MI) of a CpG site was estimated as the ratio of the methylated to the total population of molecules. Thirteen CGIs were shown to contain ≥1 CpG site completely unmethylated (MI=0.00) in maternal blood cells and methylated in the placenta (MI range 0.22-0.65). Nine CGIs were shown to contain ≥1 CpG site completely methylated (MI=1.00) in maternal blood cells and hypomethylated in the placenta (MI range 0.00-0.75). One of these placental epigenetic markers was detected in 100% of 12 maternal plasma samples and another marker in 8 samples during pregnancy. Both markers exhibited postpartum clearance. Epigenetic differences between the placenta and maternal blood cells were found on 22 of 114 (19.3%) CGIs studied on Chr21. Epigenetic alterations may therefore provide a rich source of markers for noninvasive prenatal diagnosis.

2413/T

Large scale clinical application of QF-PCR for Rapid Prenatal Diagnosis of Common Chromosome Aneuploidies. *V. Cirigliano[1,2], G. Voglino[3], A. Marongiu[3], E. Ordoñez[1,2], A. Plaja[1], C. Fuster[2], M. Adinolfi[4].* 1) General Lab, Barcelona, Spain; 2) Biologia Celular. Universitat Autònoma de Barcelona Bellaterra, Spain; 3) Promea-Day Surgery Turin Italy; 4) University College London U.K.

Rapid prenatal diagnoses of common aneuploidies can be performed using microsatellites amplified by Quantitative Fluorescent PCR (QF-PCR). The assay was introduced as preliminary investigation to remove parental anxiety while waiting for the results of cytogenetic analysis. Main advantages of the molecular assay are its low cost, speed and automation allowing large scale application. We developed a highly informative QF-PCR assay that was applied to systematically screen 38.000 consecutive prenatal samples with results issued within 24 hours. The most common referral indications were raised biochemical risk (32%) and advanced maternal age (30%), 6% of these cases were also associated with increased nucal translucency; parental anxiety generated 22% of samples and abnormal ultrasound findings were present in 7 % of fetuses. All samples were also tested by conventional cytogenetic analysis and results compared. In most cases a normal chromosome complement was correctly assessed by QF-PCR without false positive results. All 1278 non mosaic aneuploidies involving chromosomes 21, 18 and 13 were identified with 100% sensitivity and specificity. Several cases of partial trisomies and chromosome mosaicisms could also be detected. The assay proved efficient and reliable allowing early termination of affected pregnancies without waiting for cytogenetic analysis. Despite being deliberately targeted to investigate only disorders affecting three autosomes (21, 18 and 13) and the two sex chromosomes, we show that QF-PCR can detect the great majority of chromosome abnormalities in a few hours after sampling. Our results raise the possibility of reducing the load of conventional prenatal cytogenetics if all pregnancies are also monitored by careful application of biochemical and ultrasound tests in the first trimester. In countries where large scale cytogenetics is hampered by its cost and lack of technical expertise QF-PCR may be used as the only prenatal diagnostic test.

2414/T

Prenatal diagnosis of a Roberts syndrome without prenatal cytogenetics characteristics findings. *C. de La Rochebrochard[1], J. Lucas[2], S. Blesson[3], P. Poulain[4], G. Le Bouar[4], L. Pasquier[1], C. Henry[2], J. Milon[4], C. Quelin[1], L. Loeuillet[5], A. Guichet[3], B. Laudier[3], H. Richard[6], G. Haddad[3], S. Odent[1].* 1) Department of Genetics, Rennes University Hospital; 2) Laboratory of Cytogenetics, Rennes University Hospital; 3) Department of Genetics, Tours University Hospital; 4) Department of Obstetrics and Gynaecology, Rennes University Hospital; 5) Department of Pathology, Rennes University Hospital; 6) Department of Obstetrics and Gynaecology, Fougeres Hospital.

The rare, autosomal recessive Roberts syndrome (RBS) is characterized by tetraphocomelia, profound prenatal growth deficiency, craniofacial anomalies, microcephaly and mental deficiency. RBS cells are characterized by heterochromatin repulsion (HR) or premature centromere separation (PCS). Recently mutations in ESCO2 gene have been reported in RBS. We report a non consanguineous couple referred to our centre for their second pregnancy. At 22 weeks of gestation of their first pregnancy, an ultrasonographic examination detected a severe tetraphocomelia with microcephaly and profound growth deficiency. RBS was suspected and confirmed on autopsy examination and cytogenetics analysis. The foetus exhibited Pierre Robin sequence and tetraphocomelia. RHG banded foetal chromosomes exhibited characteristic PCS on fibroblasts and amniocysts. The second pregnancy was monitoring by combined ultrasound examination with CVS foetal karyotype. RHG chromosome analysis concluded no PCS on 70 cytotrophoblastic cells. At 21 weeks of gestation, bilateral radial agenesis led us to evoke a RBS recurrence. Post-mortem autopsy at 22 SA confirmed moderate growth deficiency, craniofacial anomalies, bilateral radial and thumb agenesis. Interestingly, lower limbs, macroscopic and histological brain analysis were normal. Cytogenetics analysis on 5 more tissues revealed characteristic PCS in only 14 cells in lung and cord out of 213. ESCO2 gene screening is undergoing. RBS might be under diagnosed according to extreme cytogenetics findings variability. We emphasize the importance of ESCO2 mutation screening in suspected RBS, according to genetic counselling and reliable further precocious prenatal diagnosis.

2415/T

Improving Utility of Circulating DNA for Prenatal Genetic Testing: Fragment Size and Purity. *C. Jorgez, F. Bischoff.* Department of Obstetric and Gynecology, Baylor College of Medicine, Houston, TX.

Objetive Among the pitfalls of using cell-free-fetal DNA from plasma for prenatal diagnosis is the quality of recovered DNA fragments and abundance of maternal DNA (>95%). Our objective was to explore an alternative method for achieving enrichment and high-quality fetal DNA from plasma. **Methods** Cell-free DNA from 31 pregnant-women and 18 controls (10 males and 8 females) were size separated using agarose gel electrophoresis. DNA from sections containing fragments of 100-300; 500-700; and 1500-2000bp were excised and extracted, followed by whole genome amplification (WGA) of recovered fragments. Levels of βglobin and DYS1 for total and fetal DNA, respectively, were measured. **Results** Higher quality enriched fetal DNA was obtained following electrophoresis and WGA. Distribution of βglobin size-fragments was similar among pregnant-women and controls. Among the control-male cases, distribution of size-fragments was the same for both βglobin and DYS1. However, among pregnant women smallest size fragment (100-300bp) accounted for nearly 50% (39.76±17.55%) of the recovered DYS1-DNA but only 10% (10.40±6.49%) of βglobin DNA. After WGA of plasma fragments from pregnant-women, DYS1 sequence amplification was best observed when using the 100-300 bp fragments as template. As measured by the 260/280 ratio, quality of DNA also improved after WGA (0.96±0.22 before vs. 1.60±0.14 after). **Conclusions** Combination of gel-extraction and WGA could lead to enrichment of fetal-DNA from plasma for improvement of clinical applications. These methods will better enable high-throughput screening for more comprehensive testing of prenatal genetic abnormalities.

2416/T

Identification of a novel mutation in the ARG1 gene, and prenatal diagnosis for hyperargininemia. *H. Laivuori[1], T. Linnankivi[2], P.J. Miettinen[3], J. Häberle[4].* 1) HUSLAB Department of Clinical Genetics, Helsinki, Finland; 2) Department of Pediatric Neurology, Helsinki University Central Hospital, Helsinki, Finland; 3) Department of Pediatrics, Helsinki University Central Hospital, Helsinki, Finland; 4) Universitätsklinikum Münster, Klinik und Poliklinik für Kinderheilkunde, Münster, Germany.

Hyperargininemia (OMIM 207800) is a rare (estimated incidence 1:2 000 000 births) autosomal recessive urea cycle disorder caused by a defect in the arginase I enzyme. It is characterized by predominantly neurological symptoms which usually appear between 2 and 4 years of age (slowly progressive spastic paraparesis and cognitive decline). Limitation of protein intake, supplementation with essential amino acids and sodium benzoate ameliorate the symptoms, although the spastic paraparesis may progress as patients become older. The enzyme activity can be measured in erythrocytes, but not in chorionic villous sample (CVS) or in cultured amniotic fluid cells. Prenatal diagnosis has been reported using enzyme measurement from fetal cord blood (Kamoun et al. 1995, Hewson et al. 2003) and DNA derived from CVS (Häberle & Koch 2004). Here we report a novel mutation in the ARG1 gene (Chr. 6q23) identified from the child affected with hyperargininemia (arginase enzyme activity in erythrocytes profoundly deficient: 5 IE/g Hb, normal: >35 IE/g Hb) born to consanguineous Kurdish parents (first cousins). The parents asked for the prenatal diagnosis in a subsequent pregnancy. All coding exons including the flanking intronic regions of the ARG1 gene of the index patient were sequenced. The mutation in the exon 3: c.282C>G (S94R) was found in a homozygous state with both parents being heterozygous for this mutation. The prenatal diagnosis was performed from the CVS, and the fetus was unaffected. Direct mutation analysis from CVS can be regarded as the method of choice for prenatal diagnosis in hyperargininemia.

2417/T

Insertion/Deletions as Control Markers for Fetal DNA Detection in Non-Invasive Prenatal Diagnostic Assays. *P. Mahboubi, T. Shi, B. Dragon, D. van den Boom, P. Oeth.* Research and Development, Sequenom, San Diego, CA.

The discovery of fetal DNA in maternal plasma has driven development of non-invasive prenatal diagnostic genotyping assays. A technical challenge of this method is to ensure that a negative test result of a disease linked genomic region is truly a negative, not due to insufficient fetal DNA. We have developed a method, using common insertion/deletions (I/D), coupled with allele specific PCR (ASP), to detect fetal DNA, independent of gender, in all samples. To determine how many highly polymorphic I/D assays are needed to detect at least 3 fetal identifier markers in a maternal background, probability calculations were carried out assuming a deletion allele frequency of 0.38 and that only 2 of the 9 possible genotypes of the fetus are informative (I/D where the father is a heterozygote I/D, I/D where father is homozygote I/I). Modeling using these assumptions shows that 90 assays are needed to detect at least 3 fetal identifiers with a confidence greater than 0.99. Based on these calculations 231 I/D polymorphisms, with minor allele frequencies (MAF) greater than 0.30, were obtained. PCR assays were designed into 21-plex reactions using Sequenom Assay Design 3.1 software and genotyped using the TypePLEX reaction on the MassARRAY system (Sequenom). Assays were tested on 23 CEPH DNAs and allele frequencies confirmed on the 90 CEU HapMap DNAs. Robust assays with a MAF of 0.38 or higher were redesigned into 32-plex reactions as ASP assays by overlapping the 3' end of the forward primer with the I/D region (Sequenom QGE Assay Design Software). To mimic detection of fetal DNA against a maternal DNA background, working assays were successfully tested on mixes of an insertion-containing DNA in a deletion DNA background with insertion DNA ranging from 40% to as low as 5% of the total DNA (1000 copies). Our data to date shows that these assays function properly on targets regions composed of 5% of total DNA (50 copies in a background of 950 copies) and establishes the use of common I/Ds as control markers or "Fetal Identifiers" as a viable and robust method to control for false negative results in the emerging field of non-invasive prenatal diagnostics.

2418/T

Stability of Placental RNA using Dried Maternal Blood Spots. *D. Marquez-Do, C. Jorgez, F. Bischoff.* OB/GYN, Baylor College of Medicine, Houston, TX.

Introduction Circulating plasma RNA appears to be associated with subcellular particles, rendering stability under different preanalytical conditions. Circulating levels of several transcripts in maternal plasma have been shown to correlate with poor pregnancy and/or fetal outcome. Thus, RNA analysis for prenatal diagnosis and pregnancy related complications using dried blood spots (DBS) could provide an economical and simple way to collect, ship, store, and process samples. **Objetive** Having demonstrated successful recovery and detection of placental mRNA from DBS, we further explore the role of other factors, including temperature (4°C vs. 25°C) and processing time (from 24h to 8 weeks), that may interference stability and detection of placental transcripts. **Methods** mRNA fragments encoding human GAPDH (internal control) and βhCG (placental transcript) were analyzed by real-time PCR using DBS from 7 pregnant women (GA=9.09±2.50). **Results** GAPDH and βhCG transcript were detected in all samples 24h after collection. After one week of storage, we observed decrease in the amount of RNA recovered; however, the decrease was influenced by target transcript, temperature and storage time. For GAPDH, no significant reduction at either temperature was observed when processed at 1 week. Reduction of GAPDH was only significant ($p=0.033$) after 8 weeks storage at 25°C. For βHCG, following 1 week, a 50% reduction at both temperatures was observed. After 2 weeks at 25°C, 90% reduction was reached and held constant by 8 weeks. However, βHCG transcripts were significantly more stable over 2-8 weeks at 4°C ($p=0.051$). Though βHCG levels decrease with time at 4°C, transcript levels were not significantly lower than levels measured at 1 week ($p=0.254$). **Conclusions** Although placental mRNA can be isolated from DBS, only 50% of the mRNA appears to be protected from degradation following long storage times. Further studies are warranted to identify additional fetal/placental transcripts amenable to detection and prenatal screening.

2419/T

Circulating cell-free placental mRNA in the maternal plasma as a predictive marker for twin-twin transfusion syndrome. *K. Miura[1], K. Yoshiura[2], S. Miura[1], K. Yamasaki[1], D. Nakayama[1], N. Niikawa[2], H. Masuzaki[1].* 1) Nagasaki University Graduate School of Biomedical Science, OB/GYN, Nagasaki, Japan; 2) Nagasaki Univ. Graduate School of Biomedical Science, Human Genetics, Nagasaki, Japan.

Objective:The purpose of the present study is to know whether cell-free mRNA (cf-mRNA) concentration in maternal plasma becomes a predictive marker of later Twin-twin transfusion syndrome (TTTS). Materials and Methods:The study participants included 17 pregnant women who visited Obstetrics Clinic of Nagasaki University Hospital at 12-21 weeks of gestation for management of their pregnancy with monochorionic diamniotic twins (MCDA-T). And, 135 singleton pregnant women were also included as control group. All of the participants gave written informed consent. Although all 17 cases of MCDA-T were not complicated by TTTS at the time of blood sampling, 5 cases subsequently developed TTTS (TTTS group), while the remaining 12 cases did not develop TTTS (no-TTTS group). Plasma concentrations of such cf-mRNA for human Placental Lactogen (PL) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured and converted into multiples of the median (MoM) of the controls adjusted for gestational age. Their differences between the TTTS and the no-TTTS groups were evaluated with Mann-WhitneyÅfs U test. Significant difference was defined as a p-value of less than 0.05. Results:The median (minimum-maximum) cf-PL mRNA MoM values were 1.80 (0.89-3.81) in the TTTS-group, 1.14(0.77-1.35) in the no-TTTS group and 1.00 (0.82-2.05) in the control group, respectively. The cf-PL mRNA concentration was significantly higher in the TTTS group than in the no-TTTS group at adjusted gestational age (Mann-WhitneyÅfs U test, $p=0.035$), while there was no significant difference of cf-PL mRNA concentration between the no-TTTS group and the control group ($p=0.41$) (Figure 1). In addition, the median cf-GAPDH mRNA MoM value in the maternal plasma was significantly higher in the TTTS group (2.20; range, 1.30-2.68) than in the no-TTTS group (1.09; 0.68-3.25) ($p=0.045$). Conclusion: A quantitative aberration of both the cell-free PL and cell-free GAPDH mRNA in maternal circulation may be a novel predictive marker for TTTS.

2420/T

Compelling prenatal indication of autosomal recessive primary microcephaly (MCPH). *D.B. Rogers[1], C. Coffeen[1], L. Mahon[2], N. Qin[3], L.D. Platt[4], D. Krakow[5].* 1) Genzyme Genetics, Los Angeles, CA; 2) Quest Diagnostics, West Hills, CA; 3) Genzyme Genetics, Orange, CA; 4) Center for Fetal Medicine and Women's Ultrasound, David Geffen School of Medicine at UCLA, Los Angeles, CA; 5) Div. of Medical Genetics, Dept. of OBGYN, Cedars-Sinai Medical Center, Los Angeles, CA.

Occasionally prenatal cytogenetic testing yields results that suggest a specific non-chromosomal disorder. A 29-year-old G2P0TAB1 Persian woman underwent genetic counseling and amniocentesis at 19 weeks GA because of abnormal ultrasound findings consisting of echogenic bowel and bilateral renal pyelectasis. The karyotype was reported as 46,XY, but the banding resolution was only 350. This is below the standard cytogenetic methodology. The patient declined a repeat amniocentesis and continued her pregnancy. Ultrasound follow up at 24 weeks revealed the fetal head to be growing at the fifth percentile. Second opinion ultrasonography confirmed the possible microcephaly with no structural defects. Fetal MRI was remarkable for decreased brain parenchyma volume and underdevelopment with relatively increased amount of subarachnoid fluid. The patient then sought and achieved pregnancy termination. This patient's first pregnancy was terminated in the first trimester due to the ultrasound finding of a cystic hygroma. Cytogenetic analysis on CVS revealed 46,XX with a band resolution at the 350 level

MCPH is a neurodevelopmental disorder that features microcephaly and mental retardation. The brain is small, but structurally normal, and the cerebral cortex is greatly reduced in size. One of the four identified genes implicated in MCPH is MCPH1 that encodes for microcephalin. This protein is believed to play a role in cell-cycle timing and DNA repair following ionizing radiation damage. The findings in our patient's fetal chromosome preparations from two different pregnancies and two distinct tissues are consistent with premature chromosome condensation, a feature of MCPH due to a mutation in MCPH1. Fetal cells saved from the latest pregnancy will be studied to confirm a microcephalin gene defect.

2421/T

New light on the changing epidemiology of cystic fibrosis: the 15-year experience of Brittany (western France). *V. Scotet[1], I. Duguépéroux[1], MP. Audrézet[1,2], M. Blayau[3], P. Parent[4], H. Journel[5], P. Boisseau[6], C. Férec[1,2].* 1) Inserm U613, Brest, France; 2) Dept of Genetics, Brest, France; 3) Dept of Genetics, Rennes, France; 4) Unit of Medical Genetics, Brest, France; 5) Unit of Medical Genetics, Vannes, France; 6) Dept of Genetics, Nantes, France.

This study aimed to describe 15-year experience in the field of prenatal diagnosis (PD) for cystic fibrosis (CF) of Brittany, a region where CF is frequent and where the uptake of PD is common. For this, we registered, by the genetic laboratories of our region, all the PDs performed in women living in Brittany over the period 1991-2005. First, we described the number of PDs made for each reason (way by which the one-in-four risk was identified: previous affected child, family testing, echogenic bowel, etc). We then reported the proportion of CF fetuses and of consecutive terminations, and assessed the incidence modification due to PD. Over the 15-year period, a total of 253 PDs were performed in couples living in Brittany. Most of them were done in couples already having CF child(ren) (n=167, 66.0%). Extended testing in families led to the identification of 18 new one-in-four risk couples among the relatives of CF patients who opted for PD 38 times over the study period (15.0%). The 48 other PDs were made in couples without previous history of CF. The one-in-four risk was mainly identified following the detection of an echogenic bowel during pregnancy ultrasound examination (n= 40 - 15.8%). The other PDs were consecutive to the detection of an heterozygote through newborn screening (n=6, 2.4%) or for an other reasons (n=2, 0.8%). Overall, a total of 88 CF fetuses were identified, among whom 77 were terminated (87.5%). The inclusion in the incidence calculation of these 77 pregnancy terminations led that to an incidence modification of 28.8% over the study period. This study reports the long experience of a region in the field of PD for CF. It shows that this test is commonly used in Brittany and highlights the impact of family testing and of routine ultrasound examination of pregnancies in that region. Supported by the French CF association « Vaincre La Mucoviscidose ».

2422/T

Detection of circulating fetal cells utilizing automated microscopy for non-invasive prenatal diagnosis of chromosomal aneuploidies. *A. Seppo[1], V. Frisova[2], Y. Kim[1], M.I. Evans[3], A. Antsaklis[4], K.H. Nicolaides[2], T. Tafas[1], P. Tsipouras[1], M.W. Kilpatrick[1].* 1) Ikonisys Inc, New Haven, CT; 2) Harris Birthright Research Ctr, King's College Hospital, UK; 3) Comprehensive Genetics & Mt. Sinai School of Medicine, New York, NY; 4) First Dept. of ObGyn, National University of Athens, Greece.

Our objective is to identify fetal cells in peripheral blood samples from pregnant women for detection of chromosomal aneuploidies. This could be accomplished either through detection of a fetal cell specific marker or, alternatively, through the detection of aneuploid FISH signals. To that effect we utilized an automated microscopy system developed to identify and enumerate cells based on their FISH signal complement. For FISH-based scanning, verified fetal cells are identified based on a dual FISH probe labeling approach. Previously we showed that dual labeling reduces the false positive rate below 0.00005% when scanning for rare nuclei. Fetal nuclei are identified at low mag based either on the presence of a Y chromosome signal or on aneuploid FISH signals for chromosome 21. These nuclei are verified at high mag utilizing two FISH probes for the chromosome of interest. In addition, density gradient centrifugation was investigated for enrichment of fetal cells. FISH- based scanning identified fetal cells in 28 out of 29 maternal samples, 11 first trimester and 18 second. A range of 1-10 fetal cells were detected in unenriched samples and 1-20 in enriched samples. On average 0.5 and 2.3 fetal cells per million nucleated maternal cells were detected in unenriched and enriched samples, respectively. Thus simple density gradient centrifugation achieved a 4-5 fold increase in the number of fetal cells detected. Our data demonstrate that automated microscopy was able to detect fetal cells in greater than 95% of maternal samples, both first and second trimester. This was achieved utilizing dual FISH probes for the chromosome of interest. This suggests that automated scanning for aneuploid FISH signals could form the basis of a credible clinical test for non-invasive prenatal diagnosis, eliminating the need for a fetal cell specific biomarker.

2423/T

Prenatal detection and characterization of supernumerary marker chromosomes by array-CGH. *M.J. Simovich[1], S.H.L. Kang[1], A. Patel[1], A. Pursley[1], A.C. Chinault[1], J.R. Lupski[1], A.L. Beaudet[1], I.B. Van den Veyver[1,2], S.W. Cheung[1].* 1) Dept Mol Hum Genet; 2) Dept Ob-Gyn, Baylor College of Medicine, Houston, TX.

Small supernumerary marker chromosomes (sSMC) occur in about 0.043% of newborns and in 0.076% of prenatal diagnoses. The phenotypes associated with sSMC vary substantially depending on size, gene content and chromosome origin, which cannot easily be determined by karyotype or FISH analysis. Therefore, prediction of the pregnancy outcome is difficult and genetic counseling can be a challenge. We analyzed five prenatal cases referred to our laboratory for chromosome microarray analysis (CMA) by array-CGH after karyotype analysis showed an uncharacterized sSMC. In case 1, CMA detected a gain of DNA copy number in the pericentromeric region of 12q, estimated to be approximately 5Mb in size. Metaphase FISH analysis revealed a minute ring-like sSMC in 16 of 20 cells analyzed. In case 2, array-CGH detected a gain of ~4Mb on chromosome 12p. FISH analysis showed that the marker was present in 2.5% of the cells and corroborated the chromosome 12p origin. In case 3, array-CGH detected an 18 Mb gain in the proximal region of chromosome 21q that was confirmed by interphase FISH analysis on cultured amniocytes in 100% of the cells. Postnatal follow-up by array-CGH also confirmed the results. The sSMCs in case 4 and 5 were shown to have originated from the fusion of the centromeric heterochromatin of one or both chromosomes 14 and 22 by G banded chromosome and FISH analyses. The array-CGH did not detect any abnormalities. Since the array is designed to detect unique sequences in the pericentromeric regions, these results suggest there is no apparent genetically active chromatin material present in the marker. We show that the origin of sSMC cannot be identified by conventional cytogenetic analysis alone. Their precise characterization requires high resolution genome analyses by molecular techniques, of these; array-CGH emerges as the fastest and most precise diagnostic tool to determine the chromosomal origin and approximate size of the sSMC. This improved characterization, is crucial for accurate prenatal genetic counseling.

2424/T

Ethnic adjustment factors for Black women undertaking prenatal screening for Down syndrome in Ontario. *A. Summers, T. Huang.* Genetics Program, North York General Hosp, Toronto, ON, Canada.

In prenatal screening for Down syndrome, the measurements of certain serum markers are adjusted for ethnicity as there are differences in the levels of these markers among different racial groups. In Canadian, Black women were mainly Afro-Caribbean, although there are increasing number of African women in our screening population. This study estimated ethnic differences in the levels of the first and second trimester serum markers between Black and Caucasian women in our population and explored ethnic adjustment factors for Black women. The study was based on 7361 Black and 47840 Caucasian women undertaking prenatal screening in North York General Hospital between 12/1999 and 12/2006 using screening software Alpha. Multiple pregnancies, pregnancies associated a known chromosomal anomaly or insulin dependent diabetes were excluded from the study. Black and Caucasian women were identified through screening requisitions. The study quantified the ethnic differences in the levels of serum markers between Black and Caucasian women by comparing median MoMs of serum markers between the two groups prior and after weight correction. Fixed ethnic correction factors for Black women were estimated for AFP, hCG and PAPP-A. Weight correction formulae for Black women were developed for PAPP-A through regression analysis. After allowing for maternal weight, median MoMs of AFP was 15% higher, total hCG 12% higher, uE3 3% higher, PAPP-A 57% higher, and inhibin A 5% lower in Black women. The variations in the levels of serum markers in Black women were different from those reported by the studies in UK and US, suggesting local ethnic adjustment factors may needed for screening programs in different ethno-geographic regions. The differences in the levels of AFP, total hCG and PAPP-A can be corrected by applying Caucasian based weight correction formulae and a fixed ethnic adjustment factor to Black women. The differences in the levels of PAPP-A may also be corrected by applying exponential or quadratic weight correction formulae specific for Black women. A median MoM of 1.0 was obtained for AFP, total hCG and PAPP-A in Black women after the adjustments for ethnicity.

2425/T

Utility of SNPs within restriction endonuclease sites for improved identification of circulating free fetal DNA in maternal plasma. *J.A. Tynan, M. Ehrich, E. Dragon, D. van den Boom.* SEQUENOM, Inc., 3595 John Hopkins Court., San Diego, CA 92121.

Universal methods to confirm the presence of circulating, free fetal DNA in maternal plasma would improve the clinical application of fetal genotyping assays such as those for fetal RHD and gender assessment for X-linked recessive disorders. Previously, we evaluated the detection of paternally inherited SNP alleles as a universally applicable method to detect fetal DNA in maternal plasma. Because fetal DNA constitutes only 3-6% of the DNA in maternal plasma, the utility of detecting paternally inherited fetal SNP alleles is largely dependent on methods enriching for fetal DNA. Here, we incorporate the use of restriction endonucleases (REs) to enhance the detection of specific SNP alleles present at low relative concentrations in DNA mixtures. A panel of SNPs was screened for the presence of RE recognition sites altered by one SNP allele, and for the absence of additional instances of the same RE site within +/- 50 base pairs of the SNP. Primer extension genotyping assays using mass spectrometric analysis were designed for SNPs meeting these criteria. Prior to PCR, DNA was incubated with or without RE in PCR master mix at each REs optimal temperature. DNA was amplified by PCR and genotyped with the TypePLEX assay using the Compact MassArray system. Maximal digestion of heterozygous SNP alleles in genomic DNA could be obtained using 0.25 U RE in PCR buffer with incubation for 15 minutes. Using a model system of DNA mixtures comprising 2, 5, 20, and 50 % DNA heterozygous for a given SNP in a balance of RE cleavable, homozygous DNA, RE digestion allowed detection of the non-cleavable, heterozygous derived SNP allele in all mixtures. Without RE digest, this allele was not detectable in the 2 and 5 % DNA mixtures. These results show the utility of RE enhanced detection of SNP alleles present in low relative concentration in a DNA mixture. Incorporation of this method to detect paternal SNP alleles in maternal plasma will improve confidence in negative genotype calls where the presence of fetal DNA can not otherwise be confirmed.

2426/T

Alobar holoprosencephaly presenting in a female fetus with a X;19 translocation. *A.F. Wagner[1], C. Lake[1], D. Hopcus-Niccum[2], R. Aldrich[2], E.G. Harp[3], E.D. Stolzenberg[3], G.P. Altshuler[3], P.L. Wilson[1], S. Li[2], E.J. Knudtson[1].* 1) Dept OB/GYN, MFM Section, Univ Oklahoma Health Sci Ctr, Oklahoma City, OK; 2) Dept Pediatrics, Genetics Section, Univ Oklahoma Health Sci Ctr, Oklahoma City, OK; 3) Dept Pathology, Univ Oklahoma Health Sci Ctr, Oklahoma City, OK.

Holoprosencephaly (HPE) is a malformation sequence in which the prosencephalon fails to cleave sagittally into cerebral hemispheres, transversely into telencephalon and diencephalon, and horizontally into olfactory and optic bulbs. Alobar HPE is the most severe form of cleavage failure of the prosencephalon before 6wks of gestation. It has been associated with many Mendelian conditions as well as maternal diabetes and salicylate use.

Here we present a 21yo G2P1 Caucasian/Native American female who presented at 19-3/7wks because of a positive Quad screen for trisomy 18(1:31). Ultrasound evaluation revealed alobar HPE and non-specific heart disease (left-axis shift and pericardial effusion). Amniocentesis revealed a karyotype of 46,X,der(X)t(X;19)(q10;p10). Parental karyotypes were normal. The pregnancy was otherwise uneventful.

The fetus was delivered via repeat C/S at 39-1/7wks with Apgars of $6^{1}2^{5}1^{10}$, weight of 2520g, length of 30.0 cm, and FOC of 36.0 cm. She died at 54 minutes.

On genetic exam and autopsy, there was a marked prominence of the frontal bones with a wide anterior fontanelle. The eyes showed upslanted PFs and hypertelorism. Ears were posteriorly rotated. The nasal bridge was large and pronounced with a short nose. There was retrognathia with an inverted-V cleft in the chin. Chest circumference and internipple distance were <-2SD. There was a left single palmar crease and bilateral digitalized thumbs. The heart was normal. Parathyroids were absent. There were 3 small lumbosacral dimples. Brain autopsy revealed severe hydrocephalus, flattened tissue with loss of the normal cerebral hemispheres and an exaggerated, midline ventricle with incomplete closure consistent with alobar HPE. Literature review has been unable to find HPE associated with partial trisomy 19.

2427/T

Fragile X: Risks of unstable transmissions in females with intermediate and small premutation alleles. *S.L. Nolin, A. Glicksman, T. Sukontasup, G.E. Houck, X. Ding, S.Y. Li, W.T. Brown, C. Dobkin.* Human Genetics Dept, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Fragile X screening of pregnant women to identify premutation carriers (56-200 repeats) at risk for affected offspring has become routine in many prenatal settings. While 59 repeats is the smallest allele in fragile X families to expand to full mutation in one transmission, the risks of instability for newly identified intermediate (40-55 repeats) and small premutation (56-79 repeats) alleles are not well characterized. We have performed 199 prenatal studies for mothers with 40-79 repeats and observed transmission of the larger alleles in 58% (116/199) of pregnancies. Unstable transmissions were observed in 4/21 with 40-49 repeats (19%), 6/20 with 50-54 repeats (30%), 11/29 with 55-59 repeats (38%), 12/20 with 60-69 repeats (60%) and 26/26 with 70-79 repeats (100%). Among the 59 unstable transmissions, 56 expansions and 3 contractions were observed. For expansions of 40-59 repeat alleles, most increased by 1 to 4 repeats with a range 1 to 12 repeats. In contrast, most alleles from 60 to 79, exhibited much greater size increases. Expansions to full mutations were observed in 11/26 alleles between 70-79 repeats (42%). No expansions to full mutation were observed in alleles <70 repeats. These studies indicate that while unstable transmissions with small repeats expansions are often observed in alleles <60 repeats, most expansions to full mutations occur in alleles >70 repeats.

2428/T

First prenatal detection of maternal uniparental disomy (UPD) of chromosome 6 and "rescue" of trisomy 6. *M. Haag[1], L. Beischel[2], J. Rokeach[3], D. Wallace[3], J. Knops[1], K. O'Connor[1], J. Johnson[2], J. Ibrahim[4].* 1) Genzyme Genetics,Santa Fe,NM; 2) Shodair Genetics Laboratory,Helena,MT; 3) Monmouth Medical Group,Long Branch,NJ; 4) Genetics,St. Joseph's Regional Medical Center,Paterson,NJ.

Uniparental disomy (UPD) can result from a meiotic or postzygotic nondisjunction event followed by trisomy "rescue" and is of concern when trisomy mosaicism is detected during routine prenatal diagnosis. Characteristic phenotypes resulting from UPD are emerging along with better understanding of novel mechanisms of gene expression, such as imprinting. In well documented cases of UPD 6 that are paternal in origin there has been a strong association with transient neonatal diabetes due to abnormal expression of an imprinted gene. However, maternal UPD 6 is a very rare finding and no consistent phenotypic picture has yet emerged. We report here a case of maternal UPD 6 that was ascertained through trisomy 6 mosaicism observed in cultured chorionic villi from a 45 year old patient. Trisomy 6 was not detected in a follow-up amniocentesis. Analysis of DNA polymorphisms for chromosome 6 in amniocytes and parental samples showed markers which lacked an allele of obligate paternal origin. All loci were homozygous in the amniocytes, consistent with maternal uniparental isodisomy 6. DNA marker analysis confirmed paternity. The pregnancy was monitored by serial ultrasounds and IUGR was detected at 29 weeks. Other fetal parameters were within normal limits. The patient delivered at 33 weeks with no further fetal complications noted. Full trisomy 6 is apparently lethal to human development, reported only in fetal demise. One third of trisomy rescue for chromosome 6 results in UPD, which is compatible with development to term. Almost all cases of UPD 6 (maternal and paternal) exhibit isodisomy consistent with rescue of a meiosis II error. Reports of maternal UPD 6 have shown an association with intrauterine growth retardation (IUGR). This case offers the first example of prenatal detection of maternal uniparental isodisomy 6, associated with IUGR and early delivery, but an otherwise favorable outcome, indicating that the trisomic cells were most likely confined to the placenta.

2429/F

GENOME WIDE SCAN, IDENTIFICATION OF A COMMON HAPLOTYPE CONTAINING A NON-SYNONYMOUS SNP ASSOCIATED TO SYMPTOMATIC OSTEOARTHRITIS. *I. Meulenbelt[1], J.L. Min[1], S. Bos[1], N. Riyazi[2], J.J. Houwing-Duistermaat[3], H-J. van Wijk[3], H.M. Kroon[4], A.G. Uitterlinden[5,6], J.B.J. van Meurs[5], W.M. van der Deure[5], T.J. Visser[5], A.B. Seymour[7], N. Lakenberg[1], R. ter Breggen[1], D. Kremer[1], C.M. van Duijn[6], G. Kloppenburg[2,8], J. Loughlin[9], P.E. Slagboom[1].* 1) Dept. Molecular Epidemiology, Leiden University Medical Center, Leiden, Z-H, Netherlands; 2) Dept. Rheumatology, Leiden University Medical Center, Leiden, Z-H, Netherlands; 3) Dept. Medical Statistics and Bio-informatics, Leiden University Medical Center, Leiden, Z-H, Netherlands; 4) Dept. Radiology, Leiden University Medical Center, Leiden, Z-H, Netherlands; 5) Dept. Internal Medicine, Erasmus University Medical School, Rotterdam, The Netherlands; 6) Dept. Epidemiology & Biostatistics, Erasmus University Medical School, Rotterdam, The Netherlands; 7) Pfizer Global Research & Development, Groton, CT, USA; 8) Clinical Epidemiology and Haematology, Leiden University Medical Center, Leiden, Z-H, Netherlands; 9) Dept. of Orthopaedic Surgery, Institute of Musculoskeletal Sciences, University of Oxford, Nuffield, Botnar Research Centre, Oxford, UK.

Osteoarthritis (OA) is a prevalent late-onset disabling joint disease with complex inheritance for which no drug exist which is able reverse or slow down the disease process. Genome-wide nonparametric linkage in 183 sibships from the GARP-study with a generalised OA phenotype, suggested evidence for linkage on chromosome 14q32.11 (LOD = 3.03, P = 1.9 x 10^{-4}). The location of the linkage peak revealed three candidate genes. Genotyping and joint modelling of linkage and association of tagging SNPs capturing the genetic variation of these genes revealed a non synonymous common variant in one of the genes that explained part of the linkage (P = 0.006). In our attempt to replicate this result in two additional independent studies we identified a common haplotype, exclusively containing the minor allele of this SNP, with a significant recessive association (OR = 1.71, 95% CI 1.33-2.19, P = 2.6 x 10^{-5}) in females with symptomatic hip OA. Our findings underscore the importance of a new gene and pathway in the etiology of symptomatic OA.

2430/F

Congenital hip dislocation : the report of a genome-wide linkage scan in Brittany (Western France). *K. Rouault[1], V. Scotet[1], S. Autret[1], F. Dubrana[2], B. Fenoll[3], F. Gaucher[4], D. Tanguy[5], C. Yaacoub[6], C. Férec[1].* 1) Inserm U613, CHU Morvan, University, Brest, France; 2) Department of orthopaedic surgery, CHU La Cavale Blanche, Brest, France; 3) Department of paediatric surgery, CHU Morvan, Brest, France; 4) Department of orthopaedic surgery, Hotel Dieu, Pont L'Abbé, France; 5) Department of physical medicine, Centre de Perharidy, Roscoff, France; 6) Department of orthopaedic surgery, CH Cornouaille, Quimper, France.

Congenital dislocation of the hip (CDH) is a public health matter because of its high frequency, the severe functional handicap induced if it is not treated early and its natural evolution towards hip osteoarthritis. This disease presents a mechanical component linked to the pregnancy and delivery conditions but the ethnical predispositions and the familial aggregation observed is also in favour of a genetic component. Clusters of CDH have been observed among Lapps and Navajo Indians, but this condition is also very frequent in the area of Finistère (Brittany - France). In order to identify the gene(s) responsible for CDH, we set up the first genome-wide linkage scan on this disease. To date, 38 family structures including affected sib-pairs (n=29) or at least four cases of CDH (n=9) could have been recruited. The whole scanning of the genome is carried out by typing 400 markers microsatellites (STRs) at 10 cM intervals (kit Linkage Mapping Set MD10 v2.5). The linkage analysis between the loci markers and the locus of gene responsible for pathology is carried out, in a first step, by a non-parametric method (npl) using the Merlin software. To date, the genotyping of all the markers is completed for the nine most informative family structures. The interpretation of the data is currently in process but non-parametric analyses could not yet bringing to light any area of interest. If this genome-wide linkage analysis enable to locate the area(s) of interest on the genome, it will enable to better understand the physiological basis of the CDH and should lead to earlier detection and treatment of subjects at risk to develop CDH. This work was supported by a grant from Projet Hospitalier de Recherche Clinique and from Programme de Recherche d'Initiative Régionale.

2431/F

Effect of lysosomal protein glucocerebrosidase on α-synuclein turnover. *O. Goker-Alpan[1], D. Urban[1], B. Stubblefield[1], M. Cookson[2], B. Giasson[3], E. Sidransky[1].* 1) MGB/NHGRI, NIH, Bethesda, MD; 2) LNG/NIA/NIH, Bethesda, MD; 3) Dept. of Pharmacology, UPenn, Philadelphia, PA.

The synucleinopathies which include Parkinson disease(PD), are characterized by aberrant α-synuclein fibrillization resulting in the formation of pathological inclusions. In PD, inclusions in neuronal cell bodies and processes are termed Lewy bodies(LBs) and Lewy neurites (LNs). Studies in familial PD implicate that abnormalities in protein clearance can lead to neurodegeneration. Although defects in the ubiquitin-proteosome system (UPS) may contribute to PD, alternate pathways such as lysosomal degradation are also involved in modulating α-synuclein accumulation. Recent evidence indicates an association between mutations in glucocerebrosidase(GBA), the lysosomal enzyme deficient in Gaucher disease, and PD as well as dementia with LB(DLB). We explored possible mechanisms to explain why α-synuclein might accumulate when GBA is mutated. To examine the effects of GBA mutations on the two pathways implicated in α-synuclein metabolism, brain samples from 7 subjects with PD or DLB carrying GBA mutations were studied with immunofluorescence. Ubiquitin and lysosomal markers were used with antibodies against glucocerebrosidase and α-synuclein. Although in some LBs, mutant glucocerebrosidase was present at the core, only 40-60 % of glucocerebrosidase positive LBs were ubiquinated. However, all LBs and LNs positive for both α-synuclein and glucocerebrosidase displayed antigenicity to the lysosomal markers. Proteosome function was examined using the small degron CL-1, which demonstrated no influence of either wild-type or mutant GBA on the UPS. α-synuclein solubility and turnover were studied in Cos-7 cell co-transfected with h-A53T α-synuclein and wild-type or mutant GBA. Detergent fractionation demonstrated higher levels of soluble α-synuclein in cell lines carrying wild-type GBA. In pulse-chase experiments, there was also more effective clearance of α-synuclein in the presence of wild-type GBA. These data suggest that glucocerebrosidase may affect α-synuclein catabolism, and when mutated, may interfere with the lysosomal clearance of α-synuclein aggregates.

2432/F

HLA-DRB1 alleles in MS: twin concordance and sexual dimorphism. *B.M Herrera[1,2], S.V. Ramagopalan[1,2], D.A. Dyment[1], M.R. Lincoln[1,2], G.C. Deluca[1], S. Orton[1,2], M.J. Chao[1,2], C.J. Willer[1], D.A. Sadovnick[3], G.C. Ebers[1,2].* 1) Wellcome Trust Centre for Human Genetics,Oxford University, Oxford, UK; 2) Department of Clinical Neurology, John Radcliffe Hospital, Headley Way, Oxford,UK; 3) Department of Medical Genetics, University of British Columbia Vancouver, Canada.

Population-based twin concordances in multiple sclerosis are 30% for monozygotic (MZ) and 5.4% for dizygotic (DZ) twins in Canada. Stratification by sex showed that concordance is gender and gene-interactive, being 34% for female MZ and 3.8% for female-female DZ pairs vs. male-male rates of 6.5% for MZ and 11.4% for DZ. Recent studies have revealed susceptibility (S) and resistance (R) alleles at HLA-DRB1, the only consensus locus associated with MS risk. In this investigation we analysed the frequency of S(HLA-DRB1*08, *15 and 17) and R (HLA-DRB1*01, *11 and *14) alleles and genotypes in 489 MS twins. We found that HLA-DRB1 mediated susceptibility to MS in twins is sexually dimorphic. Together with differential female sensitivity to the environment, gene-environment interactions involving the HLA-DRB1 region itself are strongly implied in MS pathogenesis.

2433/F

Use of a genetic isolate to characterize genome wide profile behind multiple sclerosis. *E. Jakkula[1,2], S. Purcell[2,3], J. Saarela[1,4], S. Kallio[1,4], P. Tienari[5], K. Koivisto[6], A. Palotie[2,7], MJ. Daly[2,3], L. Peltonen[1,2,4].* 1) Dept of Molec. Medicine, National Public Health Institute, Helsinki, Finland; 2) The Broad Institute of MIT and Harvard, Cambridge, MA, USA; 3) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; 4) Research Program in Molec. Medicine at Biomedicum Helsinki, Helsinki, Finland; 5) Dept. of Neurology, Helsinki Univ. Central Hospital, Helsinki, Finland; 6) Central Hospital of Seinäjoki, Seinäjoki, Finland; 7) The Finnish Genome Center, Univ of Helsinki, Helsinki, Finland.

Multiple sclerosis (MS) shows very high incidence in the Western Ostrobotnia sub-isolate of Finland and genealogical research suggests limited number of founders. We therefore hypothesize that one or more relatively penetrant variants predisposing to MS may be regionally enriched and that shared haplotype analysis can be used to identify MS loci using a genome wide, high density SNP screen.

Using genealogical information reaching up to 15 generations back in history, two regional "megapedigrees" were constructed and 72 MS cases (and 68 regional controls) were genotyped using the Illumina 317K HumanHap panel. A five SNP sliding window haplotype option in PLINK was used to scan each chromosome. When comparing all haplotypes between cases and controls two regions with global p-value <10^{-6} were detected (11q12.1, 12q24.33) and six regions that had single haplotype association p-values < 10^{-6}: the HLA region and regions on 1q25.3, 1q41, 11q12.1, 17q11.2 and 22q13.2. These regions are 61-500 kb in size and limited by probable recombination hotspots. We are currently following these initial findings in the Finnish study sample of 700 MS families. One region of special interest locates on 5p representing a previous linkage region identified in these MS families. A shared "risk" haplotype covers the complement component 7 gene *(C7)* and this risk haplotype has been validated in an enlarged sample from Southern Ostrobotnia. This approach should provide insight especially to the rare, high impact alleles in the genetic background of MS.

2434/F

Notch 3 gene mutation associated with Sneddon's syndrome- a complex disorder of unknown etiology with variable clinical features overlapping the phenotype of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]. *D. Kumar[1], J. Holroyd[1], I. Frayling[1], I. Ferguson[2].* 1) Clinical Geneticist,Institute of Medical Genetics,University Hospital of Wales,Cardiff,UK; 2) Dept of Neurology,Frenchay Hospital,Bristol,UK.

A 42 year old lady was referred with a clinical diagnosis of Sneddon's syndrome who has a long medical history of multiple medical problems including Raynaud's disease, one episode of major cerebrovascular accident, recurrent transient ischaemic attacks [TIAs], one emergency admission with chest pain due to pulmonary embolism, recurrent joint stiffness with non-specific musculoskeletal symptoms, recurrent migrainous headaches and skin rash in the form of livedo reticularis and erythema. The family history includes her two daughters with early onset migraine-like headaches and father who had recurrent TIAs, stroke and Alzheimer's type senile dementia. Molecular testing for mutations in the Notch 3 gene confirmed a heterozygous C>T transition at nucleotide 3646 in exon 22 (Arg1190Cys). This is a known pathogenic mutation for 'cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]'. This is probably the first report describing the pathogenic association of Notch 3 gene mutation with Sneddon's syndrome. Sneddon syndrome is a chronic complex connective and/or vascular tissue inflammatory disease of unknown etiology that usually presents with recurrent complicated migraine, skin rash (erythematous rash and livedo reticularis), non-specific musculoskeletal symptoms, TIAs, stroke and dementia. A small subset of families might have multiple affected members following autosomal dominant inheritance pattern. This is probably the first report describing Notch 3 gene mutations in Sneddon syndrome. Molecular testing should be considered in a patient/ family presenting with clinical features falling within the broad spectrum of CADASIL. More clinical and genetic data are required to establish genotype-phenotype correlations of Notch 3 gene mutations with CADASIL, Sneddon's syndrome and other similar disorders with overlapping clinical features.

2435/F

Molecular Studies of three SNPs in the regulatory region of *DYX1C1*, a candidate dyslexia gene. *I. Tapia-Páez[1], K. Tammimies[1], S. Massinen[2], J. Kere[1].* 1) Department of Biosciences , Karolinska Institute, Stockholm, Sweden; 2) Department of Medical genetics, University of Finland, Helsinki, Finland.

Dyslexia is a complex disorder characterized by reading disability despite normal intelligence, senses and proper education; it affects 5-10% of the population. Genetic studies have pointed out loci linked to dyslexia on several chromosomes including 1, 2, 3, 6, 11, 15, 18 and X. Until today, six genes have been associated with dyslexia: *DYX1C1, DCDC2, KIAA0319, ROBO1* and two genes on chromosome 2, *MRPL19* and *C2ORF3*. Four of these genes are involved in neuronal migration or brain development and their functional role in dyslexia remains to be elucidated. The *DYX1C1* gene on chromosome 15 (Taipale et al. PNAS 2003) is the first gene implicated in dyslexia, it was cloned based on a translocation t(2;15)(q11;q21) that cosegregated with dyslexia in a Finnish family. Two sequence changes in *DYX1C1* showed association with dyslexia, one introduced a stop codon truncating the protein by four amino acids, and the second in 5'UTR, close to the translation initiation site. Several groups with sample sets from different populations have attempted to replicate these results but the results have been ambiguous, prompting us to look for new SNPs in the promoter of the *DYX1C1* gene. Two new SNPs in the promoter region were found, and when combined with additional results from German samples, they showed supportive evidence for *DYX1C1* as a candidate dyslexia gene (Dahdouh F et al. manuscript). To further understand the functional consequences of these polymorphisms, we prepared constructs for the three SNPs to study in electrophoretic mobility shift assays. Allele-specific differential retardation of mobility was observed with the promoter and 5'UTR SNPs, suggesting a functional effect of these variations in the regulation of *DYX1C1 gene*. We also prepared constructs for reporter assays in pGL3 basic and promoter vectors using these polymorphisms. We could detect very significant differences in luciferase expression with all three SNPs. Further association studies are motivated to confirm the role of these SNPs in the regulation of *DYX1C1*.

2436/F

Semaphorins and *RET* are associated with Hirschsprung Disease. *S. Arnold[1], M. Guy[1], K. West[1], C. Kashuk[1], F. Lantieri[2], G. Burzynski[3], R. Fernandez[4], A. Pelet[5], Y. Sribudiani[3], S. Borrego[4], I. Ceccherini[2], R. Hofstra[3], S. Lyonnet[5], A. Chakravarti[1].* 1) IGM, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Lab di Genetica Molecolare, Ist Gaslini, Genova, Italy; 3) Dept Medical Genetics, Univ Groningen, The Netherlands; 4) UC Genética y Reproducción, HH UU Virgen del Rocio, Sevilla, Spain; 5) Dept de Génétique and INSERM U781 , Hôpital Necker Enfants Malades, Paris, France.

Hirschsprung Disease (HSCR), or aganglionic megacolon, is an oligogenic disease that varies in severity from short segment disease to total colonic aganglionosis. Current evidence suggests that down-regulation of the gene encoding the receptor tyrosine kinase *RET* is necessary, but not sufficient, for disease expression. Coding sequence *RET* mutations that inactivate the protein are most often associated with the most severe forms of disease, while noncoding variants, like the recently described *RET+3* enhancer mutation, are associated with the least severe, most common form of HSCR. In an effort to identify genes acting in concert with *RET* to explain the majority of HSCR cases, we analyzed 220 isolated short segment HSCR trios (~ 4:1 male:female) on the Affymetrix 500K SNP array platform. Transmission analysis (using the disequilibrium-based TDT) identified the *RET* locus as the most significantly associated with disease, with 10 SNPs displaying p-values ranging between 2.97×10^{-8} and 9.87×10^{-21}. A second, striking cluster of 70 SNPs was identified on chromosome 7 between Semaphorins 3A and 3D, with 15 SNPs displaying p-values between 9.64×10^{-5} and 7.10×10^{-6}. We genotyped the two chromosome 7 SNPs with lowest p-values in a broader HSCR sample for replication. In this sample, representative of all segment lengths, one SNP obtained greater significance with a p-value of 4.53×10^{-10}, while the second maintained a p-value on the order of 10^{-6}. As chemo-attractive/-repulsive molecules involved in the development of the enteric nervous system, the semaphorins are logical candidates for modification of *RET* function. Our ultimate goal is to elucidate the specific variant responsible for producing the identified association and its possible interaction with *RET*.

2437/F

Transcription Factor 7-Like 2 *(TCF7L2)* interacts with Arachidonate 5-Lipoxegenase *(5-LO)* to decrease fasting insulin (FI) in Mexican Americans (MA). *M.H. Black[1], J. Hartiala[1,2], A. Xiang[1], E. Trigo[3], M. Kawakubo[1], J. Lawrence[4], T.A. Buchanan[3], R.M. Watanabe[1], H. Allayee[1,2].* 1) Dept of Prev Med, Keck Schl of Med of USC, Los Angeles, CA; 2) Inst for Genetic Med, Keck Schl of Med of USC, Los Angeles, CA; 3) Dept of Med, Keck Schl of Med of USC, Los Angeles, CA; 4) Kaiser Permanente, Pasadena, CA.

5-LO, which generates pro-inflammatory leukotrines, has been implicated in atherogenesis and has recently been shown to play a role in adiposity and insulin homeostasis. *5-LO* -/- mice are obese and have lower insulin secretion versus wild type mice. Genome-wide studies have established *TCF7L2* as a susceptibility gene for type 2 diabetes (T2D). As both *TCF7L2* and *5-LO* are involved in Wnt signaling, we hypothesized that interaction between *TCF7L2* and *5-LO* may be associated with FI in humans. To test this hypothesis, we genotyped *TCF7L2* rs7903146 and the *5-LO* promoter-repeat variant in 143 MA families from the BetaGene study. Participants were phenotyped with oral and intravenous glucose tolerance tests. We report data from 672 subjects (41% male, 59% female) with mean age 34.0±9.1 yrs and BMI 29.3±6.0 kg/m^2. Variant interaction was tested for association with T2D-related traits using a likelihood ratio test under a variance components framework, adjusting for age and sex. Given functional data showing increasing *5-LO* expression with decreasing repeat size, we assumed an additive genetic model for *5-LO*. rs7903146 was tested under a dominant genetic model due to low minor allele frequency. Interaction between rs7903146 and *5-LO* promoter repeats was significantly associated with FI (p=0.038). Among *TCF7L2* CC subjects (n=413), FI decreased by ~1 μU/ml with decreasing *5-LO* repeat length. In contrast, among *TCF7L2* T carriers (n= 259), those with at least one *5-LO* 3 repeat had FI levels more than twice that of subjects with *5-LO* 4 or 5 repeats. This decrease in FI with increasing number of *5-LO* repeats among *TCF7L2* T carriers is consistent with reduced insulin observed in *5-LO* -/- mice. These results suggest that variation in *5-LO* and *TCF7L2* play an interdependent role, possibly through Wnt signaling mechanisms, in regulating FI levels in MA.

2438/F

Simultaneous genotyping of 51 SNPs in 35 genes encoding molecules involved in inflammation: their role in determining Crohn disease susceptibility and phenotype. *P. Borgiani[1], S. Romano[1], C. Perricone[1], L. Biancone[2], C. Petruzziello[2], L. Steiner[3], C. Ciccacci[1], F. Pallone[2], G. Novelli[1].* 1) Dept. of Biopathology, Genetics, University Tor Vergata, Rome, Italy; 2) Gastroenterology, Policlinico Tor Vergata, Rome, Italy; 3) Roche Molecular Systems, Alameda, CA, USA.

Crohn disease (CD; OMIM #266600) is a chronic inflammatory bowel disease of unknown etiology. We cooperated with Roche Molecular System to optimize a genotyping system for the simultaneous detection of 51 SNPs in 35 genes encoding for molecules involved in inflammation. These variants were studied to investigate their influence on disease susceptibility/phenotype. We also considered CARD15 CD susceptibility SNPs (R702W, G908R, L1007fsinsC). 190 CD patients were diagnosed in accordance with international guidelines. 190 Caucasian healthy subjects served as controls. The genotyping method was based on Reverse Dot Blot technique on solid support and colorimetric revelation. We demonstrated a highly statistically significant association between CD and ICAM1, G241R (P<0.001) and IL10, -571 (P<0.005). SDF1, +800 (P<0.03) and IL4R, I75V (P<0.05) are other susceptibility variants. The associations remained unchanged when stratified for the CARD15 mutations that were found highly associated with CD susceptibility (P<0.001). Multivariate analysis confirmed the high contribution of ICAM1, IL10 and CARD15 L1007fsinsC SNPs. ICAM1, IL10, TCF7, LTA, SELP, ADRB2, NOS3, C3 SNPs were found to be significantly associated with different disease phenotypes in terms of site and behaviour. When stratified for age at diagnosis we found ICAM1, IL4R, ADRB2, SELE, IL1A SNPs to be associated with early disease onset (age at diagnosis<30y), while IL10 and LTA SNPs were associated with late onset (age at diagnosis>40y). The genotyping method revealed to be easy, quick, accurate and could be useful in a variety of inflammatory diseases. CD demonstrated to have a strong genetic component, especially when early onset. ICAM1, G241R and IL10, -571 were found to be major susceptibility variants in CD, together with CARD15, L1007fsinsC. Other SNPs, independently or in cooperation with CARD15, were found to be capable of modulating the disease phenotype.

2439/F

Evidence for an etiologic role of WNT gene family in nonsyndromic cleft lip with or without cleft palate. *B.T. Chiquet[1,2], S.H. Blanton[3], D. Ma[3], S. Stal[4], J.B. Mulliken[5], J.T. Hecht[1].* 1) University of Texas Medical School at Houston, Houston, TX; 2) University of Texas Dental Branch, Houston, TX; 3) University of Miami Miller School of Medicine, Miami, FL; 4) Texas Children's Hospital, Houston, Houston, TX; 5) Children's Hospital, Boston, MA.

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common complex birth disorder with a prevalence of 1/700 live births. Genetic and environmental factors have been implicated and studies have begun to delineate genetic contributions. The Wnt gene family plays an integral role during embryogenesis, including regulating midfacial development and upper lip fusion. Also, the clf1 region in A/WyN clefting susceptible mice contains two Wnt genes. Both suggest that Wnts are biologically plausible NSCLP candidate genes. To determine if Wnt genes are associated with our NSCLP cohort, we interrogated seven Wnt genes: Wnt3, -3a, -5a, -7a, -8a, -9b, and -11. These genes are either (a) mutated in a orofacial clefting syndrome, (b) expressed in the developing craniofacial region or (c) interact with other known NSCLP candidate genes. Thirty-eight single nucleotide polymorphisms (SNPs) and two microsatellite markers were genotyped in 63 multiplex NSCLP families and 287 simplex parent-child trios. All SNPs were in HWE. Allele frequencies were significantly different between our Caucasian and Hispanic cohorts; therefore the data was stratified by ethnicity and analyzed using the pedigree disequilibrium test (PDT) and genotype-PDT. At least one SNP in each gene was significantly associated to NSCLP. The most significant results were found for SNPs in Wnt11 (p=0.002), Wnt3a (p=0.0054), and Wnt5a (p=0.0004). Wnt11 directs neural crest cell (NCC) migration and Wnt3a controls the fate of NCC migration; NCCs are part of the craniofacial processes that form the upper lip and palate. Likewise, Wnt5a is highly expressed in the developing craniofacial processes. Alteration in Wnt gene function may perturb craniofacial process formation/fusion and may predispose an individual to NSCLP. The results of this study suggest that variation in Wnt genes plays an etiological role NSCLP and gene-gene interaction studies are underway.

2440/F

***Hox* Genes and Idiopathic Talipes Equinovarus.** *A.R. Ester[1], D. Ma[2], A. Scott[3], S.H. Blanton[2], J.T. Hecht[1,3].* 1) Univ Texas Medical Sch, Houston, TX; 2) University of Miami Miller School of Medicine, Miami, FL; 3) Shriners Hospital for Children, Houston, TX.

Idiopathic talipes equinovarus (ITEV, clubfoot) is a common birth defect, occurring 1/1000 live births, with over 135,000 cases born around the world each year. Talipes equinovarus (TEV) is characterized by forefoot adductus, midfoot cavus, hindfoot adductus, and hindfoot equines, and ITEV is applied to clubfoot that is not associated with any other anomaly. Segregation analyses suggest that multigenic inheritance with environmental effects contribute to the development of ITEV. Among children that had clubfoot along with other anomalies, six overlapping chromosomal deletion regions were identified, which may contain genes that contribute to ITEV. One of these regions, 2q31-33, contains the *HoxD* gene cluster, which has been shown to be involved in limb development. Mutations in this cluster have been associated with synpolydactyly and congenital vertical talus in humans, and a knockout mouse shows rotational limb defects as well as fusion of the vertebrae. A mutation in *HoxD10* (M319K) has been associated with congenital vertical talus (CVT) and was also seen in an individual with CVT in one foot and TEV in the other. The M319K mutation was postulated to play a role in the development of ITEV in the previous study. This study interrogated the *HoxA* and *HoxD* gene clusters on chromosomes 4 and 7, using SNP genotyping including the M319K polymorphism. The population consisted of 76 Caucasian and 152 Hispanic simplex trios and 93 Caucasian and 52 Hispanic multiplex families. The M319K mutation was not present in any cases or controls. Minimally positive p values were found for one SNP in *HoxD4* (rs1867863) and two SNPs in *HoxA11* (rs3779456 and rs1859164) in the Caucasian population. These results indicate that the M319K mutation does not play a role in ITEV and suggests that variation in these *Hox* genes does not significantly contribute to the isolated clubfoot phenotype.

2441/F

Whole Genome Study of Idiopathic Talipes Equinovarus (Clubfoot) Families. *J.T. Hecht[1,2], A.R. Ester[1], X. Tang[1], F.R. Dietz[3], M.S. Bray[4], A. Scott[2], Y. Bradford[5], S.H. Blanton[6].* 1) Dept Pediatrics, University of Texas Medical School, Houston, TX; 2) Shriners Hospital for Children, Houston, TX; 3) University of Iowa, Iowa City, IA; 4) Baylor College of Medicine, Houston, TX; 5) Vanderbilt University, Nashville, TN; 6) University of Miami Miller School of Medicine, Miami, FL.

Idiopathic talipes equinovarus (ITEV), or isolated clubfoot, is a common birth defect with a prevalence of 1/1000 live births. Males are affected twice as often as females; half of the cases are bilateral, with the majority of the unilateral occurrences in the right foot. ITEV is defined by four characteristics: forefoot adductus, midfoot cavus, hindfoot adductus and hindfoot equinus. Segregation studies of ITEV show that it is a complex disorder caused by multiple genes and environmental exposures. Few gene identification studies have been performed in ITEV. The focus of this study is to identify the genes contributing to the ITEV phenotype. We conducted a genome scan using ten of our largest multiplex ITEV families and the Linkage IV genotyping panel (Illumina, Inc., San Diego, CA), which contains 6,008 single nucleotide polymorphisms (SNPs). The resulting data was analyzed using parametric and nonparametric linkage analyses (Fastlink and Allegro) and disequilibrium analysis (PDT). Multipoint linkage analysis identified four chromosomal regions with a LOD score above 1.5: 4p13-14 (LODmax=1.89), 17q22-24.1 (LODmax=2.17), 19q13.32-13.42 (LODmax=1.66) and 20q13.11-13.13 (LODmax=1.71). Interestingly, the 4p13-14 region overlaps with a previously identified chromosomal deletion region found in children with clubfoot and other anomalies. The overlapping deletion region contains several candidate genes of particular interest including WDR19 and HIP2 which reportedly suppress apoptosis. Apoptosis has been associated with ITEV. These regions provide a starting point to begin to dissect the complex etiology of ITEV.

2442/F

Analysis of a candidate region on chromosome 6 detected by genome-wide association study for human narcolepsy. *M. Kawashima[1], K. Numazawa[1], M. Honda[2], J. Ohashi[3], Y. Honda[4], T. Ebisawa[1], K. Tokunaga[3].* 1) Dept Sleep Disorder Research (Alfresa), Univ Tokyo, Tokyo, Japan; 2) Tokyo Institute of Psychiatry, Tokyo Metropolitan Institute for Medical Research, Tokyo, Japan; 3) Department of Human Genetics Graduate school of Medicine, University of Tokyo, Tokyo, Japan; 4) Sleep Disorder Clinic of Seiwa Hospital, Tokyo, Japan.

Human narcolepsy is a multifactorial disorder, involving both genetic and environmental factors. A genetic factor strongly associated has been found in the human leukocyte antigen (HLA) class II region: most Japanese narcoleptic patients possess the HLA-DRB1*1501-DQB1*0602 haplotype. To find out associated genetic factors other than HLA, a genome-wide association study using about 23,000 microsatellite markers with pooled DNAs has been performed. The subjects were all Japanese living in the Tokyo area (case: 220, control: 420). From the 1st and 2nd screening using pooled DNAs, one of the associated markers which showed difference between the allele frequencies of cases' and controls' by Fisher's exact test (P<0.005): D6S0129i, is located near the HLA region. This marker was assessed by individual genotyping and three alleles reached significant level. To avoid the effect of linkage disequilibrium with HLA-DRB1*1501-DQB1*0602 haplotype, HLA-DRB1*1501 heterozygous cases and controls were then used (case: 170, control: 112). In the analysis, one allele still showed significant association (P=0.0022). Therefore, the surrounding region of marker D6S0129i was applied to the high-density association mapping. Forty Tag SNPs and eighteen SNPs were analyzed by direct sequencing. As a result, thirty-three SNPs reached the significant level. These SNPs were subjected to the association analyses using HLA-DRB1*1501 heterozygotes, and 7 SNPs still showed significant association by permutation test (P<0.005). In contrast, the associations observed in HLA class I region disappeared when HLA-DRB1*1501 heterozygous cases and controls were used. These observations indicate that the region is one of the newly associated loci which are independent from HLA class II haplotype.

2443/F

Analysis of WDR36 gene on Finnish glaucoma families. *S. Lemmela[1], E. Forsman[2], H. Nurmi[1], A. Eriksson[2], H. Forsius[2], I. Järvelä[1].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Population Genetics Unit, Folkhälsan Institute of Genetics, Helsinki, Finland.

Primary open angle glaucoma (POAG) is a heterogeneous group of disorders that have in common a characteristic optic neuropathy with associated visual field loss. Despite many positive linkage studies only three predisposing genes for POAG have been identified. The most recently identified susceptibility gene, *WD40-repeat 36 gene* (WDR36), is located on 5q22.1 at locus GLC1G. In the original study overall 24 sequence variations were identified in WDR36; four *predicted disease causing mutations*, three *potential disease susceptibility mutations*, five amino acid polymorphisms and 12 intronic-polymorphisms. Since the original report controversial results have been reported concerning WDR36 as a susceptibility gene for POAG. The aim of the current study was to analyse the role of WDR36 in Finnish patients by sequencing all 23 exons and flanking splice sites of the WDR36-gene in 21 Finnish POAG and 8 XFG (exfoliation glaucoma) and one XFS (exfoliation syndrome) patients. Two non-synonymous (D658G, I264V), two synonymous (V714V, V727V) and one intronic (IVS5→30C>T) sequence alterations were identified. All of these variations have been reported previously. Heterozygous D658G alteration was found in 1/21 POAG patients. This variation, originally defined as a *predicted disease causing mutation*, was not present in 100 Finnish anonymous blood donors as controls. Neutral variant I264V was identified in 12/21 POAG and 3/8 XFG subjects. Heterozygous synonymous polymorphism V714V alteration was found in 5/21 POAG patients and 1/8 XFG subject. Sequence polymorphism V727V was detected in 12/21 POAG patients and 3/8 XFG subject and intronic change IVS5→30C>T was detected in 16/21 POAG patients and 6/8 XFG subjects. Non-synonymous common variant I264V, synonymous alterations and intronic variation has been reported as neutral polymorphisms, whereas contradictory results of D658G mutations role in pathogenesis of POAG have been represented. Our results do not support the crucial role of the WDR36 gene in POAG pathogenesis in Finnish patients but suggests genetic heterogeneity underlying POAG.

2444/F

Variation in Insulin-Like Growth Factor 2 mRNA Binding Protein 2 (*IMP-2*) is Associated with Adiposity in Mexican Americans (MA). *X. Li[1], H. Wijesuriya[1,2], A.H. Xiang[1], E. Trigo[3], M. Perez-Ospina[1,2], H. Allayee[1,2], J.M. Lawrence[4], T.A. Buchanan[3], R.M. Watanabe[1].* 1) Dept of Preventive Med, Division of Biostatistics, Keck Schl of Med of USC, LA, CA; 2) Institute for Genetic Med, Keck Schl of Med of USC, LA, CA; 3) Dept of Med, Division of Diabetes and Endocrinology, Keck Schl of Med of USC, LA, CA; 4) Research and Evaluation, Kaiser Permanente, Pasadena, CA.

Recent genome-wide association (GWA) studies identified *IMP-2* as a susceptibility gene for type 2 diabetes (T2D). Based on stage 1 GWA results from the FUSION study, we examined whether *IMP-2* was associated with T2D-related quantitative traits (QTs) in the BetaGene study, a family-based study to identify genes associated with T2D-related QTs. A proband with prior gestational diabetes, her siblings and first cousins were phenotyped by oral glucose tolerance test (OGTT), intravenous glucose tolerance tests with minimal model analysis, and DEXA scan for percent body fat (PBF). Our study included 716 subjects in 143 families with mean age of 34.5±8.4 years, and mean PBF of 33.5±8.5%. We genotyped 13 SNPs in a 20 Kb region around rs1470579 in *IMP-2*. Three tag SNPs (rs13060777, rs6444082, rs11705701) were identified and tested for association with T2D-related QTs. We report Bonferroni corrected P-values, adjusting for age and sex. PBF was significantly associated with rs6444082 under additive, dominant and recessive genetic models (p<0.036) and rs11705701 under additive and dominant genetic models (p<0.004). For rs6444082, PBF decreased by 1.0% with addition of one T allele and 1.3% with addition of the second T allele. The magnitude in PBF change was similar for rs11705701. rs11705701 was also associated with T2D-related QTs (30 minute and 2-hour OGTT insulin) under an additive genetic model (p=0.039 and 0.034, respectively). However, these associations became non-significant with adjustment for PBF. In conclusion, variation in *IMP-2* is associated with adiposity in MAs. The variation in *IMP-2*, which regulates insulin-like growth factor-2 mRNA, may alter insulin-like growth factor 2 levels which are known to be associated with adiposity.

2445/F

Examining age-related macular degeneration in the Amish. *J.L. McCauley[1], L. Jiang[1], N. Schnetz-Boutaud[1], P.J. Gallins[2], A.E. Crunk[1], L.L. McFarland[1], D. Fuzzell[1], C. Knebusch[1], M. Creason[2], L. Caywood[2], C.E. Jackson[3], W.K. Scott[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) University of Miami School of Medicine, Miami, FL; 3) Scott & White, Temple, TX.

Age-related ophthalmic diseases present a significant health problem with huge social and economic consequences. Over the past two years, multiple genes have been identified for age-related macular degeneration (AMD). However, these critical successes only partially explain the genetic etiology of AMD. We have undertaken a powerful complementary approach for finding additional genes involved in ophthalmic diseases by using a genetically isolated founder population, the Midwestern Amish communities of the US. These Amish communities are more homogeneous in both environmental and genetic exposures. We have ascertained nearly 1600 Amish individuals for participation in studies of diseases prominently seen in older populations. We have identified 118 individuals who have self-reported AMD. Taking advantage of ongoing work within our group, we have genotyped 58 of these individuals, along with 614 additional Amish individuals, using the Illumina Linkage Panel IVb. We performed 2-pt linkage analysis, using both dominant and recessive models, on 5,645 SNPs using the Superlink program. Initial analysis identified 177 SNPs with lod scores ≥1.0. Three SNPs (on 2p, 4q, and 1q) have lod scores ≥2.0, with the 1q variant being approximately 2 Mb from the *CFH* gene. In addition to the SNPs within this linkage panel, we genotyped two previously confirmed AMD variants: rs10490924 within *LOC387715* and rs1061170 within *CFH*. The rs1061170 variant gave a 2-pt lod score of 1.43 suggesting involvement of *CFH* in AMD risk within this isolated population. However, rs10490924 did not have strong evidence for linkage within our study (lod = 0.22). This result may not be surprising given that this variant's risk is influenced by smoking, which is rare within the Amish.

2446/F

MEP1A is a susceptibility gene for inflammatory bowel disease. *B. Oneda[1], L. Min Yap[2], F. Seibold[3], D. Jewell[2], E.E. Sterchi[1], D. Lottaz[1].* 1) Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland; 2) Gastroenterology Unit, Radcliffe Infirmary, University of Oxford, United Kingdom; 3) Department of Gastroenterology, University of Bern, Switzerland.

Crohn's disease (CD) and ulcerative colitis (UC), the two most common forms of inflammatory bowel disease (IBD), are idiopathic, chronic, relapsing, inflammatory conditions, which are characterized by overreactive immune response. Genetic and environmental factors are known to influence the development and course of the disease, although the exact cause is still not known. Several IBD-susceptibility regions (IBD1-IBD9) across different chromosomes are known from linkage studies. The IBD3 susceptibility region on chromosome 6 harbors the MEP1A gene that encodes for the metalloprotease meprin-α, which is abundantly expressed in intestinal epithelial cells and is secreted into the gut lumen. In a previous genetic association study, we have found a significant association of MEP1A in a cohort of 379 UC and 380 CD patients, compared to 372 healthy controls. One non-synonymous and three synonymous SNPs in the coding region were significantly associated with UC, but not CD, whereas one 3'UTR SNP (C2417A) was particularly strongly associated with both UC and CD ($p=2.10^{-7}$ and 3.10^{-3}, respectively). We hypothesized that the IBD-associated meprin-? 3'UTR alleles show quantitative differences. Indeed, quantitative RT-PCR showed a marked reduction of meprin-? mRNA in inflamed mucosa, as well as a trend for lower expression in non-inflamed mucosa in IBD patients. These data indicate that MEP1A is a susceptibility gene that contributes to the pathogenesis of IBD and that a reduction in expression of meprin-α can be associated with intestinal inflammation. Because the 3'UTR is a region frequently recognized by miRNAs, we did an in silico analysis to compare the effect of the observed C2417A transversion on putative miRNA bindings sites. Two miRNAs targeting this site were identified, both of which highly expressed in human colon. Ongoing studies using cell culture models and reporter gene assays are now aimed to reveal the function of this 3'UTR polymorphism in meprin-? mRNA and protein expression.

2447/F

A new Androgenetic Alopecia genetic predisposing factor. *D.A. Prodi[1], N. Pirastu[1], G. Maninchedda[1], A. Mossa[1], A. Sassu[1], A. Picciau[1], M.A. Palmas[1], G. Biino[1,2], L. Casula[2], M. Adamo[1], A. Angius[1,2], M. Pirastu[1,2].* 1) Shardna Lifesciences, Pula, Cagliari, Italy; 2) Inst Population Genetics, Alghero, Italy.

Androgenetic alopecia (AGA) is a common disorder which affects mostly men. Despite a clear genetic predisposition, the aetiopathogenesis remains still unknown. An epidemiological survey of 7 genetic isolates in the secluded region of Ogliastra (Sardinia) demonstrates high prevalence of AGA (47.7%). It also clusters very well within families and has usually an early age of onset (70% <35 yrs). Candidate gene approach has been carried out analyzing several polymorphisms (CAG and GGN repeats and StuI RFLP rs6152) in the X-linked androgen receptor gene which have been previously associated with AGA in different populations, although these results are still controversial. For our study we selected 500 cases (age of onset <30,IV degree of AGA Norwood scale) and 500 controls (age>40 and no sign of AGA). For statistical analysis we used CC-QLS which can correct association values based on the kinship coefficient. When tested on the whole sample, StuI gave a pvalue of 2x10-19 (OR= 4.2) while the repeats gave a weaker association (GGN pval=0.007,CAG =2.2x10-4). StuI showed the strong association in all the villages separately but one (Triei). LD pattern revealed that AR is in strong LD with a gene 900kb centromeric:EDA2R. We chose to test the only validated EDA2R nsSNP rs1385699 in our samples. This SNP reached an even stronger association than StuI (pval=8.9 x 10-31; OR=5.5) and gave a positive result on all populations including Triei. EDA2R, through TRAF3-6, activates JNK which can stimulate c-juns' expression which is important for AR trans-activation. The absence of the predisposing allele in the African HapMap samples may justify the low prevalence of AGA in men of African descent. However, the AR and EDAR2R genes do not explain all the genetic susceptibility to AGA. In order to find other genetic factors involved we selected 25 families coming from the village of Talana for linkage analysis both with 1000 microsatellites and 16k SNP genomewide. This way we found 3 loci on Chr 1, 6 and 7 which contain candidate genes related to the same pathway as both AR and EDA2R.

2448/F

Filaggrin mutations confer susceptibility to atopic dermatitis but not to asthma. *A.J. Rogers[1,2,4], J.C. Celedón[1,2,4], J.A. Lasky-Su[1,3], B.J. Klanderman[1], E.T. Bevilacqua[1], L.M. Catalano[1], S.T. Weiss[1,2,4], B.A. Raby[1,2,4].* 1) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Harvard School of Public Health, Boston, MA; 4) Pulmonary Division, Brigham and Women's Hospital, Boston, MA.

Background: Loss-of-function mutations in the filaggrin gene (FLG) have been strongly associated with atopic dermatitis and allergic phenotypes in multiple populations. The role of these mutations in relation to the development of asthma is less clear, particularly in patients who do not have coincident atopic dermatitis.

Objective: To determine whether FLG mutations are associated with asthma and/or asthma-related intermediate phenotypes.

Methods: We genotyped two loss-of-function FLG mutations (R501X and 2282del4) in white children (ages 5-12 years) with mild to moderate asthma who participated in the Childhood Asthma Management Program (CAMP). We assessed the relationship of these mutations to asthma and allergy-related phenotypes in children with and without atopic dermatitis using both population-based and family-based tests of association.

Results: One third (174/611) of the participating children had concurrent atopic dermatitis. Although strong associations were observed between FLG mutations and atopic dermatitis (OR 2.39, $p = 7 \times 10^{-5}$ for combined mutation) and total serum Immunoglobulin E levels (p= .006 in the atopic dermatitis cohort), these mutations were associated with neither asthma nor asthma-related phenotypes (p >0.1 for all tests).

Conclusion: Although FLG loss-of-function mutations are consistently associated with atopic dermatitis and other allergic phenotypes, these mutations do not appear to influence susceptibility to asthma, nor do they influence asthma severity phenotypes.

This work was supported by grants: T32 HL007427, U01 HL065899, and P01 HL083069.

2449/F

Genetic Testing in 323 cases of Fatal Pulmonary Thromboembolism in the City of New York Revealed Racial Stratification. *Y. Tang[1], E.T. Bieschke[1], S.J. Jeudy[1], S. Sainte-Marie[1], Y.A. Kim[1], S. Pack[1], B.A. Sampson[2], M. Prinz[1].* 1) Forensic Biology; 2) Forensic Patholgy, Office of Chief Medical Examiner, New York, NY 10016.

Fatal pulmonary thromboembolism (PE) is a common cause of death encountered in the forensic pathology setting and usually presents as a complication of deep venous thrombosis (DVT). The pathogenesis of venous thrombosis is multifactorial and requires interaction between both inherited and acquired risk factors. Heterozygous or homozygous Factor V Leiden (G1691A) or prothrombin (G20210A) mutations, and homozygous MTHFR (C677T) variant have been recognized as common independent genetic risk factors in DVT. In order to investigate the frequency of these genetic risk factors in fatal PE and to understand the genotype and phenotype correlation, we have validated a genetic testing method to detect the three common mutations, using multiplex PCR-SNaPshot technologies, on postmortem tissue and blood samples. Between March 2005 and May 2007, we have tested 323 cases of fatal PE in the Office of Chief Medical Examiner in the City of New York. We found that 48 of the 323 cases were positive for at least one mutation. The genetic testing results were categorized by the demographic data and acquired contributing factors. We found the overall frequency of three mutations in PE cases is highest in Whites (34.15%), followed by Hispanics (28%), very low in Blacks (3%), and zero in Asian cases; in contrast, the number of fatal PE instances in our study is highest in Blacks (54.8%), followed by Whites (25.4%), and Hispanics (15.5%), and very rare in Asians (1.5%). Blacks were also associated with high percentage of idiopathic PE with unknown acquired contributing factors. This study suggests that there are racial disparities in genetic risks contributing to fatal PE. Further research focused on delineating the genetic risks in black populations is warranted. Detailed characterization of the mutation spectrum in fatal PE is vital for providing accurate diagnosis of cause of death and efficient preventative treatment to the high-risk family members.

2450/F

ACDC protective haplotype in a black South African cohort: Perspectives on the phenotypic context. *A. Olckers[1, 2], G.W. Towers[1], A. van der Merwe[2], A.E. Schutte[3], P. Rheeder[4], P.E.H. Schwarz[5].* 1) Centre for Genome Research, North-West University (Potchefstroom Campus), Pretoria, South Africa; 2) DNAbiotec (Pty) Ltd, Persequor Park, Pretoria, South Africa; 3) School for Physiology, Nutrition and Consumer Sciences, North-West University (Potchefstroom Campus), Potchefstroom, South Africa; 4) Division of Clinical Epidemiology, University of Pretoria, Pretoria, South Africa; 5) Department of Endocrinopathies and Metabolic Diseases, Medical Faculty Carl Gustav Carus, Technical University Dresden, Dresden, Germany.

Consisting of an array of clinically heterogeneous disorders, type 2 diabetes mellitus (T2D) is one of the fastest growing non-communicable diseases in the world, with the developing regions such as sub-Saharan Africa being at greatest risk. T2D affects circa 4% of the general population and is caused by the pathogenic interaction between insulin resistance and secretion.

During this investigation a diabetic (n=227) and control cohort (n=226) of adult black South African individuals were screened for the reported single nucleotide polymorphisms (SNPs) termed C-11377G and G-11391A, within the promoter of the adiponectin (ACDC) gene. Genotyping was achieved via a real time PCR method.

In a previous investigation it was determined that the 12 (haplotype structure = C-11377G; G-11391A) haplotype was significantly associated with a protective effect against T2D (OR = 0.16, 95% CI 0.03-0.72, p<0.01). In this investigation, individuals harbouring the 12 haplotype were compared to individuals that did not contain this haplotype for certain clinical parameters. This investigation was undertaken to elucidate the biological origin of the change in the risk phenotype.

The study strengthens the conclusions drawn previously that the genetic risk towards T2D is population dependent. Furthermore it highlights the fact that therapeutic regimens should be developed in a population dependent manner in order for these treatments to be effective on a global scale.

2451/F

CAPN10 haplotypes detected in high frequencies in type 2 diabetes patients and controls in the black South African population, in contrast to non-African populations. *G.W. Towers[1], A. van der Merwe[2], P.E.H. Schwarz[3], A. Olckers[1, 2].* 1) Centre for Genome Research, North-West University (Potchefstroom Campus), Pretoria, South Africa; 2) DNAbiotec (Pty) Ltd, Persequor Park, Pretoria, South Africa; 3) Department of Endocrinopathies and Metabolic Diseases, Medical Faculty Carl Gustav-Carus, Technical University Dresden, Dresden, Germany.

Objective: To elucidate the role of three reported calpain 10 (CAPN10) single nucleotide polymorphisms (SNPs) i.e. University of Chicago (UC) SNP-43, -56 and -63, in genetic type 2 diabetes (T2D) susceptibility within the black South African population.

Methods: Using a case-control strategy to determine association to T2D, a diabetic cohort (n=218) and a control cohort (n=217) of adult black South African individuals were screened for the aforementioned SNPs via real-time polymerase chain reaction. Genotypes were evaluated for adherence to Hardy Weinberg equilibrium and association testing was achieved by standard statistical methods. A subset of 100 female individuals underwent a two hour oral glucose tolerance test (OGTT) with measurements of glucose, insulin, c-peptide, proinsulin, free fatty acids and adiponectin to determine the biochemical effects of the CAPN10 SNPs.

Results: Protection towards T2D was observed in the black South African cohorts harbouring the wild type homozygote at the UCSNP-56 locus. Individuals harbouring the 2,2 genotype at this locus presented with increased c-peptide levels. Furthermore it was determined that the previously reported "at-risk" haplotypes in the Caucasian population were at much lower frequencies in the black South African cohort. This implies that alternative T2D susceptibility factors are present in these two populations.

Conclusions: This investigation highlights the importance of population history in the investigation of T2D genetic susceptibility. This is most likely due to the very different evolutionary pressures that these populations have experienced. Therapeutic strategies for T2D should therefore be developed in a population specific manner.

2452/F

Profiles of Resistance to Insulin in Multiple Ethnicities and Regions (PRIMER) study. *A. van der Merwe[1], G.W. Towers[2], A. Olckers[1, 2].* 1) DNAbiotec (Pty) Ltd, Pretoria , Gauteng, South Africa; 2) Centre for Genome Research, North-West University (Potchefstroom Campus), Pretoria, South Africa.

In order to elucidate the genetic, biochemical and physiological determinants of insulin resistance (IR) and impaired glucose tolerance (IGT) and in turn the effect of these factors on susceptibility towards type 2 diabetes (T2D) and the Metabolic Syndrome, the PRIMER study was undertaken. The ultimate aim of this investigation is the elucidation of the biochemical and genetic profiles of disease risk in the black South African population. By investigating the interplay between these two factors it will be possible to describe the underlying mechanisms of T2D pathogenesis.

The PRIMER study is a multi-disciplinary longitudinal prospective study over 12 years. Sample collection is scheduled for years 0, 5 and 10. The first collection phase of the PRIMER study was conducted from August to November 2005. Circa 500 individuals were collected with informed consent. It consisted of individuals from both rural and urban environments of the North West province and the cohort is defined as follows: predominantly Tswana individuals; 36% male and 64% female; 65% younger than 45 years, 34% between 45 and 54 years, and 1% between 55 and 64 years of age.

Individuals collected were "apparently healthy". Each individual had to have been fasting for at least 10 hours prior to a two hour oral glucose tolerance test (OGTT), with sampling and glucose measurement at 0', 30', 60', 90' and 120'. Glycosylated haemoglobin (HbA1c) was measured via high performance liquid chromatography (HPLC) to determine an individual's glycaemic control. Participants completed a questionnaire to determine individual risk towards the Metabolic Syndrome. Whole blood, plasma, serum and urine were collected via appropriate methodologies. This investigation consists of the largest cohort of 5-point OGTT's in the black South African population.

2453/F

Microrearrangements could be the major molecular mechanism in isolated holoprosencephaly: array CGH detects gains and/or losses in 24% of the patients. *C. Bendavid[1,2], J. Seguin[1], C. Dubourg[1,2], I. Gicquel[1], L. Pasquier[1,4], M.R. Durou[2], S. Jaillard[1,3], C. Henry[3], J. Mosser[1], S. Odent[1,4], V. David[1,2].* 1) UMR 6061 CNRS, Univ de Rennes1, Rennes, France; 2) Molecular Genetics, CHU Pontchaillou, Rennes, France; 3) Cytogenetics, CHU Pontchaillou, Rennes, France; 4) Medical Genetics, Hopital Sud, Rennes, France.

Holoprosencephaly (HPE) is the most common developmental brain anomaly in humans, usually associated with facial features. Our group focuses on patients with HPE and normal karyotype. Genetics of holoprosencephaly is complex: in our experience, mutations (18%) or deletions (8%) in the four main genes (SHH, ZIC2, SIX3 and TGIF) can explain about 26% of HPE cases. MLPA subtelomeric screening revealed 4% of additional complex rearrangements. In order to identify new candidate loci and thus novel candidate genes, we decided to screen HPE patients using Agilent CGH-array technology. 74 samples (47 fetuses and 27 live-borns children), with no karyotype alterations, were tested using a unique male or female DNA as control. Out of these 74 samples, 18 presented with new rearrangements involving known or new potential HPE loci located on different chromosomes but with poor redundancy. We observed 11 isolated deletions, 5 isolated duplications and 2 associated genomic losses and gains, the latters suggesting an unbalanced translocation from parental origin. Detected alterations ranged from less than 100 kb to 16 Mb and were not further considered if they involved less than 3 consecutive spots on the array. None of these regions matched against copy number variations described in databases (TCAG database). The few observed redundancies encompassed chromosomal regions on 21q and 20p. Frequencies of alterations were higher in foetuses (32%) than in live-borns (11%), but no correlations could be made between the size of the defects and the severity of the phenotypes. Added to the previous microdeletion findings in known HPE genes and subtelomeres, our data show that microrearrangements could be the major molecular mechanism in HPE and strongly reinforce the multigenic origin in this developmental disorder.

2454/F

Correlation of genotype/phenotype in different ethnic groups with primary Congenital Glaucoma. *D. Bercovich[1,2], C. Shochat[1], O. Geyer[3].* 1) The Human Molecular Genetics , Migal - Galilee Bio-Technology Center, Kiryat-Shmona, Israel; 2) Tel Hai Academic College; 3) Department of Ophthalmology, Carmel Medical Center, Haifa, Israel.

Mutations in the CYP1B1 gene are responsible for more than 50% of primary congenital glaucoma (PCG) and mutations in the myocilin gene (MYOC) have also been associated with this disease. The optineurin gene (OPTN), is known to be associated with primary open-angle glaucoma and low-tension glaucoma. We noticed a different clinical presentation of PCG in our patients according to ethnicity. Our goal was to find a correlation between genotype and phenotype in people with congenital glaucoma according to their ethnic origin. The medical history of patients with PCG including the numbers of affected and normal sibs available for each family were obtained by means of a questionnaire and details of the pedigree going back at least 4 generations. In the preliminary screen we screened the entire coding regions of the CYP1B1 gene in 25 individuals from five families of Israeli Moslem Arabs and one Druze family. First line screening was done by the DHPLC apparatus followed by sequencing the DNA. The screening revealed the cause for congenital glaucoma in three of these six families. The mutations includes a homozygous missense mutation R469W in exon 3 of the CYP1B1 gene. In the second family, the affected boy had the typical severe type of PCG and was compound heterozygous for two missense mutations (E229K (paternal) and R368H (maternal)). At another family the patient was also compound heterozygous, but from his mother he got two missense mutations (M1T & L432V) and from his father he got a frame-shift (Pro289 ins C). Screening the MYOC & OPTN genes in the rest of the families with no mutations in the CYP1B1 did not revile any DNA mutations. We are acquiring DNA samples from 15-20 more families from each selected ethnic groups. Establishing the genotype-phenotype correlations of PCG in our various ethnic backgrounds may add valuable knowledge for predicting the prognosis of the disease, for guiding therapeutic decision making and for genetic counseling of carriers of this cause of blindness in children.

2455/F

First results of a genome-wide association using jointly 10k and 500k Affymetrix chips in a Sardinian cohort. *M. Uda[1], S. Sanna[1,2], W-M. Chen[2], G. Albai[1], G. Usala[1], A. Maschio[1], F. Busonero[1], A. Mulas[1], M. Dei[1], S. Lai[1], A. Scuteri[1], M. Orru'[1,4], S. Naitza[1], L. Crisponi[1], M. Masala[1], E. Lakatta[3], P. Costa[3], G.R. Abecasis[2], D. Schlessinger[3], A. Cao[1].* 1) INN, CNR, Monserrato, Cagliari, Italy; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 3) Gerontology Reasearch Center, NIA, Baltimore, MD; 4) INRCA, Rome, Italy.

"The ProgeNIA Project" aims to identify the genetic components of aging-associated conditions in the Sardinian founder population. This population has been isolated since the last glaciation and developed to 1,500,000 inhabitants without appreciable admixture from in-migration. Thus, Sardinians share much of the same genetic information, which makes it easier to track genetic effects through generations. We recruited and phenotyped 6,148 individuals (aged 14-102) from a cluster of four towns. We considered and analyzed 98 quantitative traits as risk factors for several aging related complex diseases. Using Affymetrix gene chip arrays technology, we genotyped 3,329 and 1,412 individuals with 10K and 500K sets respectively. We took advantage of the relatedness between individuals so that for those genotyped with the 10K SNPs array only, we used a modified version of the Lander-Green algorithm to identify stretches of haplotype shared with close relatives who were genotyped at higher density and probabilistically infer missing genotypes. The validity of this strategy is confirmed by a significant increase in the p-values for most of the traits analyzed. For the 362,129 SNPs that passed quality control tests we performed a family-based genome-wide association analyses in order to evaluate the correspondent additive effects for the levels of 38 blood tests, 5 anthropometric measurements, 35 personality traits and 20 measurements of cardiovascular function. Genome-wide significant associations with genes were found for most traits. We present data for genes associated with levels of bilirubin, red blood cell indices, BMI, uric acid. Our approach revealed previously unmapped loci for several traits, including important regulators of fetal haemoglobin levels.

2456/F

Functional analysis of a nonsynonymous coding variant (R325W) in the pancreatic β-cell specific zinc transporter, *SLC30A8*, associated with type 2 diabetes. *M.R. Erdos[1], L. Qin[2], L.L. Bonnycastle[1], A.J. Swift[1], A.G. Sprau[1], A.U. Jackson[3], C.W. Willer[3], C.L. Yang[4], S. Humphreys[4], D.H. Ellison[4], J. Tuomilehto[5], R.N. Bergman[6], M. Boehnke[3], K.L. Mohlke[2], F.S. Collins[1].* 1) GTB, NHGRI, NIH, Bethesda, MD; 2) UNC, Chapel Hill, NC; 3) U Mich, Ann Arbor, MI; 4) OHSU, Portland, OR; 5) National Public Health institute, Helsinki, Finland; 6) USC, Los Angeles, CA.

Genome wide association studies have identified several novel susceptibility genes for type 2 diabetes (T2D) including *SLC30A8*, a pancreatic β-cell specific zinc transporter. Type 2 diabetes association with the SNP (rs13266634) that marks a non-synonymous coding substitution (R325W) in *SLC30A8* achieves genome wide significance (OR= 1.12, p= 5.3×10^{-8}) in the combined analysis of three major studies (DGI, UKT2D, and FUSION). We now report that quantitative trait analyses in ~2380 FUSION individuals also suggest association with systolic blood pressure (p= .028), pulse pressure (p= .004), triglycerides (p= .036, p= .009 in controls), fasting free fatty acids (p= .024) and BMI-related traits (BMI, waist, whr; p= .033-.05). In db/db diabetic mice, dietary zinc supplementation has been shown to attenuate hyperglycemia and hyperinsulinemia. In a pilot study, normal glucose tolerant Finns homozygous for the risk allele (C, n=16) had modestly lower, but not statistically different, plasma zinc levels (72.6 ug/dl, SD= 15.0) than those homozygous for the non-risk allele (T, n=19; 75.9 ug/dl, SD= 11.5). We have synthesized both alleles of the full length SLC30A8 cDNA and transfected these into HeLa cells. We observed similar expression levels and cellular localization for each allele, and we are now examining zinc uptake with each allele using the cell permeable zinc fluorophore, Fluozin-3. In a second model system, we are injecting *Xenopus laevis* oocytes with *in vitro* transcribed cRNA for each allele of *SLC30A8* in the presence of $^{65}Zn^{+2}$ supplemented media, and monitoring the zinc transporter activity by radioactivity uptake. These studies may define the mechanism for this newly discovered risk factor for type 2 diabetes, with the potential for future therapeutic insights.

2457/F

A Pediatric Genome-Wide Association Study Identifies A Type 1 Diabetes Locus on 12q13. *H. Hakonarson[1,2], S.F.A. Grant[1,2], J.P. Bradfield[1], L. Marchand[3], C.E. Kim[1], J.T. Glessner[1], R. Grabs[3], T. Casalunovo[1], S.P. Taback[4], E.C. Frackelton[1], M.L. Lawson[5], L.J. Robinson[1], R. Skraban[1], R.M. Chiavacci[1], C.A. Stanley[6], S.E. Kirsch[7], D.S. Monos[8,9], M. Devoto[2,10], H.Q. Qu[3], C. Polychronakos[3].* 1) Applied Genomics, Children's Hosp Philadelphia, Philadelphia, PA; 2) Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA; 3) Departments of Pediatrics and Human Genetics, McGill University, Montreal; 4) Department of Pediatrics and Child Health, University of Manitoba, Winnipeg; 5) Division of Endocrinology, Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa; 6) Division of Endocrinology, Children's Hosp Philadelphia, Philadelphia, PA; 7) Markham-Stouffville Hospital, Markham, Ontario; 8) Department of Pediatrics University of Pennsylvania, School of Medicine, Philadelphia, PA; 9) Department of Pathology and Laboratory Medicine, Children's Hosp Philadelphia, Philadelphia, PA; 10) CCEB, University of Pennsylvania, Philadelphia, PA.

Type 1 diabetes (T1D) is a common, strongly heritable disease that most often manifests in childhood. To identify novel T1D risk loci, we performed a genome-wide (GW) association study using the Illumina Infinium HH550 platform. The first stage, involving 563 T1D probands and 1,146 controls plus 483 complete T1D family trios of the same ancestry, confirmed previously known loci and identified one novel locus on Chr16p, which reached GW significance in the first stage as we previously reported; a second stage in an independent cohort of 939 nuclear families confirmed this association using TDT. While a full second stage is underway, we fast-tracked to examination in the same replication cohort an additional locus that came within an order of magnitude of statistical significance in Stage 1 (three common non-coding variants in strong LD yielded P from 4.05×10^{-6} to 7.16×10^{-7}, OR range= 1.34 - 1.40). This association was replicated in Stage 2 (P from 3.74×10^{-4} to 1.34×10^{-4}). These SNPs reside in a block of linkage disequilibrium on 12q13, containing six genes. This result provides evidence of a promising locus that could lead to new T1D therapeutics.

2458/F

Association analyses of retinol binding protein 4 (*RBP4*) genetic variants on circulating RBP4 concentration and phenotypes related to glucose metabolism in Chinese subjects. *C. Hu, W. Jia, R. Zhang, C. Wang, X. Ma, Q. Fang, J. Lu, K. Xiang.* Shanghai Diabetes Institute, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China.

Retinol binding protein 4 (RBP4) was a newly discovered adipokine that played a role in insulin resistance and obesity. Previous studies showed that its circulating concentration was significantly higher in subjects with obesity and diabetes. In this study, we investigated the relationship among genetic variants of *RBP4* gene, circulating RBP4 concentrations and phenotypes related to glucose and lipid metabolism in the Chinese population. We sequenced exons and the putative promoter region of *RBP4* gene, and identify six single nucleotide polymorphisms (SNPs) in 32 Chinese subjects. Additional, we selected four SNPs from public database to increase marker density in introns. Taking account of the pairwise linkage disequilibrium and minor allele frequencies, five SNPs were further genotyped in 627 well phenotyped individuals, including 255 type 2 diabetes patients and 372 normal controls. Phenotypes measured includes plasma glucose concentrations, serum insulin and C-peptide levels during an oral glucose tolerance test, lipid profiles, body fat distribution and circulating concentration of RBP4 and adiponectin. We found none of the individual SNPs were significantly associated with type 2 diabetes in this study. But a rare haplotype CAA formed by +5388 C>T, +8201 T>A and +8204 T>A was significantly more frequent in type 2 diabetes patients than that in the normal control subjects (P=0.0343). In both groups, several non-coding SNPs were associated with circulating RBP4 concentrations (P<0.05). In the normal glucose regulation subjects, the SNP +5388 C>T was associated with serum C-peptide levels in both fasting status and 2-hours after oral glucose tolerance tests (P=0.0162 and P=0.0075, respectively). Our findings suggest that the genetic variants in the *RBP4* gene may play a role in the susceptibility to type 2 diabetes in Chinese.

2459/F

Genetic association of insulin degrading enzyme (*IDE*) variants with type 2 diabetes in Hong Kong Chinese. *V.K.L. LAM, M.C.Y. NG, J.C.N. CHAN.* Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, nil, China.

Introduction: Type 2 diabetes (T2D) is a complex disease caused by the progressive loss of beta cell function. It is associated with elevation of islet amyloid polypeptide (IAPP) level which may result partly from the deficiency of IAPP clearance. Insulin-degrading enzyme (IDE) is a ubiquitous short peptidase responsible for IAPP degradation. Inactivations of IDE by drug and knockout mouse are associated with apoptosis and impaired glucose metabolism respectively. Genetic studies including recent genome-wide association studies have demonstrated significant association of variants at or adjacent to *IDE* gene region with T2D. This study aimed to investigate the possible association of *IDE* genetic variations with the pathogenesis of T2D.

Material and Methods: We genotyped eight tag SNPs flanking 2kb upstream and downstream of *IDE* gene using Sequenom's MassARRAY System. We tested their associations with T2D in a case-control samples consisting of 461 unrelated Chinese young onset (age at diagnosis ≤ 40 years) familial T2D patients and 419 healthy controls.

Results and Conclusion: The eight tag SNPs captured 77% of common SNPs (minor allele frequency > 5%) in HapMap Chinese database at $r^2 > 0.8$. All SNPs did not show departure from Hardy-Weinberg equilibrium in control subjects, $p > 0.05$. Case-control analysis discovered that rs6583813 was significantly associated with T2D [OR (95% CI) for C allele = 1.24 (1.01-1.51), $p = 0.04$] under an allelic model. Further haplotype analysis did not reveal more significant results. In conclusion, the present study suggests that genetic variants at *IDE* gene may contribute to susceptibility for developing T2D.

2460/F

The combined effect of multiple common type 2 diabetes variants on disease risk. *H. Lango[1], E. Zeggini[2], T.M. Frayling[1], N.J. Timpson[2], C.M. Lindgren[2], K.S. Elliott[2], J.R.B. Perry[1], N.W. Rayner[2], R.M. Freathy[1], C.N. Palmer[3], A.D. Morris[3], A.T. Hattersley[1], M.I. McCarthy[2], M.N. Weedon[1], UK Type 2 Diabetes Genetics Consortium, The Wellcome Trust Case Control Consortium.* 1) Peninsula Medical School, Exeter, UK; 2) University of Oxford, UK; 3) University of Dundee, UK.

Recently published genome-wide association studies have increased the number of confirmed common variants that influence risk of type 2 diabetes (T2D) to nine. Individually, the polymorphisms only moderately increase risk of disease (between ~10 to 40%) and they are thought to be unhelpful in assessing subjects' risk clinically; however, the combined effect of these variants may allow the identification of subgroups of the population at substantially differing risk of disease. To assess the combined impact of these variants on T2D risk we assessed the impact of these nine variants in 3005 controls and 2655 cases from the population-based GoDarts study. Risk allele frequencies ranged from 0.27 to 0.90. Individual allele odds ratios ranged from 1.05 to 1.35. We found no evidence of deviation from additivity at individual SNPs (P>0.01), and no evidence of gene-gene interaction (P>0.01). There was an approximately multiplicative increase in odds of T2D with increasing numbers of risk alleles, with each additional risk allele increasing the odds of disease by 1.15 (1.12,1.18) times. The 3% of subjects with > 12 risk alleles have an OR = 4.21 (2.80,6.33) against the 4% of subjects with < 6 risk alleles. The area under the receiver operator curve, a measure of the discriminatory ability of these variants, was 0.59. This is lower than the 0.61 from the initial GWAS, probably reflecting an upward bias from the "winners curse" and enriched sampling, and falls short of the 0.75 considered clinically useful. In conclusion, many more T2D risk variants will need to be identified before genetic testing on a population-based level will be considered clinically useful; however, combining information from several known common risk polymorphisms does allow the identification of subgroups of the population with markedly differing risks of developing T2D.

2461/F

Analysis of pooling strategies using Type II diabetes whole genome association study data. *A. Montpetit[1], R. Sladek[1,3], J. Rung[1], Y. Bosse[1], G. Rocheleau[1], F. Bacot[1], C. Polychronakos[5], D. Meyre[2], P. Froguel[2,4].* 1) McGill University and Genome Quebec Innovation Centre, Montreal Quebec, Canada; 2) CNRS 8090-Institute of Biology, Pasteur Institute, Lille, France; 3) Departments of Human Genetics, McGill University, Montreal, Quebec, Canada; 4) Section of Genomic Medicine, Imperial College London and Hammersmith Hospital, London, UK; 5) Department of Pediatrics, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Whole genome association studies have led to the identification of many new unsuspected genes and chromosomal regions involved in disease etiology. One major hurdle is the cost of those studies. Pooling samples has been frequently proposed as a way to reduce the costs. However, loss of power can limit the potential gains from this strategy. In this study, we used Type II diabetes genomewide association study data to evaluate different pooling analysis methods on the Illumina platform. We first show that the pooling protocols are able to accurately determine the minor allelic frequency (MAF) as compared to individual genotypes. On the other hand, we show that only 10% of SNPs are common between the top 5% of the hits obtained by individual genotyping or by pooling, probably indicating that both approaches give rise to a high rate of false positives that are masking the true positives. However, the correlation improves the closer you get to the top as 25% of the top 1000, 40% of the top 100 and 50% of the top 10 SNPs are common to both lists (including 4 out of 5 SNPs that were confirmed by replication in another cohort). We also present similar results from other complex traits and are currently in the process of validating many of these hits. This study shows that a pooling approach combined with a second phase in which the top hits are replicated in a second cohort can be a valuable and economic strategy for the discovery of new disease genes in complex diseases.

2462/F

FTO gene variants predispose to obesity through a metabolically neutral increase in body weight -The Lausanne CoLaus Study. *V. Mooser[1], C.K. Knouff[1], K.S. Song[1], X. Yuan[1], N. Guex[1], H.A. Stirnadel[1], F. Paccaud[2], A. Pecoud[2], D. Hayoz[2], T.M. Danoff[1], D.K. Burns[1], E.H. Lai[1], L.T. Middleton[1], P. Vollenweider[2], A.D. Roses[1], D.W. Waterworth[1], G. Waeber[2].* 1) GlaxoSmithKline R&D, King of Prussia PA, RTP, NC and London UK; 2) CHUV University Hospital Lausanne Switzerland.

BACKGROUND : FTO gene variants have recently been associated with obesity and with diabetes. One mechanism linking obesity to diabetes is insulin resistance, which clinically is often associated with hyperinsulinemia, dyslipidemia, low-grade inflammation, liver and kidney dysfunction and hypertension. Here we investigated the association between FTO gene variants and these conditions, collectively referred to as the metabolic syndrome, in a Caucasian population.

METHODS : We analyzed 60 FTO gene variants in 5641 extensively phenotyped participants (ages 35 to 75 years) of the Lausanne, Switzerland CoLaus population-based study genotyped using the Affymetrix 500 K SNP chip.

RESULTS : In line with recent reports, several variants within the FTO gene were associated with an increase in body mass index, waist circumference and body fat content. Unexpectedly, however, none of these FTO risk alleles for obesity was associated with fasting blood/plasma levels of leptin, adiponectin, glucose, insulin, lipids, C-reactive protein, liver function tests, nor with blood pressure levels, glomerular filtration rate or microalbuminuria. FTO risk alleles for obesity were associated with an increase in both fat and fat-free mass and, for a given body mass index, with a slightly better metabolic profile among obese individuals.

CONCLUSIONS : FTO gene variants predispose to obesity, but not to the metabolic syndrome, by promoting a metabolically neutral increase in fat and fat-free mass. In absence of association between FTO risk alleles and the intermediate phenotypes linking obesity to diabetes, these findings suggest that the associations of FTO variants with obesity and diabetes are due to two distinct mechanisms.

2463/F

Association patterns of 5290 SNPs in the type 2 diabetes 1q linkage region in eight populations. *I. Prokopenko[1], E. Zeggini[1], N.W. Rayner[1], C.J. Groves[1], R. Hanson[2], B. Mitchell[3], J. O'Connell[3], M. Vaxillaire[4], W. Jia[5], M. Ng[6], W. Knowler[2], L. Baier[2], P. Froguel[4], J. Chan[6], P. Deloukas[7], L. Cardon[1], C. Bogardus[3], S. Elbein[8], A. Shuldiner[3], M. McCarthy[1], Type 2 diabetes 1q consortium.* 1) WTCHG, Oxford, United Kingdom; 2) Phoenix, AZ; 3) Baltimore, MD; 4) Lille, France; 5) Shanghai, China; 6) Hong Kong; 7) Hinxton, UK; 8) Little Rock, AR.

High density linkage disequilibrium mapping of the 1q linkage region in type 2 diabetes (T2D) was performed in 8 populations (Amish, Utah, UK, French, Hong Kong, Shanghai, African Americans and Pima Indians). 5290 SNPs passed stringent quality control criteria across all datasets in the 22.7Mb candidate region (147.0-169.7Mb, NCBI35, average SNP density ~4.3kb). A total of 1527 T2D cases and 1653 controls were analysed. The proportion of common variation captured on the basis of HapMap was: 80% in CEU, 50% in YRI, 72% in CHB+JPT (pairwise evaluation at r2>=0.8). Population-specific single-point analyses and Mantel-Haenszel-based meta-analyses across 8 populations were performed under the additive and dominant/recessive models. Meta-analysis across all populations identified highly significant associations with T2D for 4 SNPs (combined p<5E-05) from two 1q subregions. Minor allele frequencies (MAF) range from 0.12 to 0.49. The first cluster (3 SNPs) resides in the region of extended LD at 152.0-152.4Mb that includes the *GBA*, *PKLR* and *ASH1L* genes. The strongest association was observed for rs11264372, in the 4th intron of *ASH1L* (OR for allele G 1.39[95%CI1.19-1.62] under the dominant model p=2.5E-05). The second cluster resides within *NOS1AP* (*CAPON*) at ~158.8Mb (allele A of rs7548169, dominant model (OR= 1.40[95%CI1.19-1.66] p=4.8E-05). This SNP lies in the 1st intron of NOS1AP, within the same LD block (27kb) as 3 further SNPs associated with p<1E-04. Although MAFs vary between datasets, the direction of the effect was the same in all populations. Association analyses of high-density genotyping in the 1q candidate region for T2D identified several common polymorphisms within two regions of high LD, with the strongest signals residing in the *ASH1L* and *NOS1AP* genes.

2464/F

New congenic strains reveal complex interaction of Cd36-deficiency with genomic background in determination of metabolic syndrome features. *O. Seda[1,2,3], M. Morysova[1], L. Sedova[1,3], L. Kazdova[2], F. Liska[1], J. Tremblay[3], D. Krenova[1], P. Hamet[3], V. Kren[1].* 1) First Faculty of Medicine, Charles University in Prague; 2) Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 3) Research Centre CHUM, Montreal, Quebec, Canada.

Deficiency of fatty acid translocase Cd36 has been shown to play major role in pathogenesis of metabolic syndrome in spontaneously hypertensive rat (SHR). We have derived 2 new congenic strains PD.SHR4 using marker-assisted approach for introgression of chromosome (chr.) 4 region of SHR origin including defective Cd36gene into genetic background of PD rat strain, highly inbred model of metabolic syndrome. We have subjected standard diet-fed adult males of PD and PD.SHR4 strains (n=8/strain) to metabolic, morphometric and transcriptomic (Affymetrix Rat 1.0 ST Exon array) profiling. The differential segment of SHR origin spans ca 20Mb between markers D4Rat139 and D4Rat125in PD.SHR4a congenic strain and ca 39Mb of telomeric chr.4 segment in PD.SHR4b. We observed significantly improved glucose tolerance and lower fasting insulin in both PD.SHR4 strains compared to PD. On the other hand, PD.SHR4a strain showed highest concentrations of LDL cholesterol compared both to PD and PD.SHRb. Both congenic strains had smaller LDL particle sizes and lower HDL cholesterol than PD. The expression profile revealed 18 transcripts with >1.5fold difference in expression after FDR correction (0.1), e.g. prostaglandin D2 synthase (2.2 fold in PD vs. PD.SHR4a). None of the differentially expressed transcripts resides in the introgressed chr.4 segment.

The transfer of chr.4 region of SHR origin, previously ascertained as a quantitative trait locus for dyslipidemia and insulin resistance, into PD genetic background resulted in paradoxical amelioration of insulin resistance and deterioration of lipid metabolism. Our results suggest that apart from mutant Cd36 allele, other genomic features present in the SHR-derived segment play a role in pathogenesis of metabolic syndrome. Their eventual phenotypic outcome is then a function of complex interactions with particular environment and genomic background, upon which they operate.

2465/F

Molecular etiopathophysiology of Diabetic Nephropathy (DN): Possible Role of SPARC and IGF2 signaling pathway. *S. Movva[1], S. Venkatasubramanian[1], K.K. Vattam[2], Y.R. Ahuja[3], Q. Hasan[1,2].* 1) Department of Genetics, Bhagwan Mahavir Hospital and Research Centre, Hyderabad-500004, Andhra Pradesh, India; 2) Department of Genetics & Molecular Medicine, Kamineni Hospitals. L.B.Nagar, Hyderabad- 500068, Andhra Pradesh,India; 3) Department of Genetics, Vasavi Hospital and Research Centre, Khairtabad, Hyderabad-500004, Andhra Pradesh, India.

Diabetic Nephropathy (DN) is a devastating complication of diabetes, which affects approximately 30- 40 percent of diabetics. However, the exact molecular pathophysiology of DN is not established. It is believed that elevated glucose damages the kidney, which is constantly repaired by modulators like Secreted protein acidic and rich in cysteine (SPARC). Hyperglycemia also increases insulin like growth factors (IGF)especially IGF2, which acts via the IGF receptors present on renal cells. Hence, it was hypothesized that SPARC and IGF2 may be playing an important role in the etiology of DN. Human renal biopsies categorized as controls, early DN and established DN by histopathology were analyzed by immunohistochemistry and real time RT PCR for the localization and expression of SPARC, IGF2, as well as it's downstream signalling protein, Akt and it's negative regulator phospahtase and tensin homolog on chromosome 10 (PTEN) using specific antibodies and primers. This is the first study, to the best of our knowledge, which has evaluated the role of these molecules in DN studying human renal biopsies . From the results obtained the following molecular etiopathophysiology of DN can be proposed: (i) Lowered expression of the repair modulator, SPARC results in development of DN (ii) Suppression of Akt in the presence of elevated IGF2 suggests that the alternative MAPK pathway may be the relevant signaling pathway in the etiology of DN (iii) Increased PTEN is responsible for down-regulating Akt (iv) Loss of negative IGF2-PTEN feedback loop may be pivotal in developing the renal changes characteristic of DN. Targetting the relevant molecular modulators like SPARC, IGF2 or it's downstream regulators may be important in the management of DN in the future.

2466/F

A whole-genome scan reveals linkage of celiac disease to 6q21-22 and 22q13 in extended pedigrees from Hungary and Finland. *E. Einarsdottir[1], L. Koskinen[1], I. Korponay-Szabo[2], K. Mustalahti[3], K. Kurppa[3], J. Partanen[3], M. Mäki[3], J. Kere[1,4], P. Holopainen[1].* 1) Medical Genetics, Helsinki University, Helsinki, Finland; 2) Heim Pal Children's Hospital, Budapest and University of Debrecen, Hungary; 3) University of Tampere and Tampere University Hospital, Finland; 4) Karolinska Institute, Huddinge, Sweden; 5) Red Cross Blood Service, Helsinki, Finland.

Celiac disease is a complex genetic disorder caused by inflammatory responses to gluten. Apart from the known susceptibility genes at HLA-DQ locus, the search for additional risk genes continues. We performed a whole-genome linkage scan in two extended four-generation pedigrees consisting of multiple individuals with celiac disease. Our aim was to identify genomic regions shared by the affected individuals within the pedigrees, regions harbouring genetic factors influencing susceptibility to celiac disease. Approximately fifty thousand single-nucleotide polymorphisms were genotyped with the Affymetrix 50K microarray system and analysed by affecteds-only non-parametric linkage analysis. We selected individuals separated by as many meiosis as possible in order to minimise random sharing of genomic segments and to narrow down the disease-linked genetic regions. Our material consisted of one pedigree from central Hungary and one from Finland. Six patients from the Finnish family were genotyped and seven from the Hungarian family. In addition to the well known HLA-DQ risk genes, we identified linkage in both families to a locus on chromosome 6q21-22 (LOD= 2,01 p=0.0012). The Finnish family also showed linkage to a locus on chromosome 22q13 (LOD= 1,29, p= 0.007). These regions have previously been suggested to be involved in celiac disease in European populations, but the primary risk genes at these loci remain unknown. Further finemapping in our larger independent family materials will be performed to narrow down the linked region and to identify the disease-associated gene(s). Characterization of novel genetic factors in celiac disease will help us understand the pathogenesis of this complex disorder.

2467/F

Risk of age-related macular degeneration is determined by genes and smoking. *A. Hughes, N. Orr, C. Patterson, H. Esfandiary, R. Hogg, V. McConnell, G. Silvestri, U. Chakravarthy.* Queen's Univ Belfast, Belfast, United Kingdom.

Age-related macular degeneration (AMD) is the major cause of blindness in the elderly. Those with the neovascular end-stage of disease have irreversible loss of central vision. AMD is a complex disorder in which genetic and environmental factors play a role. Polymorphisms in the complement factor H gene (*CFH*), *LOC387715* and the *HTRA1* promoter are strongly associated with AMD and smoking also contributes to the etiology.

We genotyped polymorphisms in *CFH*, *LOC387715* and the promoter of *HTRA1* in 401 patients with neovascular AMD and 266 controls without signs of disease, and collated genetic risk scores at these loci with risk from smoking history.

We scored risk haplotypes within *CFH* and *LOC387715*/*HTRA1* and smoking status. Each was found to exert a large effect on overall susceptibility, enabling risk scores to be generated with appropriate weighting of these three factors. Patients with severe macular degeneration had considerably higher scores than those without disease, and risk of blinding AMD rose to more than 14% in the tenth of the population with highest predicted risk.

2468/F

***MTHFR/VDR* genes variants interaction, bone mineral density and osteoporosis.** *O.M. Mutchinick, M.A. López, J.J. Morales.* Genetica, Inst Natl Ciencias Medicas Nutricion, Mexico, D.F., Mexico. E-mail: osvaldo@servidor.unam.mx.

Osteoporosis (OPS) is the bone mineral disorder most frequently found in adults, particularly in postmenopausal women (PMW), and one of the most frequent causes of morbidity and mortality in women over 50. Reduced bone mineral density (BMD) is the main metabolic feature and major determinant of the disease. Although controversy exists, VDR variants have been associated with low BMD, OPS and bone fractures. In a previous study we showed that the homozygous bb prevalence of the Bsm1 variant of VDR was significantly higher in cases than controls, though not associated to reduced BMD. Recently has been claimed that the common C677T MTHFR variant and low serum folate increase the risk of OPS and bone fractures. The very high prevalence of the T allele and TT genotype in the Mexican population and the reported low capacity to absorb folate by the elderly, prompted us to investigate the interaction effect on BMD of diverse variants of VDR with those of genes involved in folate metabolism.We studied 67 PMW with OPS. Of the VDR we studied the BsmI, FokI, ApaI and TaqI variants and the C677T and A1298C of MTHFR, and the common variants of MTR, MTRR genes. The methodology was based on DNA extraction, PCR amplification and RFLP's analysis with restriction enzymes. The results showed that the only significant gene-gene interaction associated to decreasing BMD were bb/CT and bb/TT combined genotypes of the VDR and MTHFR gene variants, and that the differences were statistically significant. Although the differences of the BMD appears small (bb/CC: 0.69; bb/CT: 0.63, bb/TT: 0.64 and for bb/CT and bb/TT together 0.64), to the knowledge of physicians specialized in OPS, they are significant. P values of the comparisons among the BMD between the different combined genotypes (bb/CC vs bb/CT; bb/CC vs bb/TT and bb/CC vs bb/CT+bb/TT) were 0.02, 0.06 and 0.005 respectively. The above findings suggest that the presence of one 677T allele of MTHFR in the presence of the homozygous bb of BsmI of VDR, insert a considerable harmful effect on the BMD of PMW resulting a risk factor for OPS, interaction to our knowledge not previously described.

2469/F

Gene-environmental interactions that affect serum IgE levels of urban school children. *Y. Suzuki[1], Y. Mashimo[1], H. Inoue[1], M. Funamizu[1], N. Shimojo[2], Y. Kohno[2], Y. Okamoto[3], A. Hata[1].* 1) Department of Public Health, Chiba University, Chiba, Japan; 2) Department of Pediatrics, Chiba University, Chiba, Japan; 3) Department of Otolaryngology, Chiba University, Chiba, Japan.

Background: Serum IgE level is determined by both environmental and genetic factors and their interactions. Little is known about the specific environmental and genetic factors that show significant interactions.

Aim: To evaluate interactions between environmental factors and interleukin 4 receptor alpha (IL4RA) gene Ile50Val polymorphism on serum levels of total and specific IgE in urban school children.

Methods: Four hundreds and seventy-three school children with 6 to 12 years of age were examined by questionnaires for their life styles in an urban area of Japan. We determined total IgE, specific IgE (mite, cat dander, alternaria, egg white, cedar pollen, orchard grass), and the genotype for IL4RA Ile50Val in the 411 children. Association between categorical data was evaluated with chi-square tests. Screening of factors that affected on IgE values was carried out with Kruskal-Wallis test. Effects on serum IgE levels of the polymorphism, environmental factors and their interactions were evaluated with logistic regression.

Results: Among environmental factors examined, daycare attendance before 2 years of age was associated with total IgE and cat dander-specific IgE; current floor type of bedroom was associated with total IgE and mite-specific IgE; consumption of yogurt was associated with total IgE, mite-specific IgE, egg white-specific IgE; raising pats was associated with cat dander-specific IgE. Significant interaction between raising pets and IL4RA Ile50Val genotype on cat dander-specific IgE level was observed. Effect of daycare attendance on total and cat dander-specific IgE levels was also modified by IL4RA Ile50Val genotype.

Conclusion: Response in total and cat dander-specific IgE serum levels to environmental alterations is affected by IL4RA Ile50Val polymorphism.

2470/F

Familial Glaucoma In Taxiarches, a Small Greek Village. *M.K. Wirtz[1], A.G.P Konstas[2], J.R. Samples[1], A. Dimopoulos[2], K. Kaltsos[2], A. Economou[2], I. Georgiadou[2].* 1) Dept Ophthalmology, CE-Res, Casey Eye Inst/OHSU, Portland, OR; 2) Glaucoma Unit, University Department of Ophthalmology, AHEPA Hospital, Thessaloniki, Greece.

Purpose: To initiate a prospective study of glaucoma in a Greek village reported over 30 years ago to have several large families with primary open angle glaucoma (POAG). Methods: Random individuals from Taxiarches were interviewed in regards to history of glaucoma in their family, examined and blood samples were drawn. Consent was obtained according to the guidelines of the University of Thessaloniki. Examinations included visual acuity, ocular nerve assessment by slit lamp, intraocular pressure and corneal thickness measurement and blood pressure. POAG was defined as characteristic optic nerve cupping, vertical cup to disc ratios > 0.6 or asymmetry > 0.2 between the fellow eyes. Affected individuals were followed up at the University of Thessaloniki to determine if they had exfoliation, a common finding in Greece and for visual field testing. Results: Twenty-two of the 127 participants were diagnosed with glaucoma and 15 were suspects. The 22 affected individuals and 11 of the suspects belonged to 11 pedigrees, the largest of which contained 6 affected individuals. Fourteen individuals, 12 of whom had glaucoma, have subsequently been examined at the University of Thessaloniki. Only 1 had exfoliation glaucoma, 12 had visual field loss consistent with POAG and no exfoliation. Screening of myocilin showed that 11 of the patients with glaucoma had the Thr377Met mutation. The remaining 11 affected individuals had no myocilin mutation. Two patients with POAG were homozygous for the Thr377Met mutation. Conclusions: The village of Taxiarches is a rich resource for studying familial glaucoma with 17% of the random individuals diagnosed with glaucoma. Eleven pedigrees were identified with two or more affected family members. The Thr377Met myocilin mutation is prevalent in the village. Future studies are planned for follow-up of a larger sampling of the village to assess environmental and genetic factors affecting POAG.

2471/F

Haplotype analysis of prostate cancer susceptibility loci at 8q24. *N. Orr[1], M. Yeager[2,3], K. Jacobs[4], R. Hayes[3], P. Kraft[5], S. Wacholder[3], R. Welch[2,3], H. Spencer Feigelson[6], D. Albanes[7], D. Gerhard[8], R. Hoover[3], D. Hunter[5], G. Thomas[3], S. Chanock[1,3], The CGEMS group.* 1) Pediatric Oncology Branch, NCI, Bethesda, MD; 2) SAIC-Frederick, Frederick, MD; 3) Division of Cancer Epidemiology and Genetics, NCI, NIH, Rockville, MD; 4) Bioinformed Consulting Services, Gaithersburg, MD; 5) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 6) Department of Epidemiology and Surveillance Research, American Cancer Society, Atlanta, GA; 7) Department of Health Promotion and Chronic Disease Prevention, National Public Health Institute, Finland; 8) Office of Cancer Genomics, NCI, NIH, Bethesda, MD.

The genetic bases for sporadic prostate cancer have been unknown until recently. Following a genome-wide association scan for prostate cancer risk variants, we identified two independent susceptibility loci at 8q24 separated by a hotspot of recombination (Yeager M, et al. Nat Genet 2007). Here we present haplotype analysis conducted at each locus using the novel approach of variable sized sliding window regularized regression (Li Y, et al. Am J Hum Genet 2007) with the aim of refining the nature of these associations.

In our initial study, the risk loci centromeric and telomeric of the recombination hotspot were defined by associations at rs6983267 and rs1447295 respectively. We genotyped a total of 34 tag-SNPs (15 centromeric and 19 telomeric) in 4137 cases and 4081 controls, drawn from 4 independent prostate cancer cohorts. The centromeric and telomeric region tag-SNPs spanned approximately 63 kb and 116 kb. We found that the most significant haplotype in the centromeric region spanned 15 kb, comprised five SNPs delineated by rs10808555 and rs7014346 ($p=1.17x10^{-11}$) and incorporated rs6983267. The most significant haplotype in the telomeric region spanned 12 kb, contained 6 SNPs and was bounded by rs4871809 and rs7837688 ($p=3.47x10^{-13}$). Interestingly, this haplotype did not include rs1447295, suggesting that it is unlikely to be the causative allele at the telomeric locus. We believe this analysis will be of great value for further fine mapping studies of prostate cancer risk at 8q24.

2472/F

Candidate gene approach to identify genetic predisposition to severe forms of dengue virus infection. *M. Yasunami[1,2], T.P.L. Nguyen[2], M. Kikuchi[1,2], N. Okuda[1,2], H. Horie[1,2], T.Q.H. Vu[3], K. Morita[2], K. Hirayama[1,2].* 1) Center for Intl Collab Res, Nagasaki Univ, Nagasaki, Japan; 2) Inst Trop Med (NEKKEN), Nagasaki Univ, Nagasaki, Japan; 3) Pasteur Inst in Ho Chi Minh City, Ho Chi Minh City, Vietnam.

Dengue fever is caused by infection of dengue virus which is classified as flaviviridae. A recent surveillance revealed that up to 30% of the patients with dengue fever (DF) develop more severe forms with hemorrhagic tendency and symptoms of plasma loss, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), in the Southeast Asian countries. Multiple factors have been proposed for the development of DHF and DSS, and host genetic variation would be one of such major determinants. To identify the host genes contributing to the development of DHF and DSS, we collected 743 patients with apparent dengue virus infection (114 patients with DF, 211 patients with DHF, and 418 patients with DSS) who were diagnosed by WHO criteria at two hospitals in southern part of Vietnam from 2002 to 2005, and 193 healthy controls matched for ethnicity and age. As a screening, we employed pooled DNA genotyping for 85 microsatellite markers physically linked to immune and inflammation-related candidate genes. Comparison of one 100-DHF-patient pool and two 100-DSS-patient pools with a control pool of 100 individuals suggested the presence of alleles in different frequency across populations at 22 out of these 85 loci. The difference in allele frequency was then confirmed at 19 loci of them by genotype data of individuals who were included in the pools, indicating that the pooled DNA genotype was reliable in terms of the specificity of detection as a screening method. We extended the genotype analysis of these 19 loci for all available samples and found the association of at lease one allele at 10 microsatellite loci with any of three form disease, DF, DHF or DSS. An allele of the microsatellite locus physically linked to CD4 gene on chromosome 12 is one of the resultants, which exhibited positive association with both DHF and DSS and significant genetic interaction with a disease-resistant factor linked to HLA class II genes.

2473/F

PINK1 mutations and the risk of Parkinson's disease in family members of Southern Italy. *V. Scornaienchi[1], I.C. Cirò Candiano[1], D. Civitelli[1], S. Carrideo[1], F. Annesi[1], P. Tarantino[1], F.E. Rocca[1], E.V. De Marco[1], G. Provenzano[1], G. Nicoletti[1], G. Salemi[2], P. Ragonese[2], V. Terruso[2], M. D'Amelio[2], G. Savettieri[2], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone, Cosenza, Italy; 2) Department of Clinical Neurosciences, University of Palermo, Italy.

Mutations in the PTEN-induced kinase 1 (PINK1) gene have been identified in recessively inherited and sporadic early-onset parkinsonism (EOP). The PINK1 gene comprises 8 exons and codes for a 581 amino acid protein (PTEN-induced kinase 1 protein) with a catalytic serine/threonine kinase domain. Functional studies have shown that PINK1 protein may have a neuroprotective role as wild-type PINK1 protects cells against proteasomal inhibition. This protective effect is abrogated by mutations in the PINK1 gene. Herein we investigated a possible association of PINK1 gene mutations in Southern Italy family members with monogenic parkinsonism. 14 family members diagnosed for PD were investigated for the presence of PINK1 mutations. Of them, 5 participants had EOP (mean age at onset 36 years); the remaining 9 had familial late-onset disease (mean age at onset 65 years). DNA was extracted from blood samples following standard procedures. All eight PINK1 exons were amplified by PCR with primers flanking intronic sequences. Sequencing was performed using BigDye Terminator V.1.1. We characterize a novel homozigous mutation (889delG, D297fsX318) in the exon 4 of PINK1 gene occurring in a patient with familiar EOP. None of the other examined patients carried homozygous or heterozygous mutations. We also identified known polymorphic intronic and exonic variants, although none seemed to be associated with disease risk. In conclusion, PINK1 mutations are rare in our Southern Italy patients with EOP and familial Parkinson's disease. However, we found a novel homozigous deletion (889delG, D297fsX318) that causes a premature stop codon and a protein lacking in most of the kinase catalityc domain confirming that recessive mutations in PINK1 might increase the risk of developing PD.

2474/F

Genome-wide association analysis identifies risk loci for obesity in the Old Order Amish. *M. Fu, E. Rampersaud, H. Shen, X. Shi, L. Zhang, J. Shelton, J. Yin, J. O'Connell, B.D. Mitchell, A.R. Shuldiner.* Dept Med, Div Endocrinology, Univ Maryland, Baltimore, MD.

Obesity is associated with an increased risk of type 2 diabetes, metabolic syndrome, cardiovascular disease, and some forms of cancer. Genetic susceptibility to obesity is well recognized, with estimates of the heritability of body mass indix (BMI) ranging from 30 to 70%. We performed a genome-wide association scan (GWAS) for obesity susceptibility genes in the Old Order Amish. We genotyped 382,935 single-nucleotide polymorphisms (SNPs) in 861 subjects from the HAPI Heart Study and prioritized 32 loci showing significant association with age-, age2- and sex-adjusted BMI ($P < 10\text{-}4$). To distinguish true associations from false positives, we compared these results to GWAS results from the nondiabetic control group of the Diabetes Genetics Initiative (DGI) and the Amish Family Diabetes Study (AFDS). We confirmed the previously reported association between BMI and SNPs in FTO ($P = 4.6\times10\text{-}5$). SNP rs9939609 in FTO was also significantly associated with type 2 diabetes ($P = 0.007$) in the Amish; this association was abolished by adjustment for BMI ($P = 0.532$) suggesting that variation in FTO increases diabetes risk through its effect on obesity. There was no evidence for association between BMI with variants in the INSIG2 gene. We identified four novel obesity susceptibility loci in and around the genes ALK, ANK2, SLC24A3, and PRKG1. PRKG1 is a cyclic GMP-dependent protein kinase (PKG) that is a major receptor for cGMP in a variety of cells and has an evolutionary conserved structure. Allelic variation in the PRKG1 homolog in Drosophila or alterations in expression in the honey bee Apis mellifera and the nematode C. elegans result in differences in food-related behaviors. Furthermore, PRKG1 is located on 10q11, under our previously reported linkage peak (lod = 2.73, $P = 0.0002$) for BMI-adjusted leptin. In summary, the results of our GWAS of BMI in the Amish identified a tractable number of novel candidate genes that warrant further investigation.

2475/F

Association of gene expression levels in small airway epithelium with genotype for adjacent SNPs. *N.R. Hackett, T.P. O'Connor, J. Salit, T. Raman, I. Dolgalev, R.G. Crystal.* Weill Cornell Medical College, New York, NY.

Smoking is the major risk factor for chronic obstructive pulmonary disease (COPD), but only 15-20% of chronic smokers develop the disease. Given that abnormalities in the small airways are the initial site of COPD, we hypothesize that gene expression pattern for protective and susceptibility genes in the small airway epithelium of smokers determines risk for COPD. Further, we propose that the gene expression level for these genes is genetically determined by single nucleotide polymorphisms (SNPs) in the vicinity of the gene. The gene expression profile of 42 subjects (14 non-smokers, 17 healthy smokers and 11 smokers with COPD) was determined on the Affymetrix Human Genome U-133 2.0 Plus array. The same subjects were then subjected to genotyping for SNPs using the Affymetrix 5.0 SNP array. For all genes expressed in >50% of the small airway epithelial samples according to the Affymetrix "P" call, the correlation of expression level with genotype was assessed for all SNPs within 25,000 bp either side of the location of the gene. A total of 294 SNPs yielded strong associations with expression levels with a p value of $<10^{-6}$. These represented 114 unique genes with 1 to 10 associated SNPs that correlate with expression level. When these genes were sorted into functional categories it was noticeable how three protease inhibitors gave multiple SNPs associated with small airway expression level, including SERPIN A6 (corticosteroid-binding globulin / transcortin), SERPIN B5 (serpin peptidase inhibitor, clade B, member 5/ maspin) and SERPIN B11 (serpin peptidase inhibitor, clade B member 11) with 3, 4 and 6 associated SNPs respectively. As an example for SERPIN A6, the mean expression level for subjects with TT as genotype for SNP rs11160168 was 0.9 ± 0.3 *vs* 2.4 ± 0.7 for heterozygotes and 3.6 ± 0.4 for CC genotype. However, the mean expression levels for smokers and non-smokers was the same ($p>0.6$). Due to the known role of α1-antitrypsin deficiency in susceptibility to emphysema, these three SERRPINs represent candidates for further investigation as susceptibility factors for smoking induced pulmonary disease.

2476/F

Genome-Wide Association Study for Longevity Using 550,000 SNPs in the Quebec Founder Population. *S. Kebache[1], J. Raelson[1], P. Van Eerdewegh[1], Q. Nguyen-Huu[1], G. Lepage[1], T. Fülöp[2], M. Dugas[3], H. Fournier[1], B. Paquin[1], J. Hooper[1], A. Belouchi[1], T. Keith[1].* 1) Genizon BioSciences, St-Laurent, QC, Canada; 2) University of Sherbrooke, Sherbrooke, QC, Canada; 3) Université Laval, Centre de recherche du CHUQ, Quebec, QC, Canada.

To identify genes involved in longevity, we performed a GWAS using 530 cases (>94 years of age) and 530 matched controls (18-65 years of age) from the Quebec founder population (QFP). Cases and controls were individually genotyped using the Hap550 chip (Illumina). 499,217 SNPs and 523,160,414 genotypes with a call rate of 99% and a minor allele frequency >4% were used in genetic analyses. Haplotype and single-marker association analyses were performed, with a sliding window defining haplotypes of 1, 3, 5, 7 and 9 markers. The genome-wide significance of the obtained P values was assessed by permutation studies. Regions with P values that met the criteria for genome-wide significance were identified both with the haplotype and single marker association tests, indicating that a well-powered GWAS with the QFP can be achieved with a relatively small sample size. Among the significant signals, haplotype analysis yielded 5 regions with P values $<10^{-7}$ including 2 with P values $<10^{-8}$, whereas single-marker association identified 7 regions with P values <10-5 including 1 with a P value $<10^{-6}$. Examples of top candidate loci are described, including information on the length of the regions and the relevance of the encoded genes in relation to longevity. Regions were well resolved with ~40% containing a single gene. About half of the regions contain genes relevant to longevity including neurological function, cardiovascular function, insulin metabolism and DNA repair. Using only the centenarians as cases (196 individuals), we identified 1 region that met the criteria for genome-wide significance ($P \sim 10^{-3}$ in the full sample to $P \sim 10^{-7.5}$, even though the sample size was drastically reduced). The identified genes have been used to build a GeneMap, consisting of networks of genes and their biological pathways relevant to ageing processes. We are currently performing genome-wide conditional analyses to detect gene-gene interactions.

2477/F

Genome-wide association study of human narcolepsy using 500,000 SNPs. *T. Miyagawa[1], M. Kawashima[2], N. Nishida[1], J. Ohashi[1], R. Kimura[1,3], A. Fujimoto[1], M. Honda[4], Y. Honda[5], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Department of Sleep Disorder Research, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Department of Forensic Medicine, Faculty of Medicine, Tokai University, Kanagawa, Japan; 4) Tokyo Institute of Psychiatry, Tokyo, Japan; 5) Sleep Disorder Clinic of Seiwa Hospital, Tokyo, Japan.

Human narcolepsy is a sleep disorder that is associated with multiple genetic and environmental factors. Narcolepsy affects 0.02-0.06% of the general population in the United State and Europe whereas 0.16-0.18% in Japan. A genetic factor strongly associated with the disorder has been found in the human leukocyte antigen (HLA) region: the HLA DRB1*1501-DQB1*0602 haplotype. However, it is suggested that narcolepsy susceptibility gene(s) other than HLA also exist because HLA DRB1*1501-DQB1*0602 haplotype carriers are about 12% in the Japanese general population, and the HLA alone cannot statistically explain all the genetic contribution. Therefore, to identify unknown narcolepsy susceptibility gene(s), we performed a genome-wide association study instead of a candidate gene approach. We genotyped approximately 500,000 SNPs in 222 narcoleptic patients and 389 healthy controls (Affymetrix GeneChip® Human Mapping 500K Array Set) and performed case-control association analyses. The subjects investigated in this study were all Japanese living in the Tokyo area. Approximately 250,000 SNPs with call rate ≥ 95%, HWE P ≥ 0.1% and MAF ≥ 5% were selected after data cleaning. Significant level (α = about 7×10^{-5}) was calculated using false positive report probability (FPRP). About 30 candidate SNPs were selected by referring to the above significant level and genetic information. A replication study is in process now.

2478/F

Whole genome association study in rheumatoid arthritis identifies TRAF1-C5 as a new susceptibility locus. *R.M. Plenge[1], E.F. Remmers[2], A.T. Lee[3], A. Liew[3], H. Khalili[3], A. Chandrasekaran[3], L. Davies[1], W. Li[3], C. Liu[4], C. Tian[7], W. Chen[5], D. Altshuler[1], J.P. Carulli[4], L.A. Criswell[6], C.I. Amos[5], M.F. Seldin[7], D.L. Kastner[2], P.K. Gregersen[3].* 1) Broad Institute; 2) National Institute of Arthritis and Musculoskeletal and Skin Diseases; 3) Feinstein Institute for Medical Research, North Shore L.I.J. Health System; 4) Biogen Idec; 5) Univ of Texas, M.D. Anderson Cancer Center; 6) Univ of California San Francisco; 7) Univ of California Davis.

BACKGROUND Rheumatoid arthritis (RA) is a common disease with a complex mode of inheritance. While HLA-DRB1 and PTPN22 are well-established susceptibility loci, and other genes conferring modest levels of risk have recently been identified, current evidence suggests additional genetic risk factors. Whole genome association is a powerful tool for systematically identifying new susceptibility loci. METHODS We applied the Illumina HumanHap550 genotyping array to RA patients seropositive for auto-antibodies against cyclic citrullinated peptides (CCP). We conducted an association study of SNP allele frequency with 502,757 polymorphic SNPs between 873 CCP+ RA cases and 1,196 controls. The most significantly associated SNPs were then replicated in an independent set of 537 CCP+ RA cases and 1,306 controls. We implemented statistical methods to correct for systematic differences in ancestry between case and control samples. RESULTS Aside from known associations with PTPN22 and the MHC region, we identified a SNP on chromosome 9 that is strongly associated with risk of developing CCP+ RA (rs3761847, OR=1.40 (95% CI: 1.27-1.54) and P=7x10-9 for all samples tested). The RA-associated SNP resides within a region of linkage disequilibrium containing two genes of known biological importance to chronic inflammatory states, TNF receptor-associated factor 1 (TRAF1) and Complement component 5 (C5). Conditional analysis across all 3,912 case-control samples demonstrates that a single allele can explain most of the genetic signal at this locus. CONCLUSIONS A common genetic variant at the TRAF1-C5 locus on chromosome 9 is associated with an increased risk of developing CCP+ RA.

2479/F

A Large-Scale Rheumatoid Arthritis Genetic Study Identifies TRAF1 Variants on Chr 9q33.2. *S.J. Schrodi[1], M. Chang[1], K.G. Ardlie[2], C.I. Amos[3], L.A. Criswell[4], D.L. Kastner[5], P.K. Gregersen[6], M.F. Seldin[7], R.E.M. Toes[8], T.W.J. Huizinga[8], A.B. Begovich[1].* 1) Celera, Alameda, CA; 2) SeraCare Life Sciences, Cambridge, MA; 3) Univ Texas, Houston, TX; 4) UC San Francisco, CA; 5) N.I.H., Bethesda, MD; 6) North Shore-LIJ Inst, NY; 7) UC Davis, CA; 8) Leiden Univ Med Centre, Netherlands.

To identify rheumatoid arthritis (RA) susceptibility loci, we carried out a multi-tiered, case-control association study by genotyping 26,764 putative functional SNPs in 475 white North American RA patients and 475 matched controls. Significant markers were genotyped in two additional, independent, white case-control data sets (661 cases/1322 controls from North America and 595 cases/705 controls from The Netherlands) identifying a SNP, rs1953126, on 9q33.2 that was significantly associated with RA (Pcomb=2.62E-06, ORcommon 1.34). Through a comprehensive fine-scale-mapping SNP-selection procedure, 137 additional SNPs across 668kb from MEGF9 to STOM on 9q33.2 were genotyped in a staged-approach. Significant single marker results (Pcomb less than 0.001) spanned a large 461kb region from PSMD5 to GSN; however, SNP association patterns surrounding TRAF1 were observed to have a higher degree of consistency across sample sets, heightened statistical significance and reduced variability between control groups when compared to other SNPs. A sliding-window haplotype analysis revealed a 29kb-wide maximum peak of global association in TRAF1 (Pcomb=4.15E-08). Further haplotype analyses demonstrated RA-associated variants extending 60-70kb from PHF19 across TRAF1 and through the region 3' of C5. Conditional analyses indicated that two TRAF1 SNPs exhibit stronger relative effects than other associated SNPs in PHF19, C5 or RAB14. TRAF1 is a member of the TNF receptor associated factor (TRAF) protein family that associates with TRAF2 to form a heterodimeric complex, which is required for TNF-alpha-mediated activation of MAPK8/JNK and NF-kappaB. In combination with the other two known genetic risk factors, HLA-DRB1 and PTPN22, the variants reported here substantially alter the risk of RA.

2480/F

A genome-wide association study in age-related macular degeneration in Mexican patients. *I. Silva-Zolezzi[1], J. Estrada-Gil[1], AV. Contreras[1], A. Hidalgo[1], L. Uribe-Figueroa[1], RA. Cano-Hidalgo[2], JC. Zenteno-Ruiz[2], R. Ayala-Ramirez[2], H. Perez-Cano[2], S. March[1], E. Graue[2,3], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine, Mexico; 2) Hospital Conde de Valenciana IAP, Mexico; 3) School of Medicine, UNAM, Mexico.

Age-related macular degeneration (AMD) is the most common cause of central blindness in the elderly population. The molecular mechanisms underlying this disease are poorly understood. Two genome-wide association studies with ≈110,000 SNPs, one in Caucasians and other in Asians, have demonstrated the association of two genes with AMD: Complement Factor H (*CFH*) and a serine protease (*HTRA1*). This study aims to search for new associated genes in an admixed population using the same platform. 100 unrelated Mexicans with advanced AMD, 90 unrelated healthy controls and 300 population controls were genotyped. Our results of GWAS replicated the association found in the Asian population (rs1040924) related to *HTRA1* (p-value<4.0E-7). To better characterize this result, we are currently re-sequencing the promoter region of *HTRA1*. Additional signals with suggestive p-values<5.0E-5 were identified in regions not previously associated to AMD (4q13, 10p13, 14q21). Association to rs380390 in *CFH* previously associated in Caucasians did not achieve statistical significance. Individual genotyping of the Tyr402His variant of *CFH* showed mild association (p-value= 3.0E-3). Our results suggest that *HTRA1* and *CFH* contribute to AMD in the Mexican population, and also support the idea that that genomic structure of admixed populations may contribute to the identification of new disease related genes. In addition, the identification of new regions associated to AMD suggests that its molecular mechanism may vary in the Mexican population.

2481/F

Genome-wide association study and targeted follow-up studies for anthropometric measures of obesity. *E. Speliotes[1,2,3], H. Lyon[1,4], B. Isomaa[1,8], T. Tuomi[1,6], M. Ridderstrale[1,6], M. Kuokanen[6,9], C. Guiducci[1], R. Hacket[1], V. Salomaa[7], L. Palotie[6,7,9], L. Groop[1,5,6], J. Hirschhorn[1,3,4].* 1) on behalf of the Diabetes Genetics Consortium, Broad Institute, Cambridge MA; 2) Massachusetts General Hosp, Boston, MA; 3) Harvard Medical School, Boston MA; 4) Children's Hosp, Boston MA; 5) Lund University, Sweden; 6) University of Helsinki, Finland; 7) National Public Health Institute, Finland; 8) Malmska Municipal HC, Finland; 9) Broad Institute, Cambridge MA.

Obesity and its complications have reached epidemic proportions. Heritable anthropometric measures of obesity include body mass index (BMI), waist circumference (WC), waist hip ratio (WHR)and predict future risk of diabetes, cardiovascular disease, and death. We genotyped over 3000 individuals from Scandinavia that were part of a case/control study of diabetes (Diabetes Genetics Initiative) matched for age, gender and BMI using the Affymetrix 500K platform. 389,869 SNPs passed quality control filters (genotyping success in >95% of individuals using the BRLMM algorithm, minor allele frequency >0.01, HWE p>10 -6). We tested SNPs for association with measures of obesity under an additive genetic model using the PLINK software package. For obesity measures, overall inflation factors were low (1.00-1.11)indicating that the study was not substantially affected by technical biases such as population stratification. For most measures of obesity, we observed an excess of low p values but none achieved genome-wide significance, consistent with a model of multiple loci with modest effects, and suggesting that our top results consist of true obesity loci hidden among a larger group of loci that represent expected statistical fluctuations. To identify these obesity loci, we have begun by testing 100 SNPs with the best evidence of association to BMI, using a ,multistage replication strategy involving over 20,000 separate individuals. Furthermore, through collaboration we will combine our genome-wide association results with additional genome-wide data to further enrich for loci that contribute to obesity. Association results available at http://www.broad.mit.edu/diabetes/scandinavs/index.html.

2482/F

Association of novel FTO variants with BMI in the isolated population of Sorbs in Germany. *A. Tonjes[1], E. Zeggini[2], P. Kovacs[1], Y. Bottcher[1], W. Rayner[2], M.I. McCarthy[2], M. Stumvoll[1].* 1) University of Leipzig, Germany; 2) WTCHG, University of Oxford, UK.

Recently the association of common variants of the FTO gene (FTO) with obesity was described and has been replicated in large cohorts in adults as well as in children. The implicated 47-kb intron region is assumed to contain the predisposing variant but the causal variant and the underlying mechanism of altered gene function remain unknown. Since the impact of isolated populations in the genetics of complex traits has been extensively documented, we performed a genome wide association study using 500K Affymetrix chips in a recently recruited and extensivly phenotyped isolate from the eastern part of Germany. Sorbs are of Slavonic origin and have lived in ethnic isolation among the Germanic majority for the past 1000 years. We used linear regression analyses to calculate the effects of genetic polymorphisms on BMI in 50 individuals with type 2 diabetes and 150 controls with normal glucose tolerance taken from a population based sample (age 61.6 ±10.2 years, BMI 29.8 ±4.98 kgm^{-2}). Despite the relatively small sample size, we found 6 FTO-SNPs (in high mutual LD, r2 0.5-1.0) significantly associated with BMI (eg rs8053740: ratio of geometric means per every additional C-allele is 1.06 (95% CI 1.03-1.09), λ-adjusted p-value 3.93×10^{-5}). These SNPs map in an intron about 60 kb away from the previously described SNPs which show moderate p-values (0.002-0.06) in our analysis. The LD structure of FTO in the Sorbs is similar to that observed in the European (UK) sample, where the association was initially described. We are extending these findings to a larger sample (N=1000). If confirmed, these findings will provide important clues to the allelic architecture of the FTO-variation effect on weight, potentially indicating alternative functional elements. Our data at this stage of the analysis provide further evidence that variation in FTO is associated with obesity. Our signal localises the effect in intron 3 and thus indicates that either different polymorphisms in FTO may influence obesity in various ethnic groups or that the causal SNP has not been discovered yet.

2483/F

Endophenotype Analysis in Migraine. *N.J. Colson, R.A. Lea, L.R. Griffiths.* Genomics Research Centre, Griffith University, Gold Coast, Qld, Australia.

Migraine is a common complex polygenic disorder demonstrating genetic and clinical heterogeneity. Numerous modest effect common genetic variants appear to be involved in migraine susceptibility. This study considered the hypothesis that the combined and interacting effect of these variants is a fundamental feature of migraine predisposition and its clinical heterogeneity. To test this, we analysed several previously identified migraine susceptibility variants in a large Australian migraine group to determine if specific genetic risk profiles associated with particular migraine sub-types, symptoms, and severity. The vascular genes under analysis were MTHFR, ACE, and MTRR. ESR1 and PGR were analysed as hormonal variants. 'Vascular risk' subjects possessed at least 2 susceptibility genotypes in the vascular genes. 'Hormonal risk' subjects possessed at least 2 susceptibility genotypes in the hormonal genes. 'No risk', subjects did not possess any risk genotypes. Of the 202 subjects, 26 had a complete 'hormonal risk' profile, 38 had a complete 'vascular risk' profile, 3 had both risk profiles and 4 were in the 'no risk' group. The remaining subjects did not fall into any category and were grouped as 'unclassified'. Examination of clinical data revealed that typical migraine symptoms were more likely in 'risk' subjects than 'no risk' subjects. Notably, subjects with both 'vascular risk' and 'hormonal risk' profiles all reported more severe migraine associated symptoms of nausea, phonophobia, photophobia, eye discomfort and pulsating head pain, and that their mother also suffered migraine. Severe migraine symptoms and a mother who suffered migraine were much less likely in the 'no risk' group. Of the subjects who suffered both MA and MO, 25 percent had a complete hormonal genetic risk profile even though just 8 percent of the total profiling group had a complete hormonal risk profile. This suggests that hormonal influences may be higher in subjects who suffer from both MA and MO. The development of polygenic endophenotypic risk profiles for migraine sufferers may be the next step in the process of unravelling the genetic basis of the disorder.

2484/F

A common polymorphism of STAT4 is associated with severe disease manifestations of systemic lupus erythematosus. *K.E. Taylor[1], W.A. Ortmann[2], A.T. Lee[3], E.F. Remmers[4], R.P. Plenge[5], S. Chung[1], J. Nititham[1], D.L. Kastner[4], M.F. Seldin[6], P.K. Gregersen[3], T.W. Behrens[2], L.A. Criswell[1].* 1) Division of Rheumatology, University of California, San Francisco, San Francisco, CA; 2) Genentech, Inc., South San Francisco, CA; 3) Feinstein Institute for Medical Research, North Shore L.I.J. Health System, Manhasset, NY; 4) National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD; 5) Broad Institute of Harvard and the Massachusetts Institute of Technology, Cambridge, MA; 6) University of California Davis, Davis, CA.

BACKGROUND: Systemic lupus erythematosus (SLE) is a genetically complex disease with heterogeneous clinical manifestations. A polymorphism in the STAT4 gene has recently been established as a risk factor for SLE and rheumatoid arthritis (RA), but the relationship with specific SLE subphenotypes has not been studied. METHODS: We studied 91 SNPs in the STAT4 region from the Illumina HumanHap550 genotyping array and clinical data from 2 independent Caucasian SLE case series (total=1026) and independent sets of controls (total=1373). We determined the most significant SNP for SLE risk and studied this SNP for association with specific SLE subphenotypes. To prevent possible type-I errors from population stratification, we reanalyzed the data using a subset of the combined groups determined to be most homogeneous based on EIGENSTRAT analysis. RESULTS: SNP rs7574865 was most strongly associated with SLE risk (MAF 30% cases, 23% controls), as seen previously for SLE and RA. This SNP is in a 70-kb moderate-high LD block; in conditional analysis rs7574865 explained all of the association within this block. Associations of this SNP with SLE characterized by double-stranded DNA (dsDNA) autoantibodies (OR=1.7, 95% CI [1.4-2.1], p=2.6e-7, MAF=34%) and severe nephritis (OR=2.1, 95% CI [1.3-3.4], p=0.001, MAF=39%) were striking. In contrast, STAT4 was less strongly associated with milder disease manifestations, such as oral ulcers (MAF=25%) and photosensitivity (MAF=28%). CONCLUSION: A common polymorphism of STAT4 contributes to the phenotypic heterogeneity of SLE, predisposing specifically to more severe disease.

2485/F

Extended identity of MHC SNP haplotypes is common and relates to type 1 diabetes risk. *E.E. Baschal, T.A. Aly, M.S. Fernando, M.M. Jahromi, S.R. Babu, M.J. Rewers, G.S. Eisenbarth.* Barbara Davis Center for Childhood Diabetes, University of Colorado at Denver and Health Sciences Center, Aurora, CO.

The extensive linkage disequilibrium throughout the MHC both confounds and aids efforts to identify diabetes-associated MHC loci (with analysis of extended conserved haplotypes). We analyzed 165 families enrolled in the DAISY prospective study supplemented with 72 HBDI families using a custom Illumina SNP panel of an extended MHC region (364 SNPs, 10.7Mb) and 751 families from the Type 1 Diabetes Genetics Consortium (T1DGC, 2918 SNPs, 4.6Mb (limited to the MHC)). In the T1DGC data, 850 case and 390 control parental chromosomes (AFBAC) had typing for HLA-A, -B, -DR. Within this dataset, 782 chromosomes were represented by 43 haplotype groups, each containing at least 5 chromosomes with identical HLA-A, -B, -DR alleles. The most common HLA haplotypes were: A1 B8 DR3 (190), A2 B15 DR4 (80), A2 B44 DR4 (59), A2 B8 DR3 (34), A2 B40 DR4 (29), A3 B7 DR2 (23), and A30 B18 DR3 (22). Each of these haplotypes had at least two chromosomes with stretches of greater than 99% SNP identity (>3Mb). Of the 43 haplotype groups, 7 were over-represented in cases ($p=0.04$ to 10^{-7}) and 6 were under-represented ($p=0.04$ to 10^{-11}). Using a custom panel with SNPs extending 6Mb telomeric of the MHC, we found that the A1, B8, DR3 (8.1) extended haplotype exhibited greater than 99% identity for 3 to 9 million nucleotides. We identified a significant SNP (rs1233478, $p=10^{-6}$), 46kb telomeric of the UBD gene (telomeric of the MHC), using case-control AFBAC analysis. We replicated this finding in the T1DGC data ($p=10^{-15}$, genotype OR=4). A 5-SNP haplotype with rs1233478 was overtransmitted to cases ($p=10^{-13}$). In a combined analysis of only the 8.1 haplotypes in the DAISY/HBDI and T1DGC data (which fixes the entire MHC and all HLA alleles), rs1233478 was still significant ($p=0.01$). Additionally, rs1233478 was statistically significant with logistic regression using HLA-A, -B and -DR alleles as explanatory variables. This SNP analysis demonstrates the remarkable long-range identity of multiple MHC diabetes-associated haplotypes, and identifies a haplotype near the UBD gene associated with additional diabetes risk.

2486/F

Combinatorial analysis of loci on chromosomes 7, 9, and 17p supports individual and interactive contributions to vitiligo susceptibility. *Y. Jin, P.R. Fain, R.A. Spritz.* Hum Med Genet Prog, Univ Colorado Hlth Sci Ctr, Aurora, CO.

Generalized vitiligo is a common, multifactorial, polygenic disease in which autoimmune loss of melanocytes results in depigmented spots of skin and overlying hair, with frequent co-occurrence of other autoimmune diseases. By genetic linkage analysis we previously mapped common vitiligo/autoimmunity loci to chromosomes 7, 9, and 17p, and recently identified the 17p gene as *NALP1*, with two high-risk loci within the gene. By genetic linkage analysis and pedigree-based association analysis of 114 multiplex families with vitiligo and associated autoimmune diseases, we have identified high-risk SNPs on chromosomes 7 and 9, though the corresponding genes are not yet known. Whereas classical linkage and association analyses can detect individual genetic locus that contribute to disease risk, deeper insights into complex patterns of inheritance can be obtained by simultaneous analysis of multiple susceptibility loci. We have carried out stepwise-conditional logistic regression analysis of the four vitiligo susceptibility loci, two in *NALP1* and one each in chromosomes 7 and 9, in cases and pseudocontrols derived from these 114 families, to analyze the individual and interactive contributions of the four loci to disease risk. We detected significant main effects for all four loci, and furthermore found that the full four-locus interaction model fitted significantly better than any of the nested three-locus interaction models. These results suggest that each of the two risk variants within *NALP1*, as well as each of the risk variants on chromosome 7 and chromosome 9, contributes to disease via both locus-specific effects and interactions with the other loci. Our findings illustrate how combinatorial analysis of multiple loci can help elucidate the complex gene interactions that contribute to susceptibility to complex diseases.

2487/F

Genome-wide association study identifies histamine receptor 4 as a novel Crohn Disease gene. *A.A. Mitchell, L. Mayer, L. Ozelius, M.T. Abreu, R.J. Desnick, NY Crohn Study Group.* Mount Sinai School of Medicine, New York, NY.

Crohn Disease (CD) is an inflammatory bowel syndrome that is more frequent among individuals of Ashkenazi Jewish (AJ) ancestry than among non-Jewish Caucasians (NJ). CD is multifactorial, requiring both environmental triggers and predisposing genetic variants. The strongest known genetic risk factors are three coding variants in the CARD15 (NOD2) gene. CARD15 population attributable risk is similar for AJ and NJ, indicating that it is unlikely to be responsible for the higher rate of CD in AJ. In the past year, genome-wide association studies of CD have implicated several new genes, including IL23R and ATG16L1.

To identify additional CD-related genes, we conducted a genome-wide association study of 113 unrelated AJ CD patients and 115 unrelated AJ controls using Affymetrix 500K Mapping Arrays. As expected, CARD15 had the strongest signal, with two SNPs at $p < 10^{-6}$ and six SNPs at $10^{-6} < p < 10^{-4}$. Consistent with previous findings, the minor allele at G1142A in IL23R was associated with protection from CD in this sample ($p = 2.6 \times 10^{-5}$, OR = 0.10). Interestingly, the protective allele is more common among AJ controls than NJ controls and less common among AJ cases than NJ cases. Thus, G1142A does not explain the increased incidence of CD in AJ.

Because CD patients without CARD15 mutations may carry risk alleles in other genes, the analysis was repeated, comparing CARD15 non-carriers (n=59) to controls. The strongest signal mapped to a 90 kb region near the histamine receptor 4 gene (HRH4), with one SNP at $p = 10^{-5}$ and seven SNPs at $10^{-5} < p < 10^{-3}$. The minor allele at each of the eight SNPs was associated with reduced risk.

HRH4 is a G protein-coupled receptor that is primarily expressed on immune cells. HRH4 antagonists modulate cytokine production and have anti-inflammatory effects *in vitro* and *in vivo*. Functional studies to determine the role of HRH4 in CD and to characterize the putative protective variants have been initiated. A replication study of 500 CD cases and 500 controls, equally divided between AJ and NJ, is underway.

2488/F

Two adjacent loci on rat chromosome 1 regulate LPS-induced TNF and IL-6 production, experimental encephalomyelitis and arthritis. *R. Nohra[1], A. Beyeen[1], J. Ping Guo[2], O. Isacsson[1], T. Olsson[1], J. Lorentzen[2], M. Jagodic[1], E. Wallström[1].* 1) Karolinska Institutet,Department of Clinical Neuroscience, Neuroimmunology Unit, CMM L8:04, Stockholm, Sweden; 2) Karolinska Institutet,Department of Medicine, CMM L8:04, Stockholm, Sweden.

A genome-wide linkage analysis previously performed in an F2 cross between the experimental autoimmune encephalomyelitis (EAE)-susceptible LEW.AV1 and the MHC identical but EAE-resistant PVG.AV1 rat strains identified a quantitative trait locus(QTL) on rat chromosome 1 regulating LPS-induced production of proinflammatory cytokines. Using a congenic line between these two rat strains, we confirmed a role of this locus in EAE and in the production of proinflammatory cytokines. Further mapping was performed in a G10 advanced intercross line between the EAE-susceptible DA (AV1) strain and the PVG.AV1 strain. The same region was also analyzed in pristane-induced arthritis (PIA) and LPS-induced IL-6 and TNF production in G12(DAxPVG.AV1) rats. The initial QTL was resolved into two loci. The first locus regulates EAE and overlaps a QTL that regulates IL-6 production. This locus harbours approximately 8 genes, including genes playing a role in neuronal development, axonal growth and spinal cord injury. The second locus regulates EAE and overlaps a QTL that regulates PIA and TNF production. We were thus able to define two adjacent QTLs in the rat that regulate encephalomyelitis, arthritis and proinflammatory cytokine production. Sequencing and expression analysis of candidate genes are ongoing.

2489/F

Linkage disequilibrium mapping of a susceptibility gene for Kawasaki disease. *Y. Onouchi[1,2], T. Gunji[1,2], J.C. Burns[3], C. Shimizu[3], J.W. Newburger[4], T. Kawasaki[5], Y. Nakamura[6], A. Hata[1,7].* 1) Lab. Gastrointestinal Diseases, SNP Research Center, RIKEN, Yokohama, Japan; 2) Dept. Hard Tissue Engineering, Graduate School, Tokyo Medical and Dental Univ., Tokyo, Japan; 3) Dept. Pediatrics, Univ. California San Diego, School of Medicine, La Jolla, CA; 4) Dept. Cardiology, Boston Children's Hospital, Boston, MA; 5) Japan Kawasaki Disease Research Center, Tokyo, Japan; 6) Lab. Molecular Medicine, Human Genome Center, Institute of Medical Science, the Univ. of Tokyo, Tokyo, Japan; 7) Dept. Public Health, Graduate School of Medicine, Chiba Univ., Chiba, Japan.

Kawasaki disease (KD) is an acute systemic vasculitis syndrome of infants and young children. Although its etiology is largely unknown, genetic factors are considered to play a significant role in the pathogenesis of KD. Our previously performed sib pair linkage study identified signals of linkage in 10 chromosomal regions (MLS 1.14 - 2.69). In one region, we identified a SNP significantly associated with KD by linkage disequilibrium (LD) mapping (637 KD v. s. 1034 control; OR=1.89, 95%CI 1.53-2.33, P=2.2x10^{-9} in dominant model). Association with KD was replicated in 209 U.S. KD patients (T:U=64:30, OR=2.13, 95%CI 1.38-3.29, P= 0.00045 by TDT). Furthermore the SNP was associated with formation of coronary artery lesions (OR=2.05, 95%CI 1.37-3.08, P=0.00044 in Japanese, OR=3.36, 95%CI 1.72-6.59, P=0.00018 in the U.S.) and with resistance to intravenous gamma globulin therapy (OR=4.67, 95% CI 1.34-16.24, P=0.0076 in the U.S.). The SNP was located in intron 1 of gene X and in vitro analysis using minigene revealed that the susceptibility allele of the SNP reduces splicing efficiency. Allele specific transcript quantification analysis showed a consistent result that the amount of the transcripts in PBMCs from the susceptibility allele was less than that of non-susceptibility allele of the gene. Interestingly, knockdown and over-expression of the gene enhances and represses the IL-2 production in stimulated Jurkat cells. The gene can be considered as a negative regulator of T-cell activation and the SNP might play a role in immune hyper-reactivity in KD by suppressing the regulating mechanism.

2490/F

Solute Carrier Family 11 member 1 linking: Infections, Autoimmunity and Cancer? *A. Awomoyi.* Microbiology & Immunology, Univeristy of Maryland Baltimore, Baltimore, MD.

Slc11a1 encodes an integral membrane protein, expressed on endosomal/lysosomal compartment of MØs and PMNs. Slc11a1 exacts pleiotropic effects on MØ function; enhanced KC, TNF- α, IL -1β , iNOS & MHC class II expression; important in induction and maintenance of autoimmunity and cancer but essential for resistance to pathogens. Slc11a1 delivers bivalent metal cations from cytosol into acidic late endosomal/lysosomal compartment by generating toxic antimicrobial radicals for direct antimicrobial activity against phagocytosed organisms. Prolonged accumulation of toxic radicals can have detrimental effects causing damage and contribute to numerous diseases. SLC11A1 associations with infections, autoimmunity and cancer are with a 5' Z- DNA repeat polymorphism. 5'UTR SLC11A1 genomic region analysis in mice and humans reveal differences between species in TF binding sites. An ATF-3 binding site, adjacent to this Z-DNA repeat, present in humans is absent in mouse. Genetic differences exist at SLC11A1 locus. SLC11A1 ATF-3 putative motif and Z-DNA promoter repeat are interrupted by mutations. My hypothesis is that homodimer ATF-3 upon binding to this motif in SLC11A1, should repress transcriptional activation of SLC11A1. I will test whether epigenetic & genetic differences at SLC11A1 locus result in altered susceptibility to diseases, disorders and therapy. Carriage of major slc11a1 allele promotes Th1-type response to vaccination whereas minor allele promotes Th2-type response. Effect of SLC11A1 alleles on immune responses could impact on vaccine delivery and efficacy. This study should provide an understanding of the mechanisms by which SLC11A1 might affect the outcome of infections, disorders, therapy and aging.

2491/F

Mutation and functional analysis of the *IRAK-M* gene in Sardinian asthmatic patients. *S. Naitza[1], L. Balaci[1], M.C. Spada[1], N. Olla[1], G. Sole[1], F. Anedda[1], M.A. Zuncheddu[1], A. Maschio[1], C. Caria[1], S. Sanna[1], S. Pilia[1], S. Sanna[1], L. Crisponi[1], G. Malerba[2], P.F. Pignatti[2], D. Schlessinger[3], A. Cao[1], M. Uda[1].* 1) Istituto di Neurogenetica e Neurofarmacologia (INN), CNR, Monserrato , Cagliari, Italy; 2) Dipartimento Materno Infantile e Biologia-Genetica, Sezione di Biologia e Genetica, Università di Verona, Verona, Italy; 3) Laboratory of Genetics, National Institute on Aging, Baltimore, MD, USA.

Asthma is a multifactorial disease influenced by genetic and environmental factors. Its prevalence in industrialized countries is now 5% and growing, with increasing associated mortality. Interest in finding etiologic factors has correspondingly intensified. To understand the genetic basis of asthma, we are conducting linkage and association analyses in the Sardinian founder population, where limited heterogeneity of pathogenetic alleles for monogenic and complex disorders as well as of environmental conditions facilitates the study of multifactorial traits. Analysing a cohort of affected Sardinian subjects, we have recently identified *IRAK-M* as a new asthma susceptibility gene in the candidate region 12q13-24. This gene, which we found associated with early-onset persistent asthma, is a negative regulator of the Toll-like receptor/ IL-1 receptor pathways and a master regulator of NF-κB and inflammation. We showed that IRAK-M is highly expressed in lung epithelial cells, suggesting a mechanistic link between hyperactivation of the innate immune system and chronic airway inflammation, and indicating IRAK-M as a potential target for therapeutic intervention against asthma. To better understand the pathogenetic mechanisms of *IRAK-M* variants in asthma, we sequenced all the coding as well as the non-coding regulatory regions of this gene in the entire cohort of affected asthmatic subjects. We detected non-sense, missense and splicing mutations in conserved domains and tested functional significance of some *in vitro*. Furthermore, we studied *IRAK-M* gene expression in monocytes derived from individuals carrying the risk vs the protective *IRAK-M* haplotype.

2492/F

Complement C3 polymorphisms associated with Dense Deposit Disease. *M.A. Abrera-Abeleda[1,2], C. Nishimura[1], S. Sethi[3], P. Zipfel[4], S. Ramaswamy[5], G. Silvestri[6], G. Hageman[7], R.J.H. Smith[1,2,8].* 1) Dept Otolaryngology, Univ of Iowa, Iowa City, IA; 2) Genetics PhD Program, Univ of Iowa, Iowa City, IA; 3) Dept Lab Med and Pathology, The Mayo Clinic, Rochester MN; 4) Leibniz-Institute for Natural Products Research and Infection Biology,Jena, Germany; 5) Dept of Biochemistry, Univ of Iowa, Iowa City, IA; 6) Department of Ophthalmology, Queen's University, Belfast, UK; 7) Dept of Ophthalmology , Univ of Iowa, Iowa City IA; 8) Dept of Internal Medicine, Univ of Iowa, Iowa City IA.

Dense Deposit Disease (or Membranoprofilerative Glomerulonephritis type II, DDD/MPGNII) is a rare cause of chronic renal dysfunction. Deficiency of Factor H (FH) in pigs and mice is associated with the development of DDD/MPGNII, suggesting that dysregulation of the alternative pathway of the complement cascade is important in its pathophysiology. Consistent with this hypothesis, we have shown that DDD/MPGNII is associated with specific polymorphisms of FH and Factor H-related 5. Because alternative pathway control is dependent on the interaction of complement proteins, we screened the coding regions and splice sites of C3, Factor B (FB), Factor I (FI) and Factor D (FD) for allele variants in 38 DDD/MPGNII patients and 103 controls. Our results showed a significant association of the R102G ($p<0.0007$) and L314P ($p<0.05$) polymorphisms of C3 with DDD/MPGNII. Both of these SNPs are located in the beta-chain of C3 and may affect the conformational structure of C3, which could change binding affinities for FH and FB, and expose novel epitopes of C3b, which may potentiate the formation of the DDD/MPGNII-specific autoantibody, C3NeF. In addition we identified an unreported missense mutation in one MPGNII/DDD patient. This K1203R change is located in the alpha-chain of C3 near to a FH and complement receptor 2 binding site, suggesting that it may affect the binding of the two proteins to C3. Several SNPs were identified in FB, FI and FD, but none was associated with the DDD/MPGNII. Our results suggest that DDD/MPGNII is a complex genetic disease and provide further evidence to implicate the alternative complement pathway in its pathogenesis. (Supported in part by NIH grant R01DK074409).

2493/F

Association of insertion-deletion polymorphism of the angiotensin-converting enzyme gene with rheumatoid arthritis. *M.Z. Haider[1], S.S. Uppal[2], G.S. Dhaunsi[1].* 1) Dept Pediatrics, Fac Medicine, Kuwait Univ, Safat, Kuwait; 2) Dept Medicine, Fac Medicine, Kuwait Univ, Safat, Kuwait.

Rheumatoid arthritis is a multifactorial disease in which environmental agents interact with genetic factors that influence susceptibility. Only 30% of the genetic contribution to RA can be attributed to HLA genes and it is suggested that other non-HLA genes may play a relevant role in RA susceptibility. Recently, Angiotensin converting enzyme (ACE), a key player in inflammatory signal transduction pathways, has been reported to be involved in pathogenesis of RA, and high levels of ACE have been documented in RA synovial fluid and RA pleural effusions. Plasma and tissue levels of ACE are regulated at the transcriptional level, we hypothesize that the genotype of ACE in RA patients may be a determining factor in the pathogenesis of this inflammatory disease. So far no studies have assessed this possibility. Sixty RA patients were recruited and clinically characterized according to disease duration, disease severity, disease activity and ACR functional class. Thirty five healthy controls (HC) were also enrolled in the study. ACE gene I/D polymorphism genotypes were determined in patients and HC. We found a significant over-representation of the DD genotype and the D allele in RA patients when compared to HC. Additionally, we also found that gender correlates significantly with genotypic and allele frequencies, with RA males exhibiting a higher frequency of the DD genotype and D allele compared to HC males. Furthermore, Arab patients show a higher frequency of D allele when compared to Arab HC. By logistic regression analysis the DD genotype confers a relative risk for development of RA of 3. Thus, our data indicate that the worst case scenario for the development of RA would be an Arab male with DD genotype. Our results also suggest a possible influence of the ACE gene on the RA disease activity, severity and functional class.

2494/F

Association of *FGF23* Genotype with Lower BMD at the Hip and Spine in the Old Order Amish. *J. Liu, D.J. McBride, B.D. Mitchell, E.A. Streeten, A.R. Shuldiner.* Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD.

As a novel phosphaturic hormone, fibroblast growth factor 23 (FGF23) is mainly produced in bone and affects bone mineral homeostasis by regulating phosphate and vitamin D metabolism. Rare mutations that increase FGF23 levels cause autosomal dominant hypophosphatemic rickets, while disorders associated with reductions in FGF23 are characterized by hyperphosphatemia, elevated production of 1, 25-dihydroxyvitamin D and hyperostosis. We thus hypothesized that common variants in *FGF23* might influence bone mineral density (BMD) and susceptibility to the common forms of osteoporosis. Five tagging SNPs were selected according to Hapmap and genotyped in individuals from the Amish Family Osteoporosis Study (AFOS) (n=1031). In addition, one SNP, rs12812339, was genotyped in a Mexican-American (Mex) cohort from the San Antonio Family Osteoporosis Study (n=857). Significant association between the promoter SNP rs12812339 (-1951C-->A) and BMD was found in the Amish. The frequency of minor allele C was 0.38. Subjects with C/C genotype had lower BMD at the femur and spine than those with C/A or A/A genotypes. In a dominant model, age-, sex- and BMI- adjusted P values at the hip were 0.003 for the intertrochanter, 0.0005 for the femoral neck, 0.006 for the trochanter, 0.001 for the total hip; for spine, 0.007. Narrow neck femur average buckling ratio(an indicator of bending strength) was also strongly associated with C/C genotype (P=0.00006). After stratifying by age or gender, the associations with BMD traits remained only in those men and women over age 50 years suggesting a role in bone loss rather than peak bone mass. The frequency of the C allele was lower in the Mex cohort (allele frequency=0.07). Although there was no statistically significant association with BMD, those few subjects with the C/C genotype tended to have lower hip and spine BMD. Our results showed that the C/C genotype of rs12812339 was strongly associated with decreased hip and spine BMD and with buckling ratio in the Amish. This promoter SNP may act by increasing FGF23 levels and accelerating the rate of bone loss.

2495/F

The Role of *FOXE1* in the Etiology of Cleft Lip. *J. Machida[1], L.M. Moreno[1], M.A. Mansilla[1], S.B. Bullard[1], T.D. Busch[1], M.K. Johnson[1], T. McHenry[3], M.E. Cooper[3], C. Valencia-Ramirez[4], M. Arcos-Burgos[2], A. Hing[5], E.J. Lammer[6], M. Jones[7], K. Christensen[8], J.C. Murray[1], M.L. Marazita[3], A.C. Lidral[1].* 1) U.Iowa,Iowa City,IA; 2) NIH,Bethesda,MD; 3) U.Pittsburgh,PA; 4) U de Antioquia,Colombia; 5) U.Washington,Seattle,WA; 6) Children's Hosp.Oakland,CA; 7) Children's Hosp.San Diego,CA; 8) U. Southern Denmark, Odense.

Cleft lip with or without cleft palate (CL/P) is a common birth defect of complex etiology. A series of genome wide studies have identified significant linkage to 9q21-q33. Our subsequent studies of candidate genes (*ROR2, BARX1, PTCH, FOXE1, TGFBR1 and ZNF189*) indicated that a 160Kb region around *FOXE1* is associated with CL/P. The **purpose** of this study is to fine map the *FOXE1* region and identify disease causing mutations. **Methods:** Families from Colombia, USA, Denmark, and the Philippines (77 extended, 481 trios; 34 extended, 256 trios; 571 trios; 307 trios respectively) were genotyped for 24 SNPs in the 160 Kb region. FBAT was used to test for association. UNPHASED was used to construct and test haplotypes for association. *FOXE1* and 7 conserved regions within 40kb 5' of *FOXE1* were sequenced on 92 and 24 affected individuals respectively. Bioinformatic tools were used to identify potential regulatory elements. **Results:** Significant association was found for 17/24 SNPs (lowest pvalue=0.000020) and haplotypes (pvalue=1.15e-07) in the *FOXE1* region in the Colombian families. Similar, but not as significant results were observed in the US, Danish and Filipino families. Sequencing identified 2 missense mutations out of 92 individuals. In addition, 4 of 9 newly detected conserved region variants are predicted to affect the transcription factor binding sites for Evi-1, Cap, CdxA, PBF, Dof1, E74A and Hb. However, all of these variants had MAFs<12% and occurred in both cases and controls. **Conclusions:** The present data supports the hypothesis that *FOXE1* has an important role in orofacial clefting. However, the lack of a common coding mutation suggests that nearby regulatory elements may be mutated in affected individuals having the common associated haplotype. NIH grants: R01-DE014667, K02-DE015291, P50-DE016215.

2496/F

Association of genetic variations in HTR2A gene with rheumatoid arthritis. *M. Seddighzadeh[1], A. Kling[2], L. Ärlestig[3], L. Alfredsson[4,5], S. Rantapää-Dahlqvist[3], L. Padyukov[1].* 1) Department of Medicine, Karolinska Institutet and Hospital, Stockholm, Sweden; 2) Division of Clinical Pharmacology, University Hospital, Umeå, Sweden; 3) Department of Public Health and Clinical Medicine, Rheumatology, University Hospital, Umeå, Sweden; 4) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 5) Stockholm Center for Public Health, Karolinska University Hospital, Stockholm, Sweden.

Background: There is wide evidence for a negative association between rheumatoid arthritis (RA) and schizophrenia. Furthermore, the serotonin receptor (HTR2A) has been demonstrated to have implications for the pathophysiology of schizophrenia. Therefore we found it relevant to investigate the association between the genetic polymorphisms within HTR2A gene and RA. Methods: The HTR2A gene polymorphisms were analysed in RA patients and controls from two Swedish cohorts using PCR based restriction endonuclease mapping or TaqMan allelic discrimination with more than 4000 individuals included in the current study. Results: At the discovery stage it was demonstrated that there is significant difference in the genotype frequency of rs6313 (T102C polymorphism) between the RA patients and controls (p=0.006). In the validation stage 6 more SNPs and extended number of samples was investigated. In this stage a trend in associations for SNPs rs6313, rs6314 and rs6311 (p=0.0088, 0.0074, 0.0069) was seen, although it was lost after correction for multi-comparison. However, haplotype frequency analysis based on these three SNPs showed significantly low representation of TTT combination in RA patients in comparison with controls (3.5% and 5.5%, p=0.0002 in Chi-square test, empirical p=0.0032 after 10 000 permutations). Conclusion: The present study demonstrates that there are genetic polymorphisms at HTR2A gene which are associated with susceptibility for RA suggesting possible links between the serotonergic system and development of the disease.

2497/F

Heritability and prevalence of migraine in the Norfolk Island population isolate. *H. Cox[1], C. Bellis[1], S. Quinlan[1], T. Dyer[2], J. Blangero[2], L. Griffiths[1].* 1) Genomics Research Centre, Griffith University, Gold Coast, Australia; 2) Southwest Foundation for Biomedical Research, San Antonio, TX, United States.

Studies have shown that the onset of migraine with and without aura is influenced by both genetic and environmental variables. Presently the type and number of susceptibility genes involved in both of these common forms of migraine are unknown. The objective of this study was to assess the prevalence and heritability of migraine in the population of Norfolk Island, a genetic isolate located several thousand kilometers off the eastern coast of Australia. Migraine was assessed by questionnaires and diagnosed using International Headache Society criterion. A total of 372 individuals with phenotypic data were assessed for migraine prevalence. These individuals comprise a complete pedigree of 6537 individuals dating back 11 generations to 12 maternal Tahitian and 6 paternal European founders. A total migraine prevalence of 23% was observed. Migraine with and without aura were reported to affect 13% and 10% of individuals, respectively. Interestingly 5% of males compared to 18% of females were affected. These results are comparable to estimates in out-bred populations, which have been reported to vary from 4-9% in males to 11-25% in females. Heritability estimates were generated using the statistical program SOLAR (v4.0.7). The heritability of migraine was significant (P<0.05) and calculated as 0.42. Heritability estimates for migraine have been reported to range from 34-57% in worldwide populations. This data supports the use of the Norfolk Island population as a potentially useful genetic isolate for gene mapping studies aimed at identifying migraine susceptibility genes.

2498/F

Is family history of osteoporosis associated with osteoporosis preventive behavior in US women? A population-based study. *J. Robitaille[1], P.W. Yoon[2], M. Irizarry-De La Cruz[2], T. Liu[2], C.A. Moore[2], M.J. Khoury[2].* 1) National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA; 2) National Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA.

Objectives: To assess the relationship between the prevalence of reported doctor-diagnosed osteoporosis and family history in a representative sample of women in the United States, examine whether this association can be explained by other risk factors for osteoporosis, and evaluate whether high-risk individuals based on familial risk are more likely to report preventive behaviors. **Research design and methods:** Prevalence of reported osteoporosis was estimated in a sample of 8073 women aged 18 years and over from the National Health and Nutrition Examination Survey (NHANES), 1999-2004. Respondents reported whether any of their 1st degree relatives and grandparents had ever been diagnosed with osteoporosis. **Results:** The overall prevalence of osteoporosis in women was 8.3%. A positive family history was reported in 19.8% of the participants and was significantly and independently associated with osteoporosis (OR, 95% CI: 2.50, 1.97-3.17). This association was stronger when participants reported having 2 or more affected relatives (OR, 95% CI: 8.31, 4.62-14.94). When stratified by age, the association between family history and osteoporosis was observed only in women aged 35 and over. Women with a positive family history of osteoporosis were more likely to report preventive behaviors such as taking a supplement containing calcium and/or vitamin D (OR, 95% CI: 1.42, 1.17-1.72), being physically active (OR, 95% CI: 1.24, 1.01-1.53) and using estrogen (OR, 95% CI: 1.24, 1.00-1.55) compared to women with no family history of osteoporosis. **Conclusion:** Findings from this study indicate that family history is a significant and independent risk factor for osteoporosis in US women aged 35 and over. In addition to general education campaigns to increase awareness about osteoporosis risk factors and prevention, public health efforts should help identify high-risk women who may benefit most from targeted prevention strategies.

2499/F

Characterization of susceptibility locus for preeclampsia on chromosome 2p25. *H. Peterson[1], H. Laivuori[2], K. Kivinen[3], E. Kerkelä[4], H. Jiao[1], V-V. Mäkelä[1], L. Hiltunen[5], R. Kaaja[6], O. Ylikorkala[6], V. Rasi[5], J. Kere[1].* 1) Dept of Biosciences and Nutrition, Karolinska Institute, Sweden; 2) HUSLAB Dept Clinical Genetics, Finland; 3) The Wellcome Trust Sanger Institute, Cambridge, UK; 4) Institute for Regenerative Medicine, University of Tampere, Finland; 5) Finnish Red Cross Blood Service, Finland; 6) Dept of Obstetrics and Gynecology, Helsinki University Central Hospital, Finland.

Preeclampsia is a pregnancy-specific, potentially life-threatening disease characterized by hypertension and proteinuria. Its cause remains unknown, but the epidemiology of preeclampsia suggests a partially genetic basis for the disorder. We have previously mapped three candidate susceptibility loci for preeclampsia on chromosomes 2p25, 4q32 and 9p13 (Laivuori et al. 2003) and verified linkage to the chromosome 2 locus by adding microsatellites at 1 cM intervals (NPL score 4.09, p= 0.00036). Our aim since has been to evaluate potential candidate genes within our linked regions as well as to assess other previously reported susceptibility loci for preeclampsia. A recent report suggested association of the STOX1 gene on chromosome 10q22.1 with preeclampsia in the Dutch population (van Dijk et al. 2005), but we were unable to validate STOX1 as a common preeclampsia susceptibility gene (Kivinen et al. 2007). Within our 1.4 Mb linkage region on 2p25, SNPs covering five genes were genotyped in a Finnish nationwide sample set consisting of 340 cases and 350 matched controls. Haploview was used to calculate single-marker and haplotype associations and three genes were selected for sequencing based on the association results and functional information. Sequencing of one gene in revealed 20 variations, five of which were selected for genotyping in the nationwide sample set and in a data set containing 100 cases and 100 controls from Finland. Unfortunately, association analyses were inconclusive and do not support strong genetic effect in this gene. However, we are underpowered to detect more common variants with weaker genetic effects and are in progress of collecting a nationwide data set with at least 1500 trios with affected mother and 1500 matched control trios.

2500/F

Optimal Control as a Tool for Drug Development. *S. Lai[1], H. Xiong[2], F.C. Arnett[3], X. Zhou[3], M.M. xiong[4].* 1) Department of Pathology, Michael E. DeBakey VA Medical Center and Baylor Collage of Medicine, Houston, TX; 2) Department of Computer Science, Texas A&M University, College Station, TX; 3) Department of Internal Medicine, University of Texas Health Science center at Houston, Houston, TX; 4) Human Genetic Center, University of Texas Health Science Center at Houston, Houston, TX.

It is increasingly recognized that understanding of biological processes and biochemical pathways at the systems-level will lead to "smarter" drug development. Model-based drug design is emerging as a new powerful tool for treatment and drug development. Model-based drug development requires mathematic models that take biological networks as a dynamic system and allow a detailed understanding of the drug mechanism of action, and strategies that optimize drug efficacy and minimizes its side effect. Mathematical models of dynamic systems and optimal control theory have been widely used in the engineering and industries for product design and plant control. The rapid development of systems biology is building momentum for application of the mathematical models and optimal control theory to the pharmaceutical industry. In this report, we propose to use state-space equations for modeling biological networks and apply EM algorithms and extended Kalman filter to the estimation of the parameters in the state-space model of the biological networks. We formulate the drug development as a multi-objective optimal nonlinear control problem and develop algorithms for solving constrained multi-objective optimal control problems. We apply the developed methods to the development of the treatment of Systemic sclerosis (SSc) that is a typical complex disease in which fibrosis occurs in multiple organs. The major source of fibrosis in SSc is over production of collagens from fibroblasts. We developed mathematical model for TGFB pathway that produces collagens. We also developed optimal control strategies which reduce the concentration of collagens to the normal level. The results are confirmed by the experiments of siRNA.

2501/F

A Proteomic Analysis of In vivo Circulating Monocytes in Chinese Pre-menopausal Females with Discordant Bone Mineral Density. *F.Y. Deng[1,3], Y.Z. Liu[3], C. Jiang[1], L.M. Li[1], S. Wu[1], Y. Chen[1], H. Jiang[1], F. Yang[1], P. Xiao[4], S.M. Xiao[1], L.J. Tan[1], X. Sun[1], J.X. Xiong[1], M.Y. Liu[1], S.F. Lei[1], X.D. Chen[1], J.Y. Xie[1], G. Xiao[1,2,5], S.P. Liang[1], H.W. Deng[1,3].* 1) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, P. R. China; 2) Key Laboratory of Protein Chemistry, College of Life Sciences, Hunan Normal University, P. R. China; 3) Departments of Orthopedics Surgery and Basic Medical Sciences, University of Missouri - Kansas City, Kansas City, Missouri, USA; 4) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University Medical Center, Omaha, NE, USA; 5) Department of Pediatrics, Harbor-University of California, Los Angeles, CA, USA.

Osteoporosis (OP) is a major public health problem and bone mineral density (BMD) is an important determinant of OP. Circulating monocytes (CMCs) may serve as progenitors of osteoclasts and produce a wide variety of factors important to bone metabolism. However, little is known about the specific roles of CMCs in the pathogenesis of OP. Our study sample was composed of a total of 42 otherwise healthy Chinese pre-menopausal females, with 21 having high BMD and 21 having low BMD (average Z score [SD]: +1.63[0.16] vs. -1.67[0.15]). CMC samples were extracted using a monocyte negative isolation kit, with purity high up to 90%. Proteomics techniques of 2-dimensional electrophoresis coupled with matrix assisted laser desorption and ionization - time of flight/time of flight mass spectrometry were used for comparative protein expression profiling of the CMCs between the two BMD subgroups. We screened out, identified, and verified with western blotting five proteins which were differentially expressed in the two subgroups of samples: RSU1, GSN, SOD2 (up-regulated in low BMD subgroup), and GPX1 and P4HB (down-regulated low BMD subgroup). These proteins might affect monocyte trans-endothelium, osteoclast differentiation, and/or osteoclast functions. Therefore, they probably contribute to differential osteoclastogenesis and BMD variation, thus be invovled in pathogenesis of human osteoporosis.

2502/F

Height as the exemplar polygenic trait: a genome-wide association study of 10,737 UK individuals reveals multiple loci of small effect. *M. Weedon[1], C. Lindgren[2], R. Freathy[1], C. Wallace[3], G. Lettre[4], D. Evans[5], M. Mangino[6], S. Stevens[6], A. Hall[6], N. Samani[6], W. Ouwehand[5], J. Hirschhorn[4], M. Caulfield[3], P. Munroe[3], A. Hattersley[1], M. McCarthy[2], T. Frayling[1], Cambridge GEM Consortium, The Height-Genetics Consortium.* 1) Peninsula Medical School, Exeter, UK; 2) University of Oxford, UK; 3) Barts and the London, UK; 4) Broad Institute, US; 5) University of Cambridge, UK; 6) Blood Services and University of Cambridge Common Controls, UK.

Human height is a classic polygenic trait but the genes responsible remain largely unknown. The recent and increasing availability of data from genome-wide association studies (GWAS) offers new opportunities to identify genes influencing height. These genes may provide important insights into how best to dissect the genetics of polygenic quantitative traits. Initial data from 4921 GWAS subjects, together with >29000 individuals in replication studies, show that rs1042725 of HMGA2 associates with height ($P<4x10^{-16}$). In the initial scan only HMGA2 had a $P < 5x10^{-7}$. To identify further genetic variants influencing adult height we extended our analyses to 10737 people from the WTCCC and an obesity case control study. We performed an inverse-variance meta-analysis of within-sex Z-score summary statistics for 432030 SNPs genotyped on the Affymetrix 500K chip. We identified eight independent signals at $P<5x10^{-7}$. Effect sizes ranged from ~0.53 to 0.65 cm per allele. The most strongly associated variant ($P=1x10^{-13}$) is located in ZBTB38. Combining information from these eight signals showed that they explained 3% of the variance of height, with a ~5cm difference in height between the 4% of people with < 5 height increasing variants compared to the 5% of people with > 10. Multiple lines of evidence, including principal components analysis, demonstrate that the associations are not due to population stratification. These results suggest that by analyzing GWAS data from many thousands of individuals, it will now be possible to dissect the genetics of this classic, highly heritable polygenic trait. Combining data from multiple GWAS is a powerful approach to identifiying polygenic variants.

2503/F

A genome-wide association scan identifies the hepatic cholesterol transporter ABCG5/ABCG8 as a susceptibility factor for human gallstone disease. *S. Buch[1,2,3], C. Schafmayer[3,4], H. Völzke[6], A. Franke[2], C. Becker, C. Kluck, I. Bäβmann[7,9], H. von Eller-Eberstein, B. Timm, C. Höll[3], M. Brosch[1], F. Lammert, J.F. Miquel[10], F. Nervi[11], M. Wittig, A. ElSharawy[2], J. Seeger[1], T. Lu[5], D. Rosskopf[12], J. Egberts, F. Fändrich[4], U.R. Fölsch[1], M. Krawczak[5], S. Schreiber[2,3], P. Nürnberg[7,8], J. Tepel[4], J. Hampe[1].* 1) Department of Medicine; 2) Institute for Clinical Molecular Biology; 3) POPGEN Biobank; 4) Department of General and Thoracic Surgery; 5) Institute of Medical Statistics and Informatics, <1-5> all at Univ. Hospital Schleswig-Holstein, Kiel / Germany; 6) Institute of Community Medicine, Univ. Hospital Greifswald / GER; 7) Cologne Center for Genomics, Univ. of Cologne / GER; 8) Center for Molecular Medicine Cologne (CMMC), Univ. of Cologne / GER; 9) RZPD,German Resource Center for Genome Research / GER; 10) Department of Internal Medicine I, Univ. Hospital Bonn / GER; 11) Depart. de Gastroenterologia, Facultad de Medicina Univ. Catolica, Santiago / Chile; 12) Institute of Pharmacology, Univ. Hospital of the Ernst Moritz Arndt Univ. Greifswald / Germany.

Cholelithiasis represents one of the most frequent health problems of industrialized countries. A 500K-association scan was performed in 280 gallstone cases and 360 controls. In a follow-up of 235 SNPs in 1105 cases and 873 controls, the disease association of SNP A-1791411 in the *ABCG8* gene was replicated (allelic $p=4.110^{-9}$). Fine-mapping of the ABCG5/ABCG8 locus, using both haplotype and logistic regression analysis, revealed that non-synonymous SNP rs11887534 in the *ABCG8* gene (D19H) was found to represent the major source of gallstone risk. Further replication was performed in 728 patients from Germany ($p=2.810^{-7}$) and in 167 patients from Chile (p=0.02). The odds ratio (OR) for D19H carriership in German Caucasians was 2.2 (95% CI 1.8-2.6). In a post-hoc logistic regression analysis, the genotypic effect was found to be independent of BMI, age and sex. Association with D19H carriership was stronger (OR=3.3) in patients with cholesterol gallstones, suggesting that 19H might be associated with a more efficient transport of cholesterol into the bile.

2504/F

High Density Association Mapping of IBD6. *C. Labbe[1,2], P. Goyette[1], C. Lefebvre[1], C. Stevens[3], T. Green[3], J. Stempak[4], S. Brant[5], R. Duerr[6], K. Taylor[7], J. Cho[8], H. Steinhart[4], M. Daly[3], M. Sylverberg[4], J.D. Rioux[1,2,3].* 1) Montreal Heart Institute; 2) Université de Montréal; 3) The Broad Institute of MIT and Harvard; 4) Mount Sinai Hospital IBD Center, University of Toronto; 5) Johns Hopkins University School of Medicine; 6) School of Medicine, University of Pittsburgh; 7) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles; 8) Yale University.

Crohn's disease and ulcerative colitis are known as the Inflammatory Bowel Disease(IBD). Variants at a few loci have been irrevocably associated with IBD.Additional genes are expected to be involved in disease susceptibility. Two of these loci(CARD15 and IBD5) were identified via association mapping of significant linkage regions. We have performed a genomewide linkage study of IBD families and identified a locus(IBD6) on 19p with genomewide significance(LOD score = 4.6). We were interested in identifying the causal variants located within this linked region. We have embarked upon a two-stage association mapping studies of the IBD6 region. In stage 1, 1530 tag SNPs were selected (using Tagger) from HapMap data phase I that would serve as proxies($r2\geq0.7$) for all other SNPs with a minimum minor allele frequency of 10%. These SNPs were genotyped on 433 trios and 328 cases/236 controls from Canada and Italy. We excluded individuals and families with low genotyping, excessive mendelian errors, and unexpected relatedness. We also excluded SNPs that had a low call rate. We performed association testing of the post-QC data and observed a higher number of associated SNPs(<0.001, 1; <0.005, 11; <0.01, 25; <0.05, 129) than would be predicted to occur at random indicating the presence of genes influencing the susceptibility to IBD. We are currently in a replication stage, where the most associated SNPs are being genotyped in an independent cohort of 4000 DNAs.We expect that this comprehensive approach will lead to the identification of a novel susceptibility locus and we will present our screening and replication results.These results as well as examination of gene-gene and gene-environment interactions will be integrated into a general risk model for susceptibility to IBD.

2505/F

Association of *NALP1* genotype with immune cell survival and autoimmune disease. *C.M. Mailloux[1], M.G. Netea[2,3], E.C. Lewis[2], Y. Jin[1], C.A. Dinarello[2], R.A. Spritz[1].* 1) Hum Med Genet Prog, Univ Colorado Hlth Sci Ctr, Aurora, CO; 2) Dept Medicine, Univ Colorado Hlth Sci Ctr, Aurora, CO; 3) Dept Medicine, Radboud Univ Nijmegen Med Ctr, Nijmegen, Netherlands.

Generalized vitiligo is a common, multifactorial, polygenic disease in which autoimmune loss of melanocytes results in depigmented spots of skin and overlying hair, often associated with other autoimmune disorders. We recently showed that vitiligo and co-occurring autoimmune diseases are associated with common high-risk genetic variants of *NALP1*. NALP1 is an NLR protein that is a key component of the inflammasome, binds the anti-apoptotic proteins Bcl-2 and Bcl-X_L, and regulates both the caspase-1 mediated inflammatory response and apoptotic pathways in response to pathogen-associated molecular patterns such as muramyl dipeptide. As some autoimmune diseases appear to involve defects of apoptosis, with elevated or prolonged expression of Bcl-2, we hypothesized that *NALP1* variants associated with vitiligo might result in defective apoptosis of immune cells. To test this hypothesis, we assayed survival in culture of unstimulated peripheral blood monocytes from patients with vitiligo-associated multiple autoimmune disease versus healthy controls. Overall, survival of monocytes from patients was significantly prolonged compared to monocytes from controls. Furthermore, this difference was directly related to carriage of high-risk *NALP1* genotypes, rather than to disease state; survival of monocytes from individuals carrying high-risk *NALP1* genotypes was significantly prolonged compared to monocytes from individuals with low-risk *NALP1* genotypes. Our findings provide a direct correlation between *NALP1* genotype and survival of immune cells that may contribute to the development of the autoimmune response.

2506/F

Pooling-based Genomewide Association Study Identifies Loci for Systemic Lupus Erythematosus. *T. Tahira[1], M. Masumoto[1], Y. Kukita[1], T. Horiuchi[2], K. Hayashi[1].* 1) Res Ctr Genetic Info, Med Inst Bioreg, Kyushu Univ. Fukuoka, Japan; 2) Med. and Biosys. Science, Grad. School of Med. Sciences, Kyushu Univ. Fukuoka, Japan.

A genetic predisposition has been implicated in the occurrence of systemic lupus erythematosus (SLE). IRF5 gene has been shown to strongly associate with the disease among Europeans. We confirmed this association in Asians, but the risk conferred by this variant is smaller in Asians compared to those in Europeans, due to lower frequency of the responsible allele. To identify other susceptibility genes, we carried out a genomewide screening by microarray genotyping analysis of pooled DNA of Japanese. Two case pools (n=264; n=183) and three control pools (n=426; n =253; n=432) were genotyped each for three times by Affymetrix 500K chip. The averaged median intensity signals for perfect-match probes were used to evaluate the allele frequency difference between cases and controls. Relative Allele Signal score (RAS) was used as quantitative major instead of absolute allele frequency, and the values for sense (RAS1) and antisense (RAS2) directions were independently evaluated. We calculated z-scores from the RAS values between case and control, and then evaluated them using a median score at sliding window sizes of 3, 5, 10, 20, and 40 SNPs. We selected 39 SNPs as candidate from the windows that showed high score. Quantitative PCR-SSCP analysis using the same set of pooled samples confirmed the association ($P < 10^{-4}$) for 12 SNPs in three regions. The strongest association was found for a SNP (rs17634369) in the intergenic region 23 kb upstream of IKZF1 (Ikaros) on chromosome 7p. This association was confirmed by individual genotyping of cases (n = 445) and controls (n = 679). Allelic OR was 1.56 (95% CI 1.32-1.84) and P-value was 1.5×10^{-7}. Ikaros proteins are zinc finger transcription factors that are considered master regulators of lymphocyte differentiation and its role in SLE pathogenesis indicated by this study should help understanding the disease mechanism. Association study using other Asian populations is in progress.

2507/W

Depletion Analysis of Ultraconserved Elements among Copy Number Variants. *C.W.K. Chiang[1], A. Derti[2], J.N. Hirschhorn[1,3,4], C.-t. Wu[5].* 1) Dept of Genetics; 2) Dept of Biol. Chemistry and Mol. Pharmacology, Harvard Medical School, Boston, MA; 3) Div of Genetics and Endocrinology, Children's Hospital, Boston, MA; 4) Broad Institute, Cambridge, MA; 5) Div of Genetics and Molecular Medicine, Harvard Medical School, Boston MA.

Alignment of the human, mouse, and rat genomes identified 481 sequences that are ≥ 200 bp in length and 100% conserved (Bejerano et al. 2004). This set of ultraconserved elements (UCEs) was later expanded to 896 elements by adding human-dog-mouse and human-chicken UCEs (Derti et al. 2006). UCEs are hypothesized to be functional, given the apparent negative selective pressure estimated for UCEs (Chen et al. 2007), and the assumption that conservation is indicative of function (Bejerano et al. 2004, Drake et al. 2005). Recently, Derti et al. (2006) reported UCEs to be depleted from segmental duplications and copy number variants (CNVs), suggesting that UCEs may be dosage sensitive. This dosage sensitivity was further proposed to be at least partly responsible for the uniqueness and ultraconservation of UCEs. Here we expand on the study by Derti et al. (2006) by analyzing several published genome-wide CNV datasets obtained from normal subjects and patients with cancer, autism, or mental retardation. Depletions of UCEs, especially of the intronic and intergenic subclasses, were observed (P ranging from < 0.0003-0.02) in several, although not all, datasets. Variability in depletion may reflect technical challenges in determining the extent of CNVs, which may improve with the use of the new Affymetrix GenomeWide 6.0 array. The variability in depletion may also be due to differences between the CNVs of patients as versus healthy individuals, differences between common as versus rare CNVs, and/or other biologically-driven features. To begin addressing these issues, we are expanding on the diversity of datasets we analyze, especially those associated with cancer, as well as generating *in silico* sets of recurrent CNVs based on published datasets. We anticipate these studies to clarify the relationships between UCEs and CNVs, and their potential implication to human variations and diseases.

2508/W

CNV INTEGRATOR: A new automated tool for processing, annotating and visualizing Copy Number Variations. *R. Goya, A. Hidalgo, L. Uribe, J. Fernandez, G. Jimenez-Sanchez.* National Institute of Genomic Medicine, Mexico.

Copy number variation (CNV) represents an important source of variation in the genome. Copy number analysis has become of key importance in biology and medicine. Current technology allows high-throughput generation of CNV data. However, definition of basic statistics derived from large amount of samples, such as determination of minimal overlapping regions, CNV prevalence and mining of gene content in the regions is a time consuming task. To facilitate CNV analysis, we have generated CNV INTEGRATOR, a new automated mining tool that uses text tables containing array probe identifiers, their physical position in the human genome and CNV data. We analyzed CNVs in 300 individuals from the Mexican Genome Diversity Project genotyped with the 500K SNP genotyping set from Affymetrix. Copy number was evaluated using Affymetrix's CNAT 4.0.1 and DChip. A set of 30 randomly selected females was used as a reference set in each case. Text tables with copy number information from all the samples were exported from both platforms and processed using CNV INTEGRATOR. This tool consists of 1) a mysql database condensing CNV data; 2) a Perl-based pipeline to process the data, and 3) a web front-end using PHP for user interaction. The database is screened for continuous sets of altered SNPs with changes in copy number, grouping them into regions, defining the minimum regions of overlap and indicating the prevalence of these changes. This process concludes with a web-based report containing the total number of CNVs, percentages of deletions and amplifications, average number of changes per sample, the shortest and longest alteration and a summary table displaying all changes above a user-determined threshold. The report also includes a graphical representation of the overlapping regions, their sizes, and a list of genes they contain and SNPs involved. In addition, it includes pertinent links to genome browsers. To increase the CNV resolution in our population, we are currently including data generated with the Illumina 550K array from the same sample. In summary, this new bioinformatic tool will improve our ability for CNV data mining using data from different sources.

2509/W

Improved specific activity of random primer DNA-labeling by optimizing the Cy-dCTP/dCTP ratio. *J.W. Ling, B. Tazon-Vega, C. Zhang, K.P. Xu.* PGD laboratory, Center for Reproductive Medicine and Infertivity, Weill Cornell Medical College, New York, NY.

To date the random primer labeling method has generally been used for array-CGH with Cy-dCTP incorporated into DNA competing with dCTP. Previous studies suggested that specific activity (SA, defined as [amount of target DNA (ng) x 1000] / [dye incorporated (pmole) x 324.5]) of 1 incorporated dye per 25 to 50 nucleotides was optimal for microarray hybridization. Only a SA of about 70 could be achieved with the commonly used 1:1 ratio of Cy-dCTP/dCTP in our preliminary experiments. We tested whether altering the Cy-dCTP/dCTP ratio could increase the labeling quality to the optimal range. Five different Cy-dCTP/dCTP ratios (1:1, 2:1, 3:1, 5:1 and 10:1) were tested with 10 samples in each group. The total amount of Cy3/5-dCTP and dCTP in every group was equivalent to each of the other three dNTPs in the reaction. For each group 4μg of human genomic DNA were labeled by the Bioprime DNA Labeling System using Cy3- or Cy5-dCTP and Exo-Klenow. Unincorporated nucleotides were removed by filtration and the labeling quality was determined by the SA of labeled DNA using a spectrophotometer (ND-1000, NanoDrop Technologies). The average SA of Cy3 labeling for each group was 70.9±7.8, 70.9±6.7, 23.6±1.9, 23.0±1.6, 18.1±1.3; and 76.2±2.1, 74.1±1.6, 36.8±0.8, 35.2±0.5, 33.8±1.4 for Cy5. For both dyes the labeling efficiency increases with the amount of Cy-dCTP in the reaction but not proportionally. The lower incorporation capacity of Cy-dCTP requires a much higher proportion to increase the labeling efficiency. However when the ratio increases to a certain extent the double strand DNA becomes unstable by the dye, preventing further increase of incorporation. Our data showed that a 3:1 ratio may be optimal for both Cy3- and Cy5- DNA labeling achieving the SA of 23 and 37 respectively. Further investigation should be performed to test the quality of this labeled DNA for arra-CGH.

2510/W

A Bayesian chromosome peeling algorithm to detect gemonic DNA copy number variations in array CGH data. *L.Y. Wu[1], K.S. Wang[2].* 1) Dept of Statistics, University of Waterloo, Waterloo, ON, Canada; 2) Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Canada.

ArrayCGH is a high-throughput technology to generate genomic DNA copy number profiles. It plays an important role in cancer research and diagnosis (Feuk et al., Nature Review Genetics 7, 85-97). We present a Bayesian chromosome peeling algorithm to detect DNA copy number variations in profiles generated by arrayCGH. The algorithm implements a flexible mean-variance shift model for chromosomal segments with different copy numbers. A peeling procedure equipped with Bayes factor was employed to estimate segments boundaries. Compared with current leading methods, it produces comparable accuracies in detecting copy number gain and loss. Furthermore, it has the advantages of ranking chromosomal segments and identifying influential observations, i.e. outliers. The algorithm is also capable of detecting copy number gain or loss at either whole chromosome level or at single probe level. We adopted a data-driven approach to choose hyperparameters in prior distributions, thus minimize the impact of the user-controlled tuning parameters that can be problematic (Lai et al. 2005 Bioinformatics). The algorithm is computationally efficient with complexity of O(n), where n is the number of probes on the chromosome. We illustrate the algorithm with real data analysis and simulation studies.

2511/W

Copy number variability in patients with Pelizaeus-Merzbacher disease. *G. Hobson[1,2], J. Garbern[3], K. Woodward[4], Y. Wu[5], M. Maisenbacher[6], M. Henneke[7], L. Banser[1], K. Sperle[1].* 1) Nemours Biomedical Research, duPont Hosp Children, Wilmington, DE; 2) Thomas Jefferson University, Philadelphia, PA; 3) Wayne State University, Detroit, MI; 4) Western Diagnostic Pathology, Perth, WA, Australia; 5) UCSF, San Francisco, CA; 6) University of Florida, Gainesville, FL; 7) University of Gottingen, Gottingen, Germany.

Duplication of a genomic region of Xq22 that includes the proteolipid protein 1 gene (*PLP1*) is the most common mutation causing Pelizaeus-Merzbacher disease (PMD), an X-linked dysmyelinating leukodystrophy. The duplications in PMD are heterogeneous in size and in location of breakpoints. They arise most frequently by a grandpaternal intrachromosomal event and have a tandem head-to-tail orientation. Our data on 59 PMD cases suggested a coupled homologous and nonhomologous mechanism for formation of the duplications in PMD. This is in contrast to other genomic disorders where recurrent rearrangements are formed by nonallelic homologous recombination. Further, we reported that patients with three or more copies of *PLP1* have a more severe phenotype than those with duplication. We have examined copy number variability in patients with PMD in the genomic region around *PLP1* by X-chromosome oligonucleotide array CGH and by semiquantitative multiplex PCR. Five patients have three copies of a genomic region that includes *PLP1*. These patients also have duplicated regions in addition to the triplicated regions. Three other patients have higher copy number regions that do not include *PLP1*, while their *PLP1* gene is duplicated. One of these patients has a classic PMD presentation like that of most *PLP1* duplication patients; one has a severe phenotype like that of patients with triplication of *PLP1*; and the third has a severe connatal phenotype. Although the locations of the proximal ends of the higher copy number regions are variable, the distal ends are all within a 200 Mb low copy repeat region (LCR) distal of *PLP1*, suggesting involvement of this LCR region in formation of these complex rearrangements. Our data underscore the importance of genome architecture in the region around *PLP1* in the formation of complex genomic rearrangements.

2512/W

Genome-wide array-based comparative genomic hybridization (array-CGH) analysis in Aicardi Syndrome. *X. Wang[1], V.R. Sutton[2], T. Eble[1], C. O'Neill[2], R.A. Lewis[2,3], I.B. Van den Veyver[1,2].* 1) Dept of Obstetrics and Gynecology; 2) Dept of Molecular and Human Genetics; 3) Dept of Ophthalmology, Baylor College of Medicine, Houston, Texas 77030, USA.

Aicardi syndrome is characterized by agenesis of the corpus callosum, chorioretinal lacunae, severe seizures (starting as infantile spasms), neuronal migration defects, mental retardation, costovertebral defects, and typical facial features.

Because Aicardi syndrome is sporadic and affects only females or rarely 47,XXY males, it is thought to be caused by *de novo* dominant mutations in an X-linked gene, but an autosomal mutation with sex-limited effects cannot be excluded. Because genetic linkage approaches to map the gene for Aicardi syndrome cannot be used, we performed high-resolution array-CGH analysis with DNA samples of subjects with Aicardi syndrome to search for segments of copy number loss or gain that may contain the mutated gene. Genomic DNA of female subjects with Aicardi syndrome and reference female DNA were labeled differentially with Cy5 and Cy3, and co-hybridized onto human whole-genome 185k or 244k oligonucleotide DNA arrays (Agilent Technologies). After slides were scanned and feature extraction performed, results were visualized and analyzed with Agilent's CGH analytics software and displayed as log2 ratios with these settings: 1-fold cut-off, ADM-2 aberration algorithm with threshold 10.0.

To date, we have tested 16 DNA samples from well-characterized females with Aicardi syndrome on the 185K array and 28 on the 244K array. We found between 7-21 copy number gains or losses per subject. There were a total of 146 unique copy number changes across the entire genome in the 44 studied samples. Of these, 124 were previously annotated copy number variants, 6 were also found in unrelated array-CGH hybridizations for other conditions or controls, and 16 (15 autosomal and 1 X chromosomal) have not been seen before. These are currently being confirmed and studied on parental DNAs, as they may represent candidate regions for the Aicardi syndrome genes.

2513/W

Mapping copy-number variation at high resolution and determining the exact breakpoint sequence by a combination of high-resolution CGH (HR-CGH) and vectorette-PCR. *F. Grubert[1], A.E. Urban[1], J.O. Korbel[1], M. Kasowski[1], R. Haraksingh[1], J. Korenberg[2], B.S. Emanuel[3], M. Gerstein[1], M. Snyder[1], S.M. Weissman[1].* 1) Yale University, New Haven, CT; 2) Mount Sinai Hospital, Los Angeles,CA; 3) Children's Hospital of Philadelphia, PA.

Copy-Number Variation (CNV) and Copy-Number Polymorphisms (CNP) are being found to be a pervasive architectural feature of the human genome and are expected to contribute significantly to phenotypic variation both in the healthy individual and in disease states. Array-CGH, the dominant methodology to determine CNV, has a typical predictive resolution of about 50 kb. CNV below that horizon will be missed as will be the actual breakpoint-sequence of the variant or aberration. We have developed HR-CGH based on high-density oligonucleotide tiling microarrays [Urban, Korbel et al. PNAS 2006; Korbel, Urban et al. PNAS 2007]. Maskless Synthesis arrays with 385 000 oligomers are constructed to represent the non-repetitive part of the genomic sequence of entire chromosomes at a tiling density of typically 1 oligomer/~100bp or better. The ratio of signal intensities from control and experimental channel are processed by our /BreakPtr/ algorithm, which predicts copy-number changes and their dosages and breakpoints while screening out false-positive calls caused by cross-hybridization. Predictions are validated with vectorette-PCR and direct sequencing of the resulting amplicons, making cloning superfluous. Using this approach we have studied CNVs, CNPs and aberrations, and their breakpoints, using oligonucleotide tiling arrays representing chromosomes 21, 22 and X, respectively. We probed the corresponding arrays with samples from probands with Down Syndrome and partial trisomy 21, Velo-Cardio-Facial Syndrome (VCFS) and from the HapMap panel. We have detected, cataloged and verified variations from 3 Mb to smaller than 1kb in size. In several cases we were already successful in determining the exact breakpoint sequence.

2514/W

R-pipeline for the robust analysis of arrayCGH data. *A. Pearlman, S. Cohen, Y. Kluger, H. Ostrer.* Dept Pediatrics, New York Univ Sch Medicine, New York, NY.

Array CGH has emerged as a very useful tool for identifying structural genomic changes that leave discriminating signatures associated with various germline and somatic stage diseases. Routinely, raw data from array CGH experiments are very noisy; especially when samples are used from less than ideal sources (e.g. paraffin embedded archived tumors). Here, we describe a series of free analytical tools that have been pieced together to achieve a robust and systematic way of calling copy number changes for samples of varying qualities. We qualify our results on a simulated benchmark dataset (Bioinformatics. 2005; 21:3763-70) that produces a range of signal to noise (e.g. 4, 3, 2 and 1) and segment lengths (e.g. 40, 20, 10 and 5 probes) and a real data set of ten varying quality replicates hybridized to a 26k bac whole genome tilling path array (Genome Research 2006; 16:1566-1574). The process begins by passing the normalized log2ratios into the DNAcopy (Biostatistics. 2004; 5:557-72) and the MergeLevels (Bioinformatics. 21(22):4084-4091) tools to segment probes on a chromosome basis and combine probes of similar magnitudes on a genome wide basis respectively. Next, the data is binned into copy number estimates of zero through five using the sm package. These three steps are run multiple times for each hybridization, only, varying the alpha parameter of the DNAcopy segmentation tool. Finally, the data is analyzed with a naïve Bayes classification function of the e1071 package with the set of outputs of each hybridization. The results of several cross-validation analyses of the simulation data at the most difficult events with signal to noise of one and aberration length of five probes showed a marked improvement in the true positive rate (from 0.2 to 0.35) while maintaining a false positive rate of less than 0.05. Our next challenge is to apply the classification approach to the real data which requires the generation of a training dataset that will mimic each samples noise structure and length distributions. These results indicate that robust and cost-effective solutions are available to optimize arrayCGH data analysis.

2515/W

Whole-genome panels for analysis of structural variation in the human genome. *D.A. Peiffer[1], L.M. Galver[1], K.A. Viaud[1], L. Zhou[2], M. Eberle[1], K. Kuhn[1], S.S. Murray[3], R. Shen[1].* 1) Illumina, Inc, San Diego, CA; 2) Prognosys Biosciences, San Diego, CA; 3) Scripps Genomic Medicine, San Diego, CA.

Structural variation throughout the genome has been shown to associate with both disease and susceptibility of disease. Therefore, studies of copy number variation (CNV) on a genome-wide scale are necessary for any extensive whole genome disease-association analysis. We have designed two whole-genome panels for this purpose utilizing the Infinium® assay, which allows examination of both SNPs and non-polymorphic probes efficiently and accurately on a single slide. The first panel contains greater than 370k markers that were selected to maximize genomic coverage for the CEPH population as well as cover the majority of known CNV regions throughout the genome. The second panel contains more than one million markers that were chosen to maximize genomic coverage in CEPH, Han Chinese, Japanese and Yoruba populations. In addition, this panel contains extensive coverage of all RefSeq genes, known CNV regions and has even spacing across the genome for efficient discovery of novel CNV regions.

As of March, 2007 there were 2,714 regions of known CNV in the Database of Genomic Variants. The 370k and one million marker panel cover these regions with 80,000 and 230,000 markers, respectively. In partnership with deCODE Genetics, an additional ~9,000 novel regions likely to represent CNVs were identified and are targeted with approximately 38,000 SNPs and 18,000 non-polymorphic probes for both panels. These regions include segmental duplications, the MHC region, megasatellites, and regions lacking SNPs. Preliminary CNV data generated from these markers using HapMap samples will be shown. We have found that this panel can accurately measure unstable regions of the genome, including megasatellites containing up to twelve copies and overall, these regions show an order of magnitude greater number of CNVs than previously described regions. Mean spacing of markers is ~7.7kb (median ~5kb) for the 370K marker panel and ~2.4kb (median ~1kb) across the genome with less than 5,000 gaps greater than 10kb for the one million marker panel. These panels provide powerful tools for whole-genome analysis utilizing both SNPs and structural variation throughout the genome.

2516/W

Novel and small copy number variant regions identified in high resolution microarray screening of healthy French Caucasian males. *A. Tsalenko[1], A. de Smith[2], N. Sampas[1], A. Scheffer-Wong[1], A. Yamada[1], P. Tsang[1], A. Ben-Dor[1], Z. Yakhini[1], L. Bruhn[1], S. Laderman[1], P. Frouguel[2,3], A. Blakemore[2].* 1) Agilent Technologies, Santa Clara, CA; 2) Genomic Medicine, Imperial College London, Hammersmith Hospital, London, UK; 3) CNRS 8090-Institute of Biology, Pasteur Institute, Lille, FR.

Recent studies identified a wide range of copy number variations (CNVs) in the human genome. Current estimates suggest that the CNV map is still quite incomplete, and that a significant number of especially smaller variations remain to be uncovered. Toward this end, we studied CNVs in a population of 50 apparently healthy, Caucasian males of northern French origin using genomic DNA derived from peripheral blood. We used high resolution arrays with 60mer oligonucleotide probes designed to enable detection of both small and large CNVs. Initial screening for putative copy number variant loci using a genome-wide array with 185K probes was followed by a focused measurement using 244K arrays with probes spaced on average 500 bp in 2475 regions of interest identified in the initial screening. In addition, we examined 2,148 regions reported in the TCAG Database of Genomic Variants (http://www.tcag.ca/). We found 1469 copy number variant regions (CNVRs) detected by multiple probes, of which 45% were observed in more than one individual. The majority of multi-probe CNVRs measured in this study were relatively small, with a median size of 4.4Kb, in contrast to the size distribution of variations in the TCAG database. 721 CNVRs did not overlap regions in the TCAG database. Half of the novel variations were smaller than 1.5Kb, however several regions were as big as 1Mb. The novel regions contain 368 genes, which were present in similar proportions in small and large variants, of which 150 genes are represented in the OMIM database. The breakpoints of many of our detected CNVs were highly conserved across the cohort. The coefficient of variation for 83% of variant breakpoints in multi-probe CNVs observed in multiple samples was less than 0.1, suggesting that there might be a higher utility for CNVs in association studies than previously thought.

2517/W

DNA Copy Number Variation in Normal Dogs. *C.E. Alvarez[1], W.K. Chen[1], L.J. Rush[2], J.D. Swartz[1].* 1) Molecular and Human Genetics, Columbus Children's Research Institute & The Ohio State University College of Medicine, Columbus, OH; 2) Department of Veterinary Biosciences, The Ohio State University, Columbus, OH.

Domestic dogs, Canis lupus familiaris, are a subspecies of the wolf, Canis lupus. They have co-evolved with humans for over 15,000 years. In the last 150 years, hundreds of pure breeds were created by selection of mostly morphological and behavioral traits. The existence of so many isolated, but related, populations makes them attractive for genetic studies. Moreover, the majority of the hundreds of diseases described in dogs are similar to ones in humans. These include diverse cancers, heart disease and many sensory or neurological disorders. Consistent with their histories of rapid selection and common population bottlenecks, specific dog breeds are predisposed to certain diseases, suggesting that a limited number of risk alleles are responsible. New tools have spawned a new era of dog genetics. Here we report on the normal DNA Copy Number Variation (CNV) of dogs. In normal humans, thousands of gene-spanning CNV regions (CNVrs) have been identified and some have strong roles in disease predisposition. We conducted CNV discovery in a small panel of normal pure bred dogs that represent the four classes of breeds: ancient/Asian, mastiff, herding and hunting. We quantified CNV by Comparative Genome Hybridization on a high resolution whole genome microarray (isothermal long-oligonucleotides at <5 kb mean spacing; Nimblegen). Selected regions were validated by other hybridization and PCR-based methods. We identified 155 variants - spanning 50 CNVrs - at high confidence. The mean number of CNVrs per animal was 17, similar to that reported for mouse (using the mouse version of the same array platform). Cluster analysis of all CNV regions showed that different breeds generally group together within their breed classes, indicating that a significant portion of this genetic variation has been evolutionarily transmitted from the founders of the four breed classes. The CNV regions generally affect multiple genes, as in humans and mice. We thus suspect that CNV contributes to breed-specific traits of dogs, including breed-specific disease predisposition.

2518/W

The Database of Genomic Variants - annotating structural variation in the human genome. *L. Feuk[1, 2], J. Zhang[2], B. Thiruv[2], J.R. McDonald[2], S.W Scherer[1, 2].* 1) Program in Genetics & Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada.

The Database of Genomic Variants (http://projects.tcag.ca/variation/) is a comprehensive and curated catalogue of structural variation in the human genome. Copy number variants (CNVs) and inversions larger than 1kb in size are included. Its current content is based on 40 publications, with 6,4820 CNVs and 77 inversions represented. Merging entries for structural variants that overlap yields 3,643 variable loci or regions. In the clinical diagnostic setting, comparative genome hybridization (CGH) has been introduced as a means to search for submicroscopic CNVs in the investigation of certain clinical phenotypes, and potential applications are expanding rapidly. In the research laboratory, screening of large human cohorts for CNVs is now common. Currently, one of the major problems lies in the interpretation of the resulting data. The cost of genome-wide screening still prohibits many researchers from running large groups of control samples. It is therefore important to have access to a comprehensive list of regions already identified as CNVs in previous studies. The Database of Genomic Variants facilitates the interpretation of studies screening for CNVs, in relation to previously published work. The data are represented in table format, genome browser format, and text files available for download. The genome browser is ideal for viewing structural variation in relation to other genomic features, such as genes, clones and segmental duplications. The database has been designed to be easily navigated and suitable for all users, independent of bioinformatics experience. Here we present an overview of the database along with our future plans for its expanded content and enhanced presentation.

2519/W

High Resolution Measurements of Copy Number Variant Regions. *A. Ben-Dor[1], N. Sampas[1], A. Tsalenko[1], A. Scheffer-Wong[1], S. Dallaire[2], J. Tchinda[2,3], P. Tsang[1], A. Yamada[1], Z. Yakhini[1], G.H. Perry[2], C. Lee[2,3], S. Laderman[1], L. Bruhn[1].* 1) Agilent Technologies, Santa Clara, CA; 2) Brigham & Women's Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA.

Genomic copy number variations (CNVs) are a major source of genetic differences between individuals. Technological improvements for the discovery and characterization of CNVs will advance our understanding of their prevalence, complexity, and association with disease-related phenotypes. In order to detect CNVs that encompass a wide range of sizes and complexities, we have developed a database of ~16 million 60mer probe sequences that cover the non-repeat masked portion of the genome at an average spacing of 100bp. These probes were selected according to stringent thermodynamic and sequence characteristics. In addition, we developed an efficient algorithmic workflow for the analysis and visualization of CNV data that includes a statistically robust approach for calling CNV intervals in individual samples as well as methods for grouping per-sample variants into CNV regions (CNVRs). We evaluated the performance of the platform by profiling DNA samples from healthy individuals, e.g. from the Hapmap set, using custom 244K feature arrays with probes focused on candidate CNVRs. CNV we detected with multiple consecutive probes range from <200bp to several Mbp, and exhibit a large spectrum of complexities, from simple CNVRs with virtually no variation in size between individuals to very complex regions (i.e. the HLA region on Chr6). Moreover, we are able to detect many regions with clearly distinct copy number states in different individuals. To estimate reproducibility rates we performed three independent measurements comparing two DNA samples previously profiled in various studies (NA15510 vs. NA10851) using a two array set comprising 470,143 probes encompassing 2192 known CNV regions. On average, 420 multi-probe CNV were called in each sample. These CNV were highly reproducible (8% false-positive rate, and 5% false-negative rate). In addition, the boundaries of the regions were called very consistently; more than 85% of the boundaries were mapped to within single probe resolution.

2520/W

A Comprehensive Whole Genome Analysis Panel Containing over 1M SNPs. *M. Eberle[1], S.S. Murray[2], K.A. Viaud[1], D. Peiffer[1], L. Zhou[3], P.C. Ng[4], K. Kuhn[1], R. Shen[1], L.M. Galver[1].* 1) Illumina Inc; 2) Scripps Genomic Medicine; 3) Prognosis Biosciences; 4) Craig Venter Institute.

Whole genome association studies are a powerful tool for detecting loci associated with complex diseases. A comprehensive whole genome panel must include markers for even coverage of the genome and tag SNPs for maximum genomic coverage and power. We have constructed a whole-genome panel containing over one million SNPs in addition to non-polymorphic probes for this purpose. SNP loci were chosen from both the HapMap and NCBI SNP databases while probes were designed in SNP-poor regions for detection of copy number variation (CNV). This panel utilizes the Infinium® assay that allows interrogation of a large number of markers efficiently and accurately on a single slide (Steemers et al., 2006). We have maximized genomic coverage for this panel by selecting tag SNPs from the HapMap data for all 4 populations. Approximately 94%, 93% and 73% of HapMap Phase II variation is captured at an r2 ≥ 0.8 in the Caucasian (CEU), Han Chinese/Japanese (CHB/JPT) and Yoruba (YRI) populations, respectively. Alternatively, coverage can be measured by examining the power to detect risk alleles under various disease models and sample sizes. We explored a wide range of disease risks and study designs to estimate the power of this panel for detecting risk alleles associated with complex diseases. Results show that in European and Asian populations these SNPs provide ~90-95% of the power that could be achieved by genotyping all ~2.2 million common (MAF > 5%) SNPs in the HapMap database in the same number of samples. There are approximately 400,000 gene-centric SNPs in this panel that map within 10kb of a gene region and cover greater than 99% of RefSeq genes at an average density of 6 SNPs per 10kb. Approximately 25,000 of these SNPs are non-synonymous. Additionally there are greater than 6,000 SNPs and indels in the gene-rich MHC region chosen from a high-density MHC map (deBakker et al., 2006), and 10,000 SNPs located within more than 200 known ADME genes. Markers were also added to maximize coverage of published and novel CNV regions as well as achieve even spacing across the genome.

2521/W

Mapping the susceptibility genes for Carney Triad: Application of original tools for SNP chip analysis of component neoplasias. *G. Assie[1], M. Muchow[2], J. Bertherat[3], J.A. Carney[4], C. Stratakis[2], C. Eng[1].* 1) Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH; 2) Sect of Endocrinology & Genetics, NICHD, NIH, Bethesda, MD; 3) Dept of Endocrinology, Institut Cochin, Paris; 4) Dept of Pathology, Mayo Clinic, Rochester.

The Carney Triad (CT) denotes the presence of paragangliomas, gastroinstestinal stromal tumors and chondromas in a single individual. The relatively rare CT occurs sporadically. To map the causal gene(s), we developed a novel program designed for SNP chip analysis of tumor tissues. Original features of the program include automated detection and report of deletions, amplifications and copy-neutral allelic imbalances; and determination of the percentage of cells affected by these alterations, enabling discrimination of germline versus somatic events and therefore reliable inference of germline genotypes from tumor samples. The algorithm relies on the SNP minor allele frequencies and intensities provided by Illumina BeadStudio 2.0. We ran Illumina Humanhap300 chips on 54 samples (42 tumors and 12 normal tissues) from 26 patients. The smallest detected deletion was 10 kb, and the smallest amplification was 7 kb. The boundaries were validated visually with BeadStudio. No obvious alterations were missed. Several of these alterations were experimentally confirmed by semi-quantitative PCR or array CGH. Agreement between germline genotype inferred from the tumors and normal tissue was > 99% of SNPs. Utilizing this approach, we mapped relevant CT loci, defined as loci of common germline chromosomal alterations across at least 3 patients and with significant association with CT cases compared to 331 population-matched controls (Haploview v3, Fisher test with Bonferroni correction). Six common loci of germline deletions and 1 common amplification were identified. We also identified previously known as well as novel somatic alteration-hotspots, some being common with germline alterations. Of note, one locus showed common germline and somatic deletions, and significant association in cases over controls, suggesting that this is a major/the susceptibility locus for CT.

2522/W

Whole genome array strategy for detection of tandem repeat length polymorphisms. *H.A. Bruce[1], A.J. Sharp[2], T.A. Richmond[4], S.G. Lin[1], C.A. Ross[1], E.E. Eichler[2], L.E. DeLisi[3], R.L. Margolis[1].* 1) Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) Department of Psychiatry, New York University, New York, NY; 4) NimbleGen Systems Inc, Madison, WI.

Recent findings using advances in array technology have shown that up to 12% of the human genome may be subject to variations in copy number. It is now possible to detect copy number variations on whole genome SNP arrays. However probes for these arrays have generally been selected to avoid repetitive regions, even though copy number variation is likely to occur in precisely these regions. We hypothesize that polymorphisms of longer tandem repeats (unit length of 50 bp to >150,000 bp), relatively unexplored features of the human genome, may also contribute to normal human variation and to disease. We developed an oligonucleotide array, using the NimbleGen Systems Inc. platform, specifically designed to detect changes in the number of repeating units in tandem repeats. The array contains 380,000 probes and targets 3430 tandem repeats with an average repeat length of 3877 bp. We tested the DNA from a series of test cases and a well characterized reference sample. We detected between 100 and 200 polymorphic tandem repeats per hybridization, using a log 2 signal ratio cut off of >0.5 or <-0.5. Selected polymorphic repeats were confirmed by PCR. This pilot experiment demonstrates that it is possible to detect polymorphic tandem repeats using a whole genome array technology. This strategy may prove of value in further understanding variation within the human genome and identifying risk factors for disease.

2523/W

New genome-wide platform for discovery and genotyping of copy number variants reveals much higher number of common CNVs in human genome. *J. Gulcher[1], D. Gudbjartsson[1], A. Jonasdottir[1], A. Gylfason[1], A. Baker[1], G. Masson[1], A. Karason[1], A. Jonasdottir[1], Á. Olafsdottir[1], S. Reynisdottir[1], S. Gudjonsson[1], D. Peiffer[2], K. Viaud[2], L. Galver[2], L. Zhou[2], K. Kuhn[2], R. Shen[2], S. Murray[2], U. Thorsteinsdottir[1], K. Stefansson[1].* 1) deCODE genetics, Reykjavik, Iceland; 2) Illumina, San Diego, CA.

CNVs may be an important source of disease-associated variations.We designed the CNV features on the Illumina Human CNV12,CNV370, and 1M BeadChips, which target 4 categories of segments that we predicted were more likely to have common structural variation:1)the unSNPable genome- defined as gaps of ≥15kb in human HapMap map or gaps ≥5kb with more than one HapMap SNP failure due to HWE or inheritance issues,2)segmental duplications-duplicons 100 bases or greater within 3 Mb,3) megasatellites-tandem repeats ≥500 base unit length,4)numerous gaps in the MHC. In total 14,000 segments were targeted for intensity measurements using 55,000 SNPs or probes of invariant bases(2/3 were SNPs to facilitate phasing),most not covered by current SNP platforms.The 11,000 segments not in the Database of Genomic Variants comprise 7% of the genome. Although the Affymetrix 6.0 has 1M extra probes for CNV analysis,7000 of our 11,000 novel segments are missed. Our hypothesis was that these segments are more likely to contain CNVs than the "SNPable" genome, and that CNVs in these segments are likely to have higher non-wildtype allelic frequencies. We have now run 3 HapMap plates along with 100 sets of trios. Normalized intensities of probes within megasatellites correlated well with copy number precisely defined by restriction fragment lengths on Southern blotting. Twenty-one novel common CNVs predicted by our CNV algorithm were tested by Southern blot and kinetic PCR- all 21 were polymorphic as predicted demonstrating high specificity of the platform. We find at least an order of magnitude increase in the number of common CNVs found within this 7% of the genome with non-wild-type frequency greater than 2% beyond those found using arrays or BACs covering the 93% of the genome.Many CNVs correlate to expression in peripheral leukocytes of genes near the CNV. ≥.

2524/W

Search for Genomic Alterations in Monozygotic Twins Discordant for Cleft lip and Palate. *J.W. Kimani[1], K. Yoshiura[2], J.C. Murray[1].* 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) Department of Human Genetics, Nagasaki University Graduate School of Biomedical sciences, Nagasaki, Japan.

Postzygotically occurring genomic alterations that result from mitotic recombination and other somatic events have been proposed to underlie monozygotic (MZ) twin discordance. We scanned for such alterations in a cohort of MZ twins discordant for isolated cleft lip and/or palate (CLP) with the aim of detecting any chromosomal abnormalities that can reveal candidate genes within altered genomic fragments. Our analyses consisted of an array comparative genomic hybridization (aCGH) target containing 2,173 genomic BAC clones (n=6 pairs), an Illumina custom genotyping array covering 1,536 SNPs derived from 350 candidate genes (n=20 pairs) and the Affymetrix GeneChip® Human Mapping 50K Xba I (n=2 pairs) and the 250K Nsp I (n=10 pairs) arrays. The aCGH provided an average resolution of 1 BAC clone every 1Mb, but no copy number changes were detected. Average twin genotype concordance for both Illumina and Affymetrix assays was >99%, and paired analyses were carried out for both platforms using the Beadstudio software and the Copy Number Analysis Tool respectively. However, we did not detect any allelic imbalances through loss of heterozygosity or copy number changes within the resolution of the respective assays. Sequencing of a subset of SNPs with discordant genotype calls in both twins and the parents verified genotype concordance and showed consistency with Mendelian inheritance. Our results demonstrate that postzygotic genomic alterations are not a probable cause of MZ twin discordance for isolated CLP. However, balanced genomic alterations, tissue-specific events and small aberrations beyond the detection level of our experimental approach cannot be ruled out.

2525/W

Detection of human copy number variations using a collection of Japanese complete hydatidiform moles. *Y. Kukita[1], K. Higasa[1], S. Ishikawa[2], T. Tahira[1], K. Hayashi[1]*. 1) Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Division of Genome Science, Research Center for Advanced Science and Technology, University of Tokyo, Tokyo, Japan.

Copy number variations (CNVs) of DNA segments in the human genome can confer phenotypic variations such as risk to complex disease traits. Because there is an abundance of CNVs with population differentiation, cataloging CNV regions for each ethnic population is biomedically important. We carried out genome-wide high resolution CNV mapping using a collection of complete hydatidiform moles (CHMs) as samples, and analyzing the intensity values of a high-density DNA oligonucleotide hybridization experiments (Affymetrix 500K SNP Array). The advantage of using CHM for CNV detection is that the relative change in the hybridization signal caused by the copy number change is expected to be larger for CHM sample than for usual diploid sample (thus, larger S/N ratio), because CHM has the genome of single sperm origin, and its genome is haploid. Our results are being compared with the CNVs reported in the "Database of Genomic Variants" and validation by wet experimental methods is ongoing. We will present an integrated haplotype map of SNPs and CNVs, and discuss about the features of analyses using CHMs.

2526/W

Copy Number Variation Analysis Using Quantitative TaqMan® Copy Number Assays. *K. Li[1], A.J. Broomer[1], Y. Wang[1], C. Xiao[1], C. Barbacioru[1], I.R. Casuga[1], F. Wang[1], A.J. Sharp[2], E.E. Eichler[2], C. Chen[1]*. 1) Applied Biosystems, 850 Lincoln Centre Dr., Foster City, CA94404; 2) USA and 2 Department of Genomic Sciences and Howard Hughes Medical Institute, University of Washington School of Medicine,Seattle, WA 98195.

Recent whole-genome studies have identified 1447 CNV regions (CNVRs) that cover about 12% of the human genome. Some of CNVR may contain disease loci/genes, whose copy number changes could impact gene activity and disease susceptibility. Copy number changes are also detected in microdeletion/microduplication syndromes, which are associated with genomic disorders. Although array-based technologies are powerful for large-scale CNV discoveries and microdeletion/microduplication syndrome screening, more quantitative technologies with higher sample throughput are required to validate newly identified CNVs and to detect deletions/duplications for a large sample size in candidate regions/genes. To meet these challenges and demands, Applied Biosystems has developed TaqMan® based real-time quantitative copy number assays. Here, we report the development of the TaqMan® copy number assay design pipeline and validation of TaqMan® copy number assays. We used this proprietary pipeline to design assays targeting the chromosomal regions associated with genomic disorders and CNV-associated OMIM genes. The assays were tested with DNA sets for validation, HAPMAP DNA collection as well as samples with known deletions/duplications. The TaqMan® copy number assay is a duplex reaction with a FAM-assay targeting the gene of interest and a VIC®-assay targeting the reference gene (two copies per diploid genome) in the same well. The copy number is determined by relative quantification using a reference sample known to have two copies of the gene of interest. Our validation data demonstrate a high success rate of assay design and excellent assay performance. TaqMan® copy number assays are quantitative and robust, with high reproducibility, specificity, and sample throughput.

2527/W

CGH microarray analyses in Proteus syndrome. *M.J. Lindhurst[1], J.J. Johnston[1], S.J. Vacha[2], L.G. Biesecker[1]*. 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) Agilent Technologies, Inc. Santa Clara, CA.

Proteus syndrome (PS) is a rare sporadic disorder that is characterized by overgrowth of multiple tissues. It is highly variable; patients have a mosaic distribution of lesions that progressively worsen with age. The hypothesis is that a genetic alteration occurs post-zygotically that results in growth dysregulation in tissues derived from the mutant cell. Because the disorder is not inherited, traditional methods for studying genetic diseases are not amenable to studying PS. We hypothesize that a subset of patients has a genomic scale duplication or deletion that causes overgrowth. We have used oligo-based CGH microarray technology to compare genomic DNA extracted from several types of patient tissue. Most comparisons were done using DNA extracted from affected and unaffected areas of the same patient using either cultured cells or DNA extracted directly from affected tissue samples. In addition, three comparisons were done between affected DNA and standard reference DNA. Initially, 14 hybridizations were performed using a CGH microarray platform containing 244K probes. Analyses of these arrays yielded no obvious aberrations, however, there were 529 regions with high LogRatio changes. To confirm these results and further characterize these regions, custom 4 x 44K oligo arrays were designed that zoomed in on each of these areas. Probes that were located within 15 Kb to either side of the probe of interest were chosen for the custom array resulting in 50-80 probes per region. Twelve hybridizations with the DNAs labeled with the opposite fluor were repeated using the custom 44K zoom-in array. Over 185 regions still remain with one or more probes that have an amplification or deletion score of 0.5 or more. Several criteria can be chosen to use for prioritizing follow up studies. However, all have caveats making the choice difficult. The sporadic, mosaic characteristics of PS provide an added challenge in interpreting high-resolution screening technologies, as any single probe outlier could be worthy of further study. We propose to share this data set collaboratively with other investigators to allow a thorough and rational approach to follow-up studies.

2528/W

Fine-scale structural anatomy of 1086 human copy number variant (CNV) regions as defined by custom high-density oligonucleotide microarrays. *C. Lee[1,2], A. Ben-Dor[3], G.H. Perry[1], A. Scheffer-Wong[3], N. Sampas[3], S. Dallaire[1], J. Tchinda[1,2], A. Tsalenko[3], P. Tsang[3], A. Yamada[3], Z. Yakhini[3], L. Bruhn[3], S. Laderman[3]*. 1) Brigham & Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Agilent Technologies, Santa Clara, CA.

Copy Number Variants (CNVs) are highly prevalent in the human genome and may play an integral role in normal human phenotypic variation and disease susceptibility. Initial screens using a variety of technologies have identified thousands of human CNV regions. However, most of these studies provide limited resolution of the fine-scale structure of these CNV regions. To define the architecture of known CNVs, we have constructed a custom oligonucleotide array containing 470,143 distinct 60mer probes, selected with an approximate 1kb spacing within and flanking 2191 human CNV regions annotated in the Database of Genomic Variants. We have interrogated the genomic DNAs from 30 HapMap individuals and observed copy number differences at 1086 of the 2191 targeted CNV regions. Many of these individual CNV regions were, in fact, comprised of multiple and non-overlapping CNVs. In total, we confidently detected (i.e. using multiple consecutive probes) 2553 CNVs, of which 68% were identified in multiple individuals. The total amount of copy number variable DNA was reduced by over 50% in 847 of the 1086 detected CNV regions. In addition, our custom array has identified CNVs with different breakpoints among unrelated individuals. We also found a surprisingly substantial number of "complex" CNV regions that reveal smaller overlapping CNVs embedded within larger CNVs, with variable combinations of gains and losses of the smaller CNVs among individuals (e.g. a 450 kb region on chr2p11.2). These data more clearly define which genes and non-coding DNA sequences are actually copy number variable among healthy individuals and highlight a previous under-appreciation for the complexity of many human CNV regions. Furthermore, these results precede the development of a targeted, CNV-enriched array, that will be useful in future disease association studies, aimed at identifying the role of CNVs in common diseases.

2529/W

High-density genome-wide array CGH in a genetically homogenous population. *J.G. Mulle[1], A.E. Pulver[2], S.T. Warren[1]*. 1) Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore MD.

Extensive copy number variation (CNV) in the human genome is an exciting new discovery with potentially major implications for complex genetic disease studies. However, robust conclusions about CNV distribution in phenotypically normal populations have been elusive. This is in part because prior studies have used outbred samples, yielding imprecise population-level inferences. Additionally, the limited resolution of previously available technologies has biased estimates of the distribution and frequency of CNV in control genomes. Furthermore, different levels of resolution among technologies have prevented comparison between studies, even when studies investigate the same samples. We sought to improve on prior efforts in two ways; first, we assess CNV in phenotypically normal individuals ascertained from a genetic isolate, the Ashkenazi Jewish (AJ) population. Secondly, we employ CGH using an oligonucleotide array with 2.1 million features, corresponding to a density of 1 oligo every 1.1 kb in non-repetitive genomic sequence, to expand the range of CNV detection. Using this study design, we detect an average of 90 deletions and 112 duplications per genome, of median size 11 kb and 28 kb, respectively. All test samples are compared to one HapMap reference individual (NA12155), in whom CNV has been partially characterized by at least three technologies (McCarroll et al., 2006, Redon et al., 2006). We detect 60 of 81 (74%) unique CNV previously reported to exist in our HapMap reference genome, including 20 of 24 variants found using Affymetrix 500k EA array data (Redon et al., 2006), 37 of 53 variants found using WGTP BAC arrays (Redon et al., 2006), and 10 of 13 deletions found using HapMap genotype data (McCarroll et al., 2006). This 75% overlap with existing datasets (despite their overlap of only 10-25% with one another) implies that as experimental resolution increases, so will detection of true CNV. This characterization of the true CNV distribution in control genomes will be an important tool for understanding susceptibility variants for common, complex disease.

2530/W

Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridization. *K. Osoegawa[1], G.M. Vessere[1], K.H. Utami[1], M.A. Mansilla[2], M.K. Johnson[2], B.M. Riley[2], J. L'Heureux[2], R. Pfundt[3], J. Staaf[4], W.A. Van der Vliet[3], A.C. Lidral[2], E.F.P.M. Schoenmakers[3], A. Borg[4], B.C. Schutte[2], E.J. Lammer[1], J.C. Murray[2], P.J. De Jong[1]*. 1) Research Inst, Childrens Hosp Oakland, Oakland, CA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA; 3) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) Department of Oncology, Lund University, Lund, Sweden.

We analyzed DNA samples isolated from individuals born with cleft lip and cleft palate to identify deletions and duplications of candidate gene loci using array comparative genomic hybridization (array-CGH). Of 83 syndromic cases analyzed we identified one subject with a previously unknown 2.7 Mb deletion at 22q11.21 coinciding with the DiGeorge syndrome region. Eighteen of the syndromic cases had clinical features of Van der Woude syndrome and deletions were identified in 5 of these, all of which encompassed the *interferon regulatory factor 6* (*IRF6*) gene. In a series of 104 nonsyndromic cases we found one subject with a 3.2 Mb deletion at chromosome 6q25.1-25.2 and another with a 2.2 Mb deletion at 10q26.11-26.13. Analyses of parental DNA demonstrated that the two deletion cases at 22q11.21 and 6q25.1-25.2 were de novo, while the deletion of 10q26.11-26.13 was inherited from the mother, who also has cleft lip. These deletions appear likely to be causally associated with the phenotypes of the subjects. *Estrogen receptor 1* (*ESR1*) and *fibroblast growth factor receptor 2* (*FGFR2*) genes from the 6q25.1-25.2 and 10q26.11-26.13, respectively, were identified as likely causative genes using a gene prioritization software. We have shown that array-CGH analysis of DNA samples derived from cleft lip and palate subjects is an efficient and productive method for identifying candidate chromosomal loci and genes, complementing traditional genetic mapping strategies.

2531/W

Sensitive and specific real-time PCR assays to accurately determine gene copy number variations (GCNVs) of human complement *C4A*, *C4B*, *C4-long*, *C4-short* and RCCX modules: elucidation of *C4* GCNVs in 50 consanguineous subjects with defined HLA genotypes. *Y.L. Wu[1], S.L. Savelli[1], Y. Yang[1], B. Zhou[1], B.H. Rovin[2], D.H. Birmingham[2], G.N. Nagaraja[2], L.A. Hebert[2], C.Y. Yu[1].* 1) Center for Molecular and Human Genetics, Columbus Children's Research Institute, Columbus, OH; 2) Department of Internal Medicine, The Ohio State University, Columbus, OH.

Recent comparative genome hybridization studies revealed hundreds to thousands of human genomic loci can have inter-individual copy-number variations (CNVs). One of such CNV loci in the HLA codes for immune effector protein complement component C4. Sensitive, specific and accurate assays to interrogate *C4* CNV and its associated polymorphisms using sub-microgram quantities of DNA are needed for high throughput epidemiologic studies of *C4* CNVs in autoimmune, infectious and neurological diseases. Quantitative real-time PCR (qPCR) assays were developed using TaqMan chemistry and based on sequences specific for *C4A* and *C4B* genes, structural characteristics corresponding to the long and short forms of *C4* genes, and the breakpoint region of *RP-C4-CYP21-TNX* (RCCX) modular duplication. Reliable assignments for gene copy-numbers (GCN) were achieved by relative standard curve method, using cloned *C4* genomic DNA covering six logs of DNA concentrations for calibrations. The accuracies of test results were cross-confirmed internally in each sample, as the sum of *C4A*+*C4B* equals to the sum of *C4L*+*C4S*, or the total copy number of RCCX modules. These qPCR assays were applied to determine *C4* CNVs from samples of 50 consanguineous subjects with defined HLA genotypes. The results revealed the presence of 8 haplotypes with single *C4* genes coding for either C4A or C4B in monomodular RCCX that are associated with multiple autoimmune and infectious diseases. There are 33 bimodular, 4 trimodular, and 1 quadrimodular RCCX haplotypes with different combinations of *C4L* and *C4S*, and *C4A* and *C4B*. Two to eight copies of *C4* genes in a diploid genome are firmly established. These *C4* qPCR assays are proven to be robust, sensitive and reliable, as they have contributed to the elucidation of *C4* CNVs in large cohorts of samples with autoimmune and neurological diseases.

2532/W

Comparing platforms to genotype human copy-number variants. *G.M. Cooper[1], J.D. Smith[1], T.R. Zerr[1], E. Tuzun[1], J.M. Kidd[1], D.A. Nickerson[1], E.E. Eichler[1,2].* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Howard Hughes Medical Institute.

Copy-number variants (CNVs) in the human genome are likely to be contributors to common traits. Recent studies demonstrate that some CNVs can be genotyped using high-throughput SNP-typing platforms. However, the power of these platforms to genotype CNVs has not been systematically evaluated. To explore this, using Illumina arrays we genotyped 8 individuals known to carry large (>100 kbp), clinically relevant deletion or duplication events; we also genotyped 8 HapMap individuals that are concurrently being analyzed by fosmid-end sequence pair mapping (FESPM) and oligo-array CGH. The latter 8 have been elsewhere subjected to CNV discovery using Affymetrix 500K SNP arrays and BAC comparative genomic hybridization (BAC-CGH; Redon et al. 2006). We find that 6/8 of the large, clinically relevant mutations are robustly detectable. We also find high sensitivity (>90%) to variants identified using Affymetrix arrays. However, we find weak sensitivity for the SNP-platforms to identify CNV regions identified through BAC-CGH and FESPM. A substantial fraction of these CNVs are not covered with enough SNPs to allow detection on any available SNP platform; also, many CNVs that do span multiple assayed SNPs exhibit intensity values that lie within the noise ranges defined by random sampling. We estimate, for example, that less than 25% of deletions detected via FESPM or BAC-CGH can be robustly genotyped on the HH300 array. Our results indicate that genotyping success rates for large, clinically relevant CNVs are likely to be high for SNP-typing platforms. However, most smaller and more common CNVs cannot be readily genotyped via the current, widely available SNP-genotyping platforms. Nucleotide-level annotation of CNVs and higher-density SNP arrays should yield significant improvements in sensitivity; preliminary analyses of upcoming array designs indicate dramatically improved coverage of CNV regions. Continued analysis of individuals with precise CNV annotations spanning a range of sizes and genomic locations will facilitate sensitivity evaluations of future array designs.

2533/W

The frequency and distribution of normal copy number variants (CNVs) in subjects with an autism spectrum disorder (ASD) and/or idiopathic intellectual disability (ID). *C. Fawcett[1, 6], Y. Qiao[1, 2, 6], C. Harvard[1, 6], C. Tyson[1, 6], X. Liu[3, 6], JJA. Holden[3, 4, 5, 6], MES. Lewis[2, 6], E. Rajcan-Separovic[1, 6].* 1) Dept Pathology, and; 2) Medical Genetics, UBC, Vancouver, Canada; 3) Dept Psychiatry, and; 4) Physiology, Queen's Univ, Kingston, Canada; 5) Autism Research Program, Kingston; 6) ASD-Canadian American Research Consortium (www.autismresearch.com).

Copy number variants (CNVs) have been shown to be widely distributed throughout the genome of neurodevelopmentally normal individuals using high resolution techniques including array CGH. However, the nature and significance of the CNVs remain unknown. Using commercial 1 MB array CGH, we assessed the frequency and type of known CNVs in 90 ASD and 84 ID subjects and compared them to the reported findings in the normal population (http://projects.tcag.ca/variation) as well as our own cohort of 27 control subjects. For the top 5 recurrent clones (each >10% in frequency), 4 of them (RP11-259N12: 1p13.3, RP11-100C24: 13q21.1, RP11-125A5: 14q12, and RP11-79F15: 19p13.2) were shared between the two patient groups and no significant difference in frequency was found, while clone RP11-88L18 (5p15.1) was more prevalent in the ASD and clone RP11-9H12 (9q32) in the ID group. The total frequency of each of the top 5 clones in both patient groups was within the range observed for these clones amongst unaffected individuals. No correlation was found between the type of CNV (gain or loss) for the top 5 clones in each group, except for clone RP11-88L18, which was seen 10 times more often as a loss than a gain only in the ASD group. RP11-125A5 was previously reported to have a high frequency of variation in neoplasia (50% compared to 10% reference), suggesting increased genomic instability associated with the disorder. Our study has found no difference in the frequency or type of copy number for the 4 of the 5 most common CNVs observed to date in our studies of ASD and ID subjects, suggesting that their link to these neurobiologic disorders is unlikely. Additional studies on RP11-88L18 are warranted to determine the significance of the prevalent loss of this region in ASD.

2534/T

SIX1 Mutation Screening in 247 Branchio-Oto-Renal Syndrome Families: A Recurrent Missense Mutation Associated With BOR. *A. Kochhar[1, 2], D.J. Orten[3], J.L. Sorensen[2], S.M. Fischer[2], C.W.R.J. Cremers[4], W.J. Kimberling[3], R.J.H. Smith[2].* 1) Doris Duke Charitable Foundation; 2) Department of Otolaryngology, Head and Neck Surgery, University of Iowa, Iowa City, IA; 3) Department of Genetics, Boys Town National Research Hospital, Omaha, NE; 4) Department of Otorhinolaryngology, University Hospital Nijmegen, Nijmegen, The Netherlands.

Branchio-oto-renal syndrome (BOR; MIM# 113650) is a clinically heterogeneous autosomal dominant form of syndromic hearing loss characterized by variable hearing impairment, malformations of the pinnae, the presence of branchial arch remnants, and various renal abnormalities. Both *EYA1* and *SIX1* are expressed in developing otic, branchial and renal tissue. Consistent with this expression pattern, mutations in both genes cause BOR syndrome. Mutations in *EYA1* are found in approximately 40% of patients with the BOR phenotype, however, the role of *SIX1* is much lower. To date only three different *SIX1* mutations have been described in BOR patients. The current screen of 247 BOR families detected five novel *SIX1* mutations (c.50T>A, c.218A>C, c.317T>G, c.329G>A, c.334C>T) and one previously reported mutation (c.328C>T), all of which are within the protein-binding Six domain. Phenotypic variability was high in these BOR families. Seven of the eight known *SIX1* mutations are missense and the one in frame deletion is predicted to be functionally similar. The wide phenotypic variability precludes making genotype-phenotype correlations at this time.

2535/T

Variations in *GRHL2* contribute to Age-Related Hearing Impairment (ARHI) in different European populations. *L. Van Laer, E. Van Eyken, E. Fransen, J.R. Huyghe, T. Wienker, I. Pyykkö, C.W.R.J. Cremers, H. Kremer, I. Dhooge, D. Stephens, E. Orzan, M. Pfister, M. Bille, A. Parving, M. Sorri, P. Van de Heyning, G. Van Camp, European ARHI Consortium.* European ARHI consortium from Antwerp (Belgium), Bonn (Germany), Cardiff (UK), Copenhagen (Denmark), Ghent (Belgium), Nijmegen (the Netherlands), Oulu (Finland), Padova (Italy), Tampere (Finland), Tübingen (Germany).

Age-Related Hearing Impairment (ARHI) is the most prevalent sensory impairment in the elderly. Approximately 50 % of 80-year-olds suffer from a hearing loss of 25 dB or more. ARHI is a complex disease caused by an interaction between environmental and genetic factors. The contribution of various environmental factors has been relatively extensively studied. In contrast, investigations to identify the genetic risk factors have only recently been initiated. So far only 2 putative susceptibility genes have been reported. Here we describe the results of an association study on 2540 ARHI samples derived from 9 centres from 7 different European countries. The degree of hearing loss was expressed with a Z-score. In 70 candidate genes, which were chosen among the monogenic hearing loss genes identified in mice and men in addition to several strong functional candidates, a total of 768 tag SNPs was selected based on Hapmap data. Genotyping was performed by Illumina. After data polishing, statistical analysis on all samples combined resulted in a p-value that survived correction for multiple testing for a *GRHL2* SNP. Other SNPs in this gene were, though less strongly, associated as well. Subsequently, analysis of each population separately was performed, resulting in significant associations in two populations and a trend towards significance in a third population. Moreover, the direction of the association was identical in all nine populations, providing further proof for the validity of this association. Subsequently, fine-mapping of this locus was performed.

2536/T

A Variant in ENPP1 is Associated with Obesity and Insulin Action in Pima Indians. *T. Guo, M. Traurig, Y. Muller, L. Ma, R. Hanson, K. Sayuko, C. Bogardus, L. Baier.* PECRB, NIDDK/NIH, Phoenix, AZ.

ENPP1, Ectonucleotide Pyrophosphatase/Phosphodiesterase 1, also known as PC-1 (Plasma Cell Membrane Glycoprotein), maps to a chromosome 6q23-24, previously shown to have suggestive linkage to type 2 diabetes (T2D) in a genome-wide linkage scan for genetic determinants of T2D among Pima Indians. ENPP1 inhibits insulin-induced conformational changes of the insulin receptor, thereby affecting its activation and downstream signaling. Prior studies have reported that a Gln121Lys in ENPP1 is associated with type 1 diabetes, T2D and obesity in some populations. To investigate the potential role of ENPP1 in the pathophysiology of T2D and obesity in Pima Indians, all 25 exons, the 5' and 3'-UTRs and more than 2kb of the putative promoter region of ENPP1 were sequenced in DNA from 24 non-first-degree related Pima Indians. Among the 21 single nucleotide polymorphisms (SNPs) that were identified, two were nonsynonymous (Gln121Lys and Phe656Leu), but neither were associated with T2D or Body Mass Index (BMI) among a population-based sample of 3500 full-heritage Pima Indians. In contrast, one common SNP (rs17060795) located in a conserved non-coding sequence (CNS) was associated with BMI in this population sample (general analysis p=0.03; within family analysis p=0.02, both p adjusted for age, sex, and birth-year). In addition, among 358 non-diabetic Pima subjects who had been studied in our Clinical Research Center, this SNP was also associated with percent body fat, fat mass and fat-free mass (adjusted p=0.02, 0.002 and 0.002, respectively) as well as measures of insulin action, where subjects with the obesity risk allele (G) had a lower mean glucose uptake rate in response to both physiologic and high dose insulin infusions during a hyperinsulinemic, euglycemic clamp (adjusted p=0.005 and p= 0.0007, respectively). The CNS encompassing this variant is a retroposed gene of LRRC8B. We determined that this retroposed gene itself is not expressed in human tissues, but propose that the retroposed sequence may have acquired regulatory activity that is affecting expression of ENPP1 or another nearby gene, resulting in variation in body composition and insulin action.

2537/T

Apo-1/Fas gene polymorphisms and multiple sclerosis in Southern Italy. *V. Andreoli[1], P. Valentino[2], F. Trecroci[1], F. Condino[1], A. La Russa[1], F. Scionti[1], R. Cittadella[1].* 1) Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone. Cosenza; Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

The pathogenesis of multiple sclerosis (MS) is under strong genetic control involving several or more genes each of modest effect. Whilst the mechanisms underlying the pathogenesis of MS remain unknown, it has been hypothesised that either decreased apoptosis of autoreactive T cells in the central nervous system (CNS), or increased apoptosis of oligodendrocytes may play an important role. Physiologic regulation of cell death is essential for removal of potentially autoreactive lymphocytes during development and excess cells after the completion of an immune response. Apo-1/Fas, an apoptosis-signaling cell surface receptor belonging to the Tumor Necrosis Factor Receptor Super Family 6 (TNFRSF6), is considered to have an important role in the regulation of the immune system by deleting autoreactive T-lymphocytes. Several studies have shown aberrant expression of this molecule in MS, correlating with a decrease in T cell apoptosis or increase in CNS tissue damage. Moreover, Apo-1/Fas maps to the long arm of chromosome 10q23/10q24.1 in humans. Positive lod scores with microsatellite markers near this region were identified in the United States and Canadian genome screens. These two criteria, pathobiological and positional, make the Apo-1/Fas antigen an interesting candidate for an association with MS. To address these findings, we have tested two SNPs in a set of 286 MS patients from Southern Italy (Calabria region) and 271 healthy controls subjects from the same geographical area: a G to A polymorphism at position (-670) in the enhancer region of the promoter and a single nucleotide change from C to T 74 nucleotides from the beginning of exon 7 in the Apo-1/Fas gene. Our results showed no significant differences in the allele and genotype distribution of the polymorphisms between MS patients and controls suggesting that, despite a biological plausibility, Apo-1/Fas gene is not significantly associated with MS in Italian patients. In conclusion, the present findings from Italian population suggest that there was no association between these polymorphisms and susceptibility to MS. Therefore, in the absence of conclusive data, we cannot exclude the possibility that our results are only partially indicative in the wish to identify Apo-1/Fas as a susceptibility-gene to MS, because the association between genetic polymorphisms and MS may also vary with ethnicity.

2538/T

Association of Interleukin-1 beta (IL1B) Gene and Risk of Intracranial Hemorrhage in Brain Arteriovenous Malformation Patients. *P.G. Hysi[1], H. Kim[1], L. Pawlikowska[1], C. McCullough[4], J. Zaroff[5], D. Marchuk[6], M. Lawton[2], P-Y. Kwok[3, 7], W.L. Young[1].* 1) Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, UCSF, San Francisco, CA; 2) Departments of Neurological Surgery, UCSF, San Francisco,CA; 3) Cardiovascular Research Institute, UCSF, San Francisco, CA; 4) Departments of Epidemiology and Biostatistics, UCSF,San Francisco,CA; 5) Cardiology Department, San Francisco, CA Kaiser Permanente San Francisco, CA; 6) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 7) Cardiovascular Research Institute, UCSF, San Francisco, CA.

Polymorphisms in the proinflammatory cytokine interleukin-1 beta (IL1B) gene have been associated with increased risk of brain ischemic injury and subarachnoid hemorrhage. IL1B expression levels are also upregulated in primary intracerebral hemorrhage patients. To determine if genetic variants in IL1B are associated with intracranial hemorrhage (ICH) in the clinical course of brain arteriovenous malformation (BAVM) patients, we genotyped two promoter SNPs (-511C>T and -31T>C) and one synonymous coding SNP in exon 5 (+3953C>T) in 414 BAVM patients. Kaplan-Meier survival analysis censoring patients at first treatment, death or last follow-up, and Cox regression analysis adjusting for age, white race, and gender effects were performed. BAVM patients with the -31 CC genotype (HR=2.8, 95%CI=1.2-6.7, p=0.02) or -511 TT genotype (HR=2.7, 95%CI=1.1-6.6, p=0.03) had a greater risk of subsequent ICH compared to reference genotypes, whereas the +3953C>T SNP was not associated with ICH in the clinical course. These promoter polymorphisms were in strong linkage disequilibrium (r2=0.95) and were also associated with AVM susceptibility among Caucasians. The -511 C>T SNP has been shown to influence IL1B protein production in vitro and the -31 T>C SNP lies in a TATA box transcription initiation site. Our data suggest that functional promoter variants in IL1B may play a role in ICH and BAVM pathogenesis, and warrants further investigation.

2539/T

***ATP1B1*, a hypertension candidate gene, has a conserved and polymorphic 3'UTR element that regulates the selective polyadenylation of its mature mRNA.** *K. Bhalla[1], Z. Pan[2], A. Chakravarti[3], A.R. Shuldiner[1], B. Tian[2], Y-P.C. Chang[1].* 1) Division of Endocrinology, Diabetes and Nutrition, University of Maryland, Baltimore, MD; 2) Department of Biochemistry and Molecular Biology, New Jersey Medical School, Newark, NJ; 3) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Essential Hypertension, defined as chronically elevated blood pressure (BP) with no identifiable cause, is a significant risk factor for coronary heart disease, stroke, and renal disease. A previous genome-wide linkage scan for BP-related QTLs, followed by candidate gene association studies, identified several SNPs associated with BP levels in *ATP1B1*. This gene encodes the regulatory β subunit of Na,K-ATPase, a key enzyme in maintaining renal sodium reabsorption and vascular smooth muscle tone. The most significant association signals came from 2 SNPs located in the highly conserved 3'UTR of *ATP1B1*. This 3'UTR contains multiple potential polyadenylation sites and adenylate/uridylate-rich elements (AREs) that reduce mRNA stability. Hence, the selective use of one poly(A) site over another can lead to mRNAs that differ in length, stability, and translation efficiency. By sequencing *ATP1B1* 3' UTR, we have identified a polymorphic and highly conserved T-rich track that is a putative downstream regulatory element important for cleavage and polyadenylation of mRNA. Alleles of this T-rich element can be grouped into 2 categories: $T_{21\text{-}24}$ and $T_{11\text{-}12}GT_3GT_6$. Using lymphocyte mRNA, we found that the ratio of *ATP1B1* mRNAs using the 5' versus 3' poly(A) signal depends on the genotype of this T-rich element (13.2 and 2.3 for $T_{21\text{-}24}$ homozygotes and $T_{21\text{-}24}/T_{11\text{-}12}GT_3GT_6$ heterozygotes, respectively). *In vitro* polyadenylation assays also showed that the $T_{21\text{-}24}$ motif has a lower polyadenylation activity than elements with $T_{11\text{-}12}GT_3GT_6$ (3.2 fold). In summary, we have identified a novel polymorphic element that regulates the expression of *ATP1B1*. However, whether variants in this T-rich element are directly responsible for the association between 3'UTR SNPs with BP levels is unclear and requires additional studies.

2540/T

Variants in the FANCF gene are associated with Type 2 Diabetes in Pima Indians. *L. Bian, Y.L. Muller, R.L. Hanson, S. Kobes, C. Bogardus, L.J. Baier.* PECRB, NIDDK, NIH, PHOENIX, AZ.

A genome-wide association (GWA) study with the Affymetrix Mapping 100K chip was used to identify susceptibility genes for type 2 diabetes mellitus (T2D) in Pima Indians. Results from this GWA showed that rs10500938 on Chr11p14.3 was associated with early-onset T2D (onset age less than 25 yrs) in both a case-control analysis (N= 300 cases and 329 controls; p = 0.001 adjusted for age and sex) and a within-family analysis (N= 482 discordant siblings; p = 0.04 adjusted for age and sex). This variant is positioned within the 3'UTR of FANCF. FANCF encodes the Fanconi anemia (FA) complement group F, and patients with Fanconi anemia have been reported to have endocrine and metabolic abnormalities, such as abnormal glucose/insulin metabolism and obesity. In addition, FA patients also have an increased prevalence of diabetes mellitus. Therefore, the FANCF gene was directly analyzed as a positional candidate gene for T2D. The coding region, UTRs and 2 kb of the promoter region of the FANCF gene were sequenced in 24 non-first degree related Pima Indians, and 10 SNPs were identified, including rs10500938. Among these SNPs, SNP1 (rs7109087), SNP4 (rs7112345) and SNP6 (rs2307895) were in perfect linkage disequilibrium (LD) defined as D'=1 and r2 = 1. SNP2 (novel), SNP3 (rs4442551) and SNP10 (rs10500938) were also in perfect LD. Therefore SNPs 1 (rs7109087), 5 (novel), 7 (novel), 8 (novel), 9 (rs4447177) and 10 (rs10500938) were selected as representative SNPs and were further genotyped for association analysis in a population-based sample of 3230 full-heritage Pima Indians. The 3' UTR variant rs10500938 remained associated with T2D in the larger population sample using either a general analytical model (OR = 1.36, 95% CI: 1.18-1.58, p = 0.00004, adjusted for age, sex, and birth year) or a within-family model (OR = 1.41, 95% CI: 1.11-1.79, adjusted p = 0.005). Since this 3'UTR variant is in perfect LD with the SNP2 (novel) and SNP3 (rs4442551) which map to the promoter region of FANCF, in vitro expression studies are ongoing to determine the functional variant. An additional Native American cohort is also being genotyped to attempt replication of these associations.

2541/T

TCF7L2, a risk gene for type 2 diabetes, shows association with cystic fibrosis-related diabetes. *S. Blackman[1], S. Hsu[1], S.E. Ritter[2], K.M. Naughton[2], A. Bowers[2], G.R. Cutting[2], CF Twin and Sibling Study group.* 1) Division of Pediatric Endocrinology, Johns Hopkins University, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

About 20% of adults with cystic fibrosis (CF) have a form of diabetes with features of type 2 diabetes (T2DM) but with earlier onset and 5-10-fold greater prevalence in comparable age ranges. To test whether modifier genes play a significant role in CF-related diabetes (CFRD), we determined concordance rates in monozygous CF twins (75% in 12 pairs) and dizygous CF twins and CF siblings (14% in 71 pairs). These concordance rates generate heritability estimates of ~1.0, indicating a significant role for modifier genes in the development of CFRD. Heritability estimates were the same after controlling for age, sex, and CF-causing mutation. To assess whether genetic variants that cause diabetes in the general population contribute to CFRD, we examined three-generation pedigrees of CF families and determined that family history of diabetes (≥1 first-degree or ≥2 second-degree relatives) correlated with increased rates of CFRD (20 of 69 vs. 34 of 271 with no family history; OR=2.84; p=0.001). Variants in TCF7L2, a transcription factor involved in Wnt signaling, have repeatedly associated with T2DM. We hypothesized that these variants may predispose to CFRD. Genotyping of TCF7L2 SNPs and transmission disequilibrium testing (TDT) of 53 trios revealed overtransmission for rs7903146 (p=0.03), rs12243326 (p=0.05) and rs12255372 (p=0.03). In every case, the TCF7L2 allele overtransmitted in CFRD is the same allele that increases risk of T2DM. In a separate analysis of 109 CFRD cases and 37 CF controls (including affected individuals from the trio analysis), association was suggestive at both the genotype (p=0.07, p=0.09, p= 0.05) and allele (p=0.12, p=0.047, p=0.02) levels. Finally, individuals with CFRD who were homozygous for risk alleles were diagnosed at a significantly earlier age (average 14.6 vs. 20.1; p=0.03). These data support a key role for modifier genes in development of CF-related diabetes, and suggest that CFRD and type 2 diabetes share disease mechanisms such as alteration in Wnt signaling.

2542/T

Evaluation of the Klotho and GAS6 positional candidate genes on chromosome 13 for association with non-diabetic end-stage renal disease in African Americans. *M.A. Bostrom[1], P.J. Hicks[1], D.W. Bowden[1,2], B.I. Freedman[3].* 1) Department of Biochemistry, Wake Forest University, Winston-Salem, NC; 2) Center for Human Genomics,Wake Forest University, Winston-Salem, NC; 3) Department of Internal Medicine, Wake Forest University, Winston-Salem, NC.

African Americans have increased susceptibility to hypertensive (non-diabetic) end-stage renal disease (H-ESRD) compared to Caucasians and extensive evidence supports a genetic contribution. A genome-wide scan in African American families with H-ESRD revealed evidence for linkage in two regions on chromosome 13, 13q13.1 (LOD = 3.90) and 13q33.3 (LOD = 5.20). We evaluated 310 non-diabetic African Americans with H-ESRD and 353 African American healthy controls to investigate positional candidate genes in these regions. Using a case control design, we tested polymorphisms in the candidate genes: Growth Arrest Specific factor 6 (GAS 6) at 13q34 (a vitamin K-dependent growth potentiating factor upregulated in mice with glomerulonephritis) and Klotho on 13q13.3 (a type I membrane β-glucuronidase-like protein highly expressed in the kidney and which has reduced mRNA expression in chronic renal failure). A total of 13 tagging SNPs in GAS6 were genotyped in the African American H-ESRD case control collection, 3 of which, (rs7333857, rs9577924, rs11842990), did not conform to Hardy Weinberg expectations. Genotypic association analyses demonstrated minor evidence for association at one SNP, rs11842990, (p = 0.023). Of the 24 tagging SNPs genotyped in the Klotho gene, one SNP (rs564481) did not conform to Hardy Weinberg expectations. Evidence for association was observed at 2 SNPs, rs564481 and rs650439, (p = 0.0003 and p = 0.01, respectively). The rs564481 SNP is located in an exon that is not translated in the truncated Klotho isoform b and further molecular genetic analysis is underway. These data suggest that at least one gene on chromosome 13 is associated with susceptibility to H-ESRD in African Americans.

2543/T

Examination of the IL-4R gene in families with Multiple Sclerosis. *Y. Bradford[1], R.L. Zuvich[1], J.L. McCauley[1], B.M. Anderson[1], N. Schnetz-Boutaud[1], J.R. Oksenberg[2], L.F. Barcellos[3], S.L. Hauser[2], M.A. Pericak-Vance[4], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN, USA; 2) University of California at San Francisco, San Francisco, CA, USA; 3) University of California at Berkeley, Berkeley, CA, USA; 4) University of Miami School of Medicine, Miami, FL, USA.

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system with a complex etiology. While the contribution of the Major Histocompatibility Complex (MHC) to genetic susceptibility for MS has been long established with strong associations to the HLA-DRB locus (primarily to the HLA-DRB1*1501 allele), identifying other risk genes has been problematic. However, we recently identified and confirmed a strong association with a coding SNP within the IL-7R gene. Given these strong findings along with the nature and interplay of cytokines and their central involvement within the body's immune response, we hypothesized that additional cytokines or other immunity-mediated mechanics may play a substantial role in MS susceptibility. One such putative candidate gene, interleukin-4-receptor (IL-4R), is known to stimulate B-cell and T-cell development and differentiation. Additionally, IL-4R has been associated with other autoimmune diseases such as asthma and type-1 diabetes, and we had previously identified an association of IL-4R with MS in African-Americans. To investigate the possible association of the IL-4R gene with MS in a Caucasian population, we have initially genotyped 8 SNPs across an approximately 44kb region in 170 Caucasian multiplex families with 186 affected sib-pairs and 101 other affected relative pairs. However, our initial analysis shows no strong evidence of association between MS and IL-4R, suggesting that the IL-4R association may be specific to African-Americans.

2544/T

The role of HLA-DRB5 and -DRB1 loci in susceptibility to multiple sclerosis: a study of 769 African-American cases and 751 controls. *F. Briggs[1], S.J. Caillier[2], L.F. Barcellos[1], B.A.C. Cree[2], S.L. Hauser[2], J.R. Oksenberg[2].* 1) School of Public Health, Univ of California,Berkeley, CA; 2) Department of Neurology, Univ of California, San Francisco, CA.

Genetic susceptibility to multiple sclerosis (MS) is associated with the major histocompatibility region (MHC) region located on chr. 6p21. A strong consistent signal maps to a 200 kb region encompassing the HLA-class II loci and segregates with the HLA-DQB1*0602, DQA1*0102, DRB1*1501, DRB5*0101 haplotype. Very strong linkage disequilibrium between these loci (and therefore lower haplotype diversity) in northern Europeans has made it difficult to fully characterize individual genetic contributions of this region to MS risk. A recent study in African-Americans (greater MHC haplotype diversity), however, has demonstrated selective MS associations with HLA-DRB1*1501 and *1503 independent of DQB1*0602, confirming the power of this approach to fine-map susceptibility loci. While HLA-DRB1 appears to mark the centromeric border of the class II association in MS, the telomeric border has not been defined. This haplotype carries two functional DR beta chain genes, DRB1 and DRB5, and two different DR dimers can thus be formed by pairing with the non-polymorphic DR alpha chain; results suggesting an important functional role for HLA-DRB5 in MS have been reported. In this study, molecular typing of HLA-DRB5, -DRB1 and seven informative SNPs was performed in 769 African-American MS cases and 751 controls to define the telomeric border of this region. Extended MHC haplotypes were characterized and compared in cases and controls using Fisher's exact testing and logistic regression modeling. While strong associations were observed for HLA-DRB1, DRB5 and two SNPs, our results are consistent with a primary role for the HLA-DRB1 gene in conferring susceptibility to MS.

2545/T

Potential role of *p63* in human bladder exstrophy. *B.J. Ching[1], M. Ludwig[2], H. Reutter[3], C. Nauta[1], J.P. Gearhart[4], S.A. Boyadjiev[1,4].* 1) Section of Genetics, Dept. of Pediatrics, University of California Davis, Sacramento, CA; 2) Dept. of Clinical Biochemistry, University of Bonn, Bonn, Germany; 3) Dept. of Human Genetics, University of Bonn, Bonn, Germany; 4) Dept. of Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University, Baltimore, MD, United States.

The Bladder-Exstrophy-Epispadias-Complex (BEEC) represents a spectrum of urogenital anomalies in which part or all of the distal urinary tract fail to close and are exposed on the outer abdominal wall. Clinically, this rare congenital anomaly ranges from epispadias (EP) to classic bladder exstrophy (CBE), to its most severe form - cloacal exstrophy (CE). *p63*, a homolog of the *p53* tumor-suppressor gene, encodes multiple tissue-specific isoforms acting as transcription factors vital for correct embryologic development. Δ*N-p63-/-* null mice manifest bladder exstrophy in addition to severe craniofacial, limb, and skin anomalies. Human *p63* mutations are associated with at least five autosomal dominant genetic syndromes with anomalies of the urogenital system, but not BEEC. We have initiated *p63* analysis in a cohort of 15 CBE and five CE patients. Direct sequencing of the entire coding region of *p63* from genomic DNA did not yield obvious mutations. RT-PCR of COOH-terminal cDNA fragments derived from normal and exstrophic human bladder and lymphoblast RNA did not identify abnormal p63 expression and several novel isoforms were identified and validated. Sequencing of the COOH-terminal isoform specific RT-PCR products did not show nucleotide changes. N-terminal isoform specific RT-PCR, northern blot and quantitative real-time PCR analyses are underway to further assess if *p63* plays causal role in BEEC.

2546/T

S-nitrosoglutathione Reductase and β_2-Adrenergic Receptor Gene-Gene Interaction is Associated with Asthma in Latinos. *S. Choudhry[1], L.G. Que[2], L. Liu[1], C. Eng[1], S. Nazario[3], J. Casal[3], A. Torres[3], J. Salas[4], R. Chapela[4], J. Rodriguez-Santana[5], P.C. Avila[6], W. Rodriguez-Cintron[3], E.G. Burchard[1].* 1) University of California, San Francisco, CA; 2) Duke University, Durham, NC; 3) VAMC, San Juan, PR; 4) INER, Mexico City, Distrito Federal, Mexico; 5) Pediatric Pulmonary Program, San Juan, PR; 6) Northwestern University, Chicago, IL.

S-nitrosoglutathione (GSNO), an endogenous bronchodilator present in airway lining fluid (ALF) of healthy subjects, is depleted from ALF of subjects with asthma. GSNO reductase (GSNOR) is the enzyme that metabolizes S-nitrosothiols in vivo. Deletion of the GSNOR gene protected mice from experimental asthma and β_2-receptor desensitization to β_2-agonists. We hypothesized that genetic variants in the GSNOR gene may be associated with asthma. We also reasoned that there may be biological interactions between GSNOR and β_2-adrenergic receptor (β_2AR), which modulate response to bronchodilators. To test our hypotheses we performed family-based and gene-gene interaction analyses in Puerto Rican (n=386) and Mexican (n=300) trios with asthma. Five SNPs, one in the promoter region and four in the 3' UTR, were tested for association with asthma and related phenotypes. Family-based analyses demonstrated that several GSNOR SNPs, including the SNP in the promoter region ($p = 0.05$) and 3 SNPs in the 3' UTR ($p = 0.03$ to 0.008), and haplotype ($p = 0.02$) were significantly associated with asthma in Puerto Ricans. No association between GSNOR SNPs and asthma was found in Mexicans. However, we found evidence of GSNOR-β_2AR interaction and its association with bronchodilator response in both Mexican and Puerto Rican asthmatics (combined $p = 0.001$). Our data suggest a genetic association of GSNOR with asthma diagnosis and a GSNOR-β_2AR gene-gene interaction associated with bronchodilator response. These results further implicate GSNO in asthma pathogenesis and bronchodilator responsiveness.

2547/T

***PON2* Polymorphisms, PON Activity, and Systemic Lupus Erythematosus (SLE).** *S. Dasgupta[1], F.Y. Demirci[1], A.H. Kao[2], E.Y. Rhew[3], F. Bontempo[4], C. Kammerer[1], R. Ramsey-Goldman[3], S. Manzi[2], M.I. Kamboh[1].* 1) Dept. of Human Genetics, Univ Pittsburgh, GSPH, Pittsburgh, PA; 2) Lupus Center of Excellence, Univ. of Pittsburgh, Pittsburgh, PA; 3) Div. of Rheumatology, Northwestern Univ., Chicago, IL; 4) Dept. of Medicine, Univ. of Pittsburgh, Pittsburgh, PA.

SLE is a multisystem autoimmune disease that predominantly affects the women at childbearing age. The risk of coronary heart disease (CHD) in SLE women is up to 50 times higher than in the general population. Several studies have implicated the association of low paraoxonase (PON) activity with CHD and our studies have demonstrated that low PON activity is independently associated with SLE. Two SNPs in the PON1 gene (codon 55 and codon 192) are known to be major regulators of serum PON activity, though the extent of contribution from subsequently characterized PON2 and PON3 genes remain to be determined. The purpose of this study was to determine the impact of *PON2* polymorphisms on PON activity and risk for SLE. Eleven *PON2* Tag SNPs, including two non-synonymous SNPs, were genotyped in 350 Caucasian SLE patients and 454 Caucasian healthy control women using RFLP, Pyrosequencing, or TaqMan allelic discrimination methods. Haploview analysis using our data revealed pairwise LD ($D' > 0.8$) for several SNP pairs, however, strong correlation ($r^2 > 0.8$) were observed only for two SNP pairs (rs9641164 & rs1639 and rs3735586 & rs6954345) therefore one SNP from each SNP pair (rs9641164 and rs3735586) were excluded from haplotype analysis. Haplotype analysis revealed significant association with SLE risk ($P < 0.001$). Five SNPs revealed significant association ($P < 0.05$) with age, BMI, and smoking-adjusted serum PON activity in single-site analyses in both cases and controls. The effects of 2 SNPs (rs6954345 and rs11545491) remained significant in a multiple linear regression model that also included the two *PON1* SNPs (codon 55 and codon 192). We identified specific haplotypes significantly associated ($P < 0.05$) with either low or high serum PON activity. These results indicate that in addition to the known effect of *PON1* on PON activity, *PON2* genetic variation also contributes towards PON activity and SLE risk.

2548/T

***TNFSF13* (*APRIL*) Polymorphisms and Systemic Lupus Erythematosus.** *F.Y. Demirci[1], S. Manzi[2], R. Ramsey-Goldman[3], A.H. Kao[2], E.Y. Rhew[3], F. Bontempo[4], C. Kammerer[1], M.I. Kamboh[1].* 1) Dept of Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Lupus Center of Excellence, Univ of Pittsburgh, Pittsburgh, PA; 3) Div of Rheumatology, Northwestern Univ, Chicago, IL; 4) Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with a broad range of clinical manifestations. It predominantly affects women of childbearing age and its prevalence varies across different ethnic groups. SLE has complex genetic basis and is caused by complex interaction of unknown environmental factors and multiple genetic susceptibility loci on different chromosomes. *TNFSF13* (also known as *APRIL*) and *BLyS* (B lymphocyte stimulator) are members of the tumor necrosis factor superfamily. BLyS/APRIL pathway has been strongly implicated in autoimmunity as suggested by mouse studies and observation of increased protein levels in serum and synovial fluids of patients with SLE or rheumatoid arthritis. *TNFSF13* is located on chromosome 17p13.1 (positional candidate for SLE) and its variants have recently been reported to be associated with SLE in Japanese. The purpose of this study was to replicate the reported association of two *TNFSF13* coding SNPs, rs11552708 (Gly67Arg) and rs3803800 (Asn96Ser), in our Caucasian American SLE case-control cohort. DNA samples from 409 SLE-affected female subjects and 509 healthy female controls were genotyped using TaqMan SNP genotyping assays (ABI). The genotype frequencies were in Hardy-Weinberg equilibrium in both case and control groups. None of the two *TNFSF13* coding SNPs showed a statistically significant association with SLE in our Caucasian cohort. Our results do not indicate a major impact of these putative functional *TNFSF13* SNPs on the susceptibility to (or protection from) SLE in Caucasians.

2549/T

Common sequence variation in genes that cause hypogonadotropic hypogonadism and association with age at menarche. *Z. Gajdos[1,2], K. DeLellis Henderson[3], J. Butler[1,2], P. Clayton[5], L. Le Marchand[6], L. Kolonel[6], B.E. Henderson[3], M.R. Palmert[4], J.N. Hirschhorn[1,2].* 1) Children's Hospital, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Univ. Southern Calif., Los Angeles, CA; 4) Case Western Reserve Univ., Cleveland, OH; 5) Univ. of Manchester, Manchester, UK; 6) Univ. of Hawaii, Honolulu, HI.

Background: Genetic factors are estimated to account for more than half of the population variation in the timing of puberty, yet the specific genes responsible are unknown. At the extreme, patients with hypogonadotropic hypogonadism (HH) have absent or severely delayed puberty. Genes that carry severe mutations that cause HH have been identified (*FGFR1*, *KAL1*, *KISS1*, *GNRHR*, *GPR54*, *LEP*, *LEPR*, *PROK2*, *PROKR2*, and *FGF8*). We hypothesized that common sequence variants in these genes might influence the normal spectrum of variation in pubertal timing. **Results:** We genotyped 393 SNPs (217 in all subjects, 176 additional in the African-American subjects) in 8 HH genes or their ligands (*FGFR1*, *KAL1*, *KISS1*, *GNRHR*, *GPR54*, *LEP*, and *LEPR*) in 1801 women with early (age < 11 years, N= 909) or late (age > 14 years, N=892) menarche drawn from the Hawai'i and Los Angeles Multiethnic Cohort. SNPs were selected using HapMap genotype data and the Tagger software package as implemented in Haploview. We performed an association analysis, stratified by self-reported ethnicity, using the Cochran-Mantel-Haenszel test in the software package PLINK to test SNPs for association with age at menarche. Nominally significant associations were identified in *KISS1*, *GPR54*, and *LEPR*. Interactions between SNPs were also tested for association, with several nominally significant interactions observed. To further control for effects of ancestry on menarche, we genotyped 69 ancestry informative markers specific for all HapMap populations. We will also analyze these SNPs to test for the association of ancestry with age at menarche. **Conclusion:** Although the HH genes tested here are important for pubertal development, we have not found strong evidence that common variants in these genes substantially regulate the timing of puberty.

2550/T

Association of polymorphisms in folate pathway genes (MTHFR, CBS, MTRR and GCPII) and risk for neural tube defects in the State of Yucatan, Mexico. *L. Gonzalez-Herrera, I. Castillo-Zapata, M.G. Garcia-Escalante, D. Pinto-Escalante 1, T. Canto-de Cetina.* Dept Genetica, Univ Autonoma de Yucatan, Meridal, Yucatan, Yucatan, Mexico.

Neural tube defects (NTD) are prevalent congenital malformations in the state of Yucatan, Mexico (22.31 per 10,000 births). Several candidate genes have been derived from the folate pathway including MTHFR, MTRR, CBS and GCPII, since their allelic variants might increase the risk for NTD. Frequency of polymorphisms C677T and A128C in MTHFR, ins68bp in CBS, A66G in MTRR and C1561T in GCPII, was evaluated for an association with the risk for NTD in the State of Yucatan, Mexico. 96 newborn patients with non-syndromic NTD, as well 82 of their mothers and 52 of their fathers were analysed and compared with an ethnically matched control group of 115 healthy volunteers. Genotypification was performed by PCR-RFLPs. Allelic and genotypic frequencies were compared between cases and controls for gender and phenotype stratification in EpiInfo software (OR, IC 95%;). Genotypic frequencies in control group for the five polymorphisms were according to Hardy-Weinberg expectations ($p > 0.33$). Polymorphisms C677T-MTHFR, ins68bp in CBS, and C1561T in GCPII did not show significant differences between cases and control ($p > .05$), suggesting that these variants are not associated risk factors for NTD in Yucatan. Frequency of polymorphism A66G-MTRR were significantly higher in controls (50.8%;) than in cases (8.4%;) ($p < 0.0001$), suggesting that this allele might be associated as a protection factor in the population (OR: 0.10, IC 0.05-0.17). The variant A1298C-MTHFR showed a gender specific distribution and its frequency was significantly higher in male ($p = 0.017$). A1298C-MTHFR was associated with NTD in female affected newborns (OR= 2.62 IC: 0.92-7.62) and with mothers of NTD (OR 3.01, IC: 1.18-9.75). According to phenotype stratification, mothers of anencephaly offspring showed the strongest association for the allele A1298C (OR= 4.33 IC:1.23-15.29), suggesting that this polymorphism is an associated risk factor in female for both to develop an NTD and for having offspring with anencephaly in the Yucatan population.

2551/T

Genetic evidence that insulin secretion plays a role in the development of polycystic ovary syndrome: the *FEM1B* gene. *M.O. Goodarzi[1], J.F. Maher[2], H.J. Antoine[1], J. Cui[1], Y-D.I. Chen[1], W.A. Hsueh[3], X. Guo[1], J.I. Rotter[1], R. Azziz[1].* 1) Cedars-Sinai Med Ctr, Los Angeles, CA; 2) UT Southwestern Med Ctr, Dallas, TX; 3) UCLA Med Ctr, Los Angeles, CA.

Polycystic ovary syndrome (PCOS), the most common endocrine disorder of reproductive age women, is characterized by infertility, hyperandrogenism, and hyperinsulinemia. The human *FEM1B* gene is a homolog of *fem-1*, a sex-determination gene of *C. elegans* that controls masculinization. Herein, we consider *FEM1B* as a candidate gene for PCOS, a disorder of masculinization. Mice with knockout of the *fem1b* gene displayed abnormal glucose tolerance and impaired acute phase insulin secretion. To first confirm a role of *FEM1B* in human insulin secretion, we studied 804 individuals from 191 families (Mexican-Americans from Los Angeles, CA). Insulin secretion (insulinogenic index at 30 minutes, IGI30) was quantified by oral glucose tolerance test in 518 subjects. We genotyped 3 single nucleotide polymorphisms (SNPs) in *FEM1B*; all were in the same haplotype block. Generalized estimating equation methods were used in the association analysis. Haplotype GGA (frequency 22%), which is identified by the minor allele of SNP rs10152450, was associated with decreased insulin secretion ($P=0.02$). We then evaluated the role of *FEM1B* in PCOS. We genotyped 287 women with PCOS and 187 controls (all non-Hispanic Whites from Birmingham, AL). Association with PCOS was evaluated with logistic regression; association with quantitative traits was tested using ANCOVA. Carriers of the minor allele of rs10152450 had a reduced frequency of PCOS (odds ratio 0.52, $P=0.01$). Minor allele carriers of this SNP also had lower insulin secretion ($P=0.01$) calculated as HOMA-%B (index based on fasting glucose and insulin). Haplotype GGA exhibited the same associations. This haplotype occurred with a 28% frequency in PCOS and 37% in controls ($\chi^2=7.2$, $P=0.007$). These results implicate *FEM1B* in insulin secretion in two distinct populations, consistent with the mouse data, and suggest that inherited reduction in insulin secretion can protect against PCOS. The latter is consistent with *in vitro* data that insulin promotes ovarian androgen secretion.

2552/T

Genetic Variants in the Lipoprotein Lipase Gene Are Associated with Both Liver Enzyme Levels and Insulin Resistance. *X. Guo[1], J. Cui[1], M.O. Goodarzi[1], K.D. Taylor[1], F-C. Hsu[2], S. Haffner[3], J.M. Norris[4], L. Wagenknecht[2], Y-D.I. Chen[1], J.I. Rotter[1].* 1) Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Wake Forest University, Winston-Salem, NC; 3) University of Texas Health Science Center, San Antonio, TX; 4) University of Colorado Health Sciences Center, Denver, CO.

Elevated liver enzyme (LE) levels have been related to insulin resistance (IR) and the metabolic syndrome. Heritability and co-heritability analyses indicate significant evidence for a genetic contribution to LE levels. We have previously reported that LE levels share common genetic determinants with IR. The lipoprotein lipase (LPL) gene is a potential candidate for LE levels as it has been shown to be associated with IR in two different cohorts of Hispanic Americans (HA). We evaluate here the role of genetic variants in the LPL gene on LE levels. 1017 non-diabetic individuals from 88 large HA families were recruited through the Insulin Resistance Atherosclerosis Study Family Study at two clinical sites (San Antonio, TX and San Luis Valley, CO). Three liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were measured. None of the subjects self-reported a high alcohol consumption. Twelve single nucleotide polymorphisms (SNPs) in the LPL gene (all in the same block) were genotyped on these samples. The generalized estimating equation methods were used in the association analysis. After adjusting for age, sex, and body mass index, the second most common haplotype, which accounts for 18.8% of the sample and is identified by the minor alleles in SNP rs8292 and rs3200218, was significantly associated with increased GGT (40.8±2.1 vs 35.8±1.3, p=0.009), ALT (11.9±0.5 vs 10.4±0.3, p=0.010), and AST/ALT ratio (p=0.007). This is the same haplotype that we had previously reported being significantly associated with increased fasting insulin and triglycerides in this HA sample (Goodarzi MO, et al. J Clin Endocrinol Metab 92: 293-296, 2007). These results suggest that the LPL gene is a common genetic determinant for LEs and IR in the Hispanic American population, and that variation in LEs is a genetically determined component of the metabolic syndrome.

2553/T

Association between TGFB1 and age-related cortical cataract. *C.J. Hammond[1,2], F. Zhang[1], T.D. Spector[1].* 1) Twin Research Unit, Kings College London School of Medicine, London, United Kingdom; 2) West Kent Eye Center, Bromley Hospitals NHS Trust, Orpington, UK.

Purpose: Our twin studies have previously shown a significant heritability for age-related cataract: cortical cataract (0.58) and nuclear cataract (0.48). TGFB1, a gene containing 7 exons and spanning 23.5kb on chromosome 19q13 (MIM 190180), has been linked with aging traits including osteoporosis, cerebrovascular disease and cancer. It has also been associated with anterior subcapsular cataract and posterior capsule opacification in animal studies, by promoting epithelial to mesenchymal transition. We performed an association study to examine whether TGFB1 might be involved in age-related cataract.

Methods: 1012 twin subjects (mean age 63, range 50-79 years) from the TwinsUK Adult Twin Registry were phenotyped for cataract with lens photography (Scheimpflug and retroillumination images) and graded automatically. Some twins underwent genotyping of 6 TGFB1 SNPs (2 promoter region, 3 exonic, 1 intronic) which had previously been shown to be of functional importance. Logistic regression including subjects' age was used to calculate associations, with family cluster analysis to take into account twin association.

Results: 327 subjects were genotyped, and of these 71 were cases (>5% lens area cortical cataract) and 256 controls. All SNPs were in Hardy-Weinberg equilibrium. There was no association with nuclear cataract for any SNPs in TGFB1. Significant association with cortical cataract was found for 2 SNPs in the promoter region of TGFB1 (p=0.001 and p=0.017), but there was no association for 4 exonic SNPs examined.

Conclusions: TGFB1 may play a role in susceptibility to age-related cortical cataract. Replication of these results is required, and further investigation including the possible role of TGFB1 in racial differences of prevalence of cortical cataract.

2554/T

Gene-gender interactions in Systemic Lupus Erythematosus. *S. Han[1], I. Harley[1], A.L. Sestak[1], X. Kim-Howard[1], K.M. Kaufman[1,2,3], G. Bruner[1], J.M. Guthridge[1], G. Gilkeson[4], J.B. Harley[1,2,3], J.A. James[1,2], S.K. Nath[1].* 1) Oklahoma Medical Research Foundation; 2) VA Medical Center; 3) University of Oklahoma Health Sciences Center, Oklahoma City, OK,USA; 4) Medical University of South Carolina, USA.

Osteopontin (SPP1) gene polymorphisms have been shown to associate with SLE in small cohorts. This study tested association between SPP1 polymorphisms and SLE in a large, multi-ethnic cohort of 1488 unrelated SLE patients [707 European-American (EA), 549 African-American (AA), 232 Hispanics (HIS)], including 153 males, and 2321 unrelated controls (1309 EA, 834 AA, 178 HIS). To control for potential population stratification, admixture adjusted logistic regression, genomic control (GC), structured association (STRAT) and principle components analysis (PCA) were applied. Twelve SNPs out of 33 were in HWE and used for analysis. The pooled analysis of 3 ethnic groups revealed significant gene-gender interactions. Initially, 4 SNPs (SNP1, 6, 10, 12) showed significant association with SLE in males, but not in females. In accordance with the result, the interactions between the 4 SNPs and gender were also significant (P=0.02, 0.001, 0.005, 0.003, respectively). Further haplotype analysis of 3 SNPs (SNP10, 11, 12) demonstrated a significant association in males (P=0.0002) and interaction with gender (P=0.005). Subgroup analysis with single SNP and haplotype also identified the same pattern of gene-gender interactions in AA and EA. In AA males, 4 SNPs (SNP4, 6, 10, 12) demonstrated significant associations and a strong haplotype association (P=0.00004). In EA, 3 SNPs (SNP10, 11, 12) showed significant associations in males and a haplotype association (P=0.02). Additionally, haplotype interaction analysis with gender of the 3 SNPs was significant in AA and EA (P=0.006, 0.02, respectively). Although, for HIS, 2 SNPs (SNP2, 6) were associated with SLE, no gender effects were detected. All the associations remained consistent with GC, STRAT or PCA in subgroup analysis. Therefore, our data suggest SPP1 is associated with SLE in males. To our knowledge, this report serves as the first description of a human male lupus genetic risk.

2555/T

Association of IGF2 gene mutation with Type 2 diabetes mellitus and Diabetic Nephropathy. *Q. Hasan[1,2,3], S. Movva[2], S. Saharia[2], Y.R. Ahuja[3].* 1) Department of Genetics and Molecular medicine, Kamineni Hospital, Hyderabad, Andhra Pradesh, India; 2) Department of Genetics, Bhagwan Mahavir Hospital and Research Centre, A.C.Guards, Hyderabad- 500004, India; 3) Department of Genetics, Vasavi Hospital and Research Centre, Lakdi ka-pool, Hyderabad-500004, India.

Numerous metabolic pathways and associated groups of genes have been proposed as candidates having a role in the genetic susceptibility to type 2 diabetes mellitus (DM) and its devastating complication diabetic nephropathy (DN). Products of a wide range of genes might mediate the onset of DM and the renal changes resulting in DN. IGF2 is a widely expressed peptide that is essential for normal development. It is a growth promoting polypeptide that shares a high degree of structural homology with insulin. IGF2 is synthesized primarily by the liver, but it is also produced locally by many tissues, where it acts in an autocrine or paracrine manner. It is a 67 amino acid neutral polypeptide and is the major IGF present in human plasma. Animal studies have suggested that activation of a number of growth factor systems, including the insulin-like growth factors, may be involved in the development of DN. However, to date there are no studies of this gene in relation to DM or DN. Hence a 100bp region in exon 7 was PCR amplified and screened by SSCP in 336 individuals, which included DN, DM and controls. 11 percent of DN cases and 5 percent of diabetics showed a mobility shift compared to none of the controls. Sequence analysis identified a novel mutation which causes a deletion of 'A' at 827 bp position, resulting in a frame shift mutation, which affects the entire sequence and may be affecting the structure or the activity of IGF2. This newly identified mutation in the exon 7 of IGF2 gene could be associated with early onset of DM symptoms and early development of DN in diabetics, who carried this mutation. More studies in different ethnic groups and larger sample size are warranted with this IGF2 mutation to establish it's association with diabetes and its complications and also to establish it as a diagnostic or prognostic marker.

2556/T

Polymorphism in the CYSLTR1 gene is associated with asthma in a Chinese population. *X. Hong[1], H. Zhou[2], H.J. Tsai[3], X. Xu[2], X. Wang[3], X. Xu[1].* 1) Center for Population Genetics, School of Public Health, University of Illinois at Chicago, chicago, IL; 2) Program for Population Genetics, Harvard School of Public Health, Boston, MA; 3) Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center; Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Asthma is a heterogeneous respiratory disease characterized by chronic inflammation of the airways, reversible brochoconstriction, airway hyperreactivity, eosinophilia, and musus hypertecretion. We conducted a genetic study among 174 asthmatic cases and 347 matched controls using a candidate-gene association approach in a Chinese population. A total of 129 SNPs of 113 asthma-related candidate genes, which are either located in the coding regions or in the exon-intron junction regions, were genotyped using Sequenom MassArray technology. A significant association was found between SNP rs320995 in the CYSLTR1 gene and physician-diagnosed asthma after adjusting age, age squared, height, height squared, weight, smoking status and gender. Under the recessive model, subjects with GG genotype in SNP rs320995 had 2.8 times higher risk of developing asthma than those with AA or AG genotype (OR=2.8; 95% CI=1.7-4.6; p = 0.00007). We also observed significant associations of SNP rs320995 with baseline FEV1/FVC (p =0.00001), and eosinophil counts (p = 0.00002). Of note, all the associations remained statistically significant after Bonferroni correction for multiple tests. Since this SNP is located on X chromosome, we performed association tests stratified by gender. But no gender effect was found on the association between rs320995 and asthma-related phenotypes. The CYSLTR1 receptor, when activated by cysteinyl leukotrienes, can mediate proliferation and contraction of smooth muscle and eosinophil migration to the lung. Our results provided strong evidence that genetic predisposition of rs320995 in the CYSLTR1 gene may play a role in the development of asthma. Further replication in an independent population is needed to validate these results.

2557/T

Common variant of SOSTDC1 is associated with increased risks of fractures and osteoporosis--A novel candidate gene revealed by fine mapping from Anhui genome-wide scan. *Y. Hsu[1,2], T. Niu[1], H. Terwedow[1], C. Rosen[3], J. Brain[1], X. Xu[4].* 1) Mol & Integ Physiol Sci Pgm, Harvard Sch Public Health, Boston, MA; 2) Hebrew SeniorLife and Harvard Medical Sch, Boston, MA; 3) Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME; 4) Sch Public Health, UIC, Chicago, IL.

Previously, we have revealed 3 novel QTLs (LODs >3.65) on Chr7p21, Chr2q24 and Chr5q21 for bone mineral density (BMDs) in a genome-wide scan of 3093 adult Chinese siblings selected based on their extreme hip BMD. To narrow down the QTL region, we first genotyped 10-20 microsatellite markers in each of the QTLs in the same 3093 siblings. Fine mapping of the Chr7p21 QTL narrowed to a 8 cM region (LOD=3.72). Among 23 known genes in this region, twist homolog 1 (TWIST1) and sclerostin domain-containing protein 1 (SOSTDC1) have been known functionally relevant to bone metabolism. To test whether polymorphisms in the TWIST1 and SOSTDC1 are associated with osteoporosis, we genotyped tag SNPs in an independent set of 2392 extreme low FN BMD cases (T-score <-1) and extreme high FN BMD controls matched by age and sex, selected from a study of 23,327 Chinese. Multiple logistic regression with additive genetic model was used. First, the adjusted ORs for extreme low FN BMD was associated with 3 adjacent SNPs located in exon1 and intron1 of SOSTDC1 (ORs 1.4-1.6, p<0.00004, permutation test p<0.0005) in men. A weak association was found in women for the SNP located in exon1 (p=0.017). No association was found for TWIST1 SNPs. Second, the ORs(95%CI) for men carrying the polymorphic allele A for the strongest associated SNP (rs16878762, MAF=0.29) of SOSTDC1 were 1.7(1.3-2.4) for osteoporosis, and 2.0(1.1-3.7) for osteoporotic fractures. Third, SNP rs16878762 was associated with SOSTDC1 gene expression in men(p=0.004) and women(p=0.049). Notable, despite the close homologue to sclerostin (SOST), no association was found for SOST polymorphism (SRP9) in our study population. In sum, results from linkage, population-based association, and gene expression studies all suggest SOSTDC1 variants may causally reduce BMDs and increase risks of osteoporotic fractures.

2558/T

Adiponectin gene *ADIPOQ* tagging SNP haplotype associations with serum adiponectin and modulation of gene expression by promoter SNPs. *T. Kyriakou[1], L.J. Collins[1], X. Wang[2], H. Snieder[2,3,4], R. Swaminathan[5], D.J. Hart[4], T.D. Spector[4], S.D. O'Dell[1]*. 1) Nutritional Sciences Division, King's College London, United Kingdom; 2) Department of Pediatrics, Medical College of Georgia, Augusta, GA, USA; 3) Department of Epidemiology, University of Groningen, The Netherlands; 4) Twin Research and Genetic Epidemiology Unit, King's College London, London, UK; 5) Department of Clinical Chemistry, King's College London, London, UK.

Adiponectin is a potent insulin sensitizer in muscle and liver and low serum levels are associated with obesity and insulin resistance. We selected 8 tagging SNPs representing 12 common variants in the adiponectin gene (*ADIPOQ*) and tested their effect on serum adiponectin and measures of body fat in two independent samples of Caucasian females: the Chingford Study (n=808, mean age 62.8±5.9 years) and Twins UK (n=2718, mean age 47.4±12.6 years). In the Chingford cohort, tSNPs rs17300539 (-11391 G/A), rs182052 (-10068 G/A), rs16861209 (-7734 C/A), rs1501299 (+276 G/T) and rs1063537 (+3228 C/T) were significantly associated with fasting serum adiponectin levels (Ps=0.0001 to 0.014), explaining between 1.0% and 1.7% of the variance. Associations with all except rs1063537 were replicated in the Twins UK cohort (Ps=3.19 x 10^{-9} to 0.006), explaining between 0.93% and 1.88% of the variance. In addition rs16861209 was associated with BMI, weight, total fat mass, % fat, central fat mass and waist circumference (Ps=0.003 to 0.021). In order to investigate potential functional effects of SNP -11391 G/A, we cloned 1.2 kb of the *ADIPOQ* promoter region, which included SNPs -11391 G/A and -11377 C/G (rs266729), in a luciferase reporter plasmid. The four possible haplotype promoter constructs were transfected in differentiated 3T3-L1 adipocytes. Reporter gene assays showed that the -11391 G/A SNP had an effect on promoter activity only in the presence of the -11377 G-allele. The -11391G / -11377G promoter construct had approximately 2-fold higher promoter activity than the other three haplotypes tested. It therefore remains to be elucidated whether -11391 G/A or -11377 C/G is the causative SNP associated with serum adiponectin levels in the cohort studies.

2559/T

Glucose homeostasis, adiposity, and Insig2: genetic analysis in the IRASFS. *C.D. Langefeld[1], M.E. Talbert[2], J.M. Norris[4], S.M. Haffner[3], D.W. Bowden[2]*. 1) Public Hlth Sci, Wake Forest U School of Med,Winston Salem,NC; 2) Cntr for Human Genomics,Wake Forest U School of Med,Winston Salem,NC; 3) Dept of Preventive Med,U of Colorado Health Sci Cntr,Denver,CO; 4) Dept of Med,UT Health Sci Cntr,San Antonio,TX.

The Insulin-induced gene 2 (Insig2) mediates feedback inhibition of cholesterol synthesis by inhibiting Sterol Response Element Binding Proteins (SREBPs). Insig2 has been the subject of intensive genetic association analysis following strong association of SNP rs7566605 with BMI in Caucasians and African Americans (Herbert Science. 312:279-284, 2006). Insig2 genomic variants may promote abnormal SREBP activation, which could cause altered expression of cholesterol synthesis and glucose homeostasis genes. Additionally, an Insig1 SNP was reported associated with plasma glucose/post-load glucose (Krapivner Diabetologia 50:94-102, 2007). We genotyped rs7566605 and 15 additional Insig2 SNPs in 1425 Hispanics of the Insulin Resistance Atherosclerosis Family Study (IRASFS). CEU HapMap tagSNPs with MAF>5% and r2 threshold of 0.8 were selected and supplemented with HapMap genotyped SNPs, as well as those of dbSNP. SNPs were tested for association with measures of adiposity (BMI, waist circumference, waist to hip ratio, visceral adipose tissue, subcutaneous adipose tissue) and glucose homeostasis (fasting insulin, fasting glucose, insulin sensitivity, disposition index, acute insulin response) using SOLAR. No association was observed between rs7566605 and any adiposity or glucose homeostasis traits. SNPs rs17047718 (Promoter) and rs12623648 (3'UTR), however, were consistently associated with glucose homeostasis measures: fasting insulin, fasting glucose, insulin sensitivity, and HOMA (P=.0007-.04). These SNPs were also associated with obesity measures, visceral adipose tissue (P=.002-.04) and subcutaneous adipose tissue (P=.01-.04). Additional association with adiposity measures was observed with 3 SNPs, 2 of which were in the same LD block as rs7566605 (P=.002-.04). These analyses support a role for Insig2 in the regulation of adiposity in Hispanics, but also provides evidence of involvement in glucose homeostasis.

2560/T

The -A2518G polimorphism of Monocyte Chemoattractant Protein 1 (MCP-1) is associated with Crohn's disease. *A. Latiano[1], O. Palmieri[1], E. Salvatori[2], M.R. Valvano[1], F. Bossa[1], T. Latiano[1], G. Corritore[1], A. Andriulli[1], V. Annese[1]*. 1) IRCCS CSS Hospital, San Giovanni Rotondo, Italy; 2) Angelini Farmaceutici ACRAF S.p.A., Pomezia, Italy.

MCP-1 is a chemokine able to promote monocytes migration in sites of chronic inflammation. The -A2518G variation in the MCP-1 gene has recently been implicated in the pathophysiology of many autoimmune diseases. Aim: To investigate MCP-1 SNP and protein plasma levels in patients with inflammatory bowel disease (IBD). Methods: The -A2518G SNP was genotyped by RFLP in 671 IBD patients (435 with Crohn's disease [CD], 236 with ulcerative colitis [UC]), 151 CD trios, and in 310 controls (HC). Family-based (TDT) and case-control association analyses were performed. Plasma levels of MCP1 protein in 105 CD patients (48 with active and 57 with inactive disease), 39 UC (19 with active and 20 with inactive disease), and 37 HC were assessed by ELISA. The R702W, G908R, and L1007finsC variants of the CARD15 gene were also genotyped by pyrosequencing. Results: Compared to the frequency in HC (29.5%), the frequency of the risk allele (G) was significantly decreased in the IBD population (24.6%; p=0.02), and more specifically, in the subset with CD patients (23.2%; p=0.006)(TDT: p=0.004). Homo and heterozygous carriers of risk allele were significantly less frequent in IBD (44.1%; p=0.01), and in CD patients (41.6; p=0.003), than in HC (52.6%). No significant difference for the allele (27.3%) and risk genotype (48.7%) frequencies was found in UC. Mean (and median) plasma levels of MCP-1 were not significantly different in IBD patients and controls, irrespective of different genotypes and disease activity. All UC patients had their MCP-1 levels within normal ranges. Conversely, 19 CD patients (18%) had a higher (2 SD) MCP-1 plasma levels, irrespective of disease activity, localization, or MCP-1 genotypes. Conclusion: The investigated variant of MCP-1 gene is a protecting factor for CD. No interaction with CARD15 SNPs, nor a correlation with any clinical sub-phenotypes was found. CD patients carriers of the G allele have a significantly higher MCP-1 plasma levels, which may deserve therapeutic implication.

2561/T

Preliminary Screening for Susceptibility Genes for Congenital Anomalies of the Genitourinary System at 22q11.2. *J. Li-Ling[1], Y. Zhao[1], J. Zhang[1], Y. Fu[1], A. Qian[1], B. Wu[2]*. 1) Department of Medical Genetics, China Medical University, Shenyang 110001, China; 2) Department of Urological Surgery, Second Affiliated Hospital, China Medical University, Shenyang 110004, China.

Substantial proportions of 22q11.2 microdeletion carriers have been observed to have congenital malformation of the genitourinary system ranging from renal malformations, metanephric duct/urinary bladder blockage/backflow to hypospadia and/or cryptorchidism. Although the etiology still remains unclear, it has been postulated that particular gene(s) from the DiGeorge syndrome critical region (DGCR) may predispose to such malformations in both syndromic and isolated forms. Using semi-quantitative real-time PCR, we have systematically analyzed expression of murine homologs of 29 DGCR genes within kidney tissues obtained at days 13, 15, 17 and 19 of mouse development as well as adulthood. Through K-means analysis, expression pattern of these genes were clustered into six groups. Notably, certain genes, known to locate within close proximity, e.g., Serpind1 and Lztr1; Pnutl1 and Tbx1; Pcqap and Pik4ca, respectively, showed similar expression patterns, which seems to suggest that they share common regulatory mechanisms. It was discovered that, nine genes have no expression at all time points. For the remainders, most have very low level of expression during development but not at the early stages. Whilst Pnutl1, Ranbp1 and Mapk1 have relatively higher level of expression at all time points, four genes, including Cdc45l, Hira, Snap29 and Ube2l3 only expressed at critical stages of kidney development. Preliminary mutation screening in 10 patients with isolated genitourinary malformations has revealed that, in one patient featuring isolated cryptorchidism, there have been frequent mutations within exon 2 of SNAP29 gene, affecting codons 5, 6, 9, 40, 44 and 48, among which the one in codon 6 was of nonsense type. Conclusion: A number of DGCR genes, in particular CDC45L, HIRA, SNAP29 and UBE2L3 may play important roles in the development of genitourinary system. In addition to its roles in the pathogenesis of syndrome, the SNAP29 gene may also be involved in the pathogenesis of genitourinary malformations.

2562/T

Genetic evaluation of CNDP1 and CNDP2 polymorphisms in Diabetic Nephropathy. *C.W. McDonough[1], P.J. Hicks[2], B.I. Freedman[3], D.W. Bowden[1-3]*. 1) Molecular Medicine, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

The 5L allele of an SSTR marker in the carnosinase 1 gene (CNDP1) on chromosome 18q22.3 is associated with protection from diabetic End-stage renal disease (ESRD) in Europeans and European Americans (EA). CNDP1 encodes a secreted serum carnosinase which degrades carnosine; potentially predisposing to ESRD via oxidative injury and advanced glycation end-product formation. We identified 55 SNPs by sequencing the exons, promoter and 3' UTR of CNDP1 and the adjacent CNDP2 gene in DNAs from 6 African Americans (AAs) and 6 EAs. 46 SNPs were genotyped in 300 EA subjects with type 2 diabetes mellitus (T2DM)-ESRD) and 310 controls, and in 380 AA subjects with T2DM-ESRD and 364 controls. Three SNPs in CNDP1; two intronic: rs4892247, rs11659237, and one in the 3' UTR: rs2887; were significantly associated in multiple genotypic models in both populations. In EAs the 3 SNPs were associated with risk of T2DM-ESRD in a recessive model: odds ratios OR (p value) 1.95(0.023), 2.03(0.015) and 2.22(0.007) respectively. In AAs, rs4892247 was protective in an additive model: OR (p value) 0.73(0.002); and rs11659237 and rs2887 were associated with risk in an additive model: OR (p value) 1.37(0.003) and 1.33(0.007) respectively. If the EA population was stratified by 5L status, no risk association was seen in 5L/5L homozygotes, but the 3 SNPs were still significantly associated with risk in those with one or no copies of the 5L allele in a recessive model: OR (p value) 2.1(0.022), 2.05(0.024) and 2.27(0.01) respectively. When AAs were stratified by 5L status, rs11659237 and rs2887 showed significant association in 5L homozygotes in an additive model: OR (p value) 1.49(0.027) and 1.45(0.032) respectively; and rs4892247 and rs11659237 were significantly associated in those with one or no copies of 5L allele: OR (p value) 0.72(0.012) and 1.32(0.04) respectively. The 3 SNPs are in high LD These results suggest additional genetic variants in CNDP1 influence susceptibility to T2DM-ESRD.

2563/T

Polymorphisms in coagulation and fibrinolytic pathway genes mark the evolution of host-defense response. *K.P. Mooder[1], A. Siddiqui[1], A. Gordon[1], M. LeBlanc[1], H. Wellman[1], X. Zhang[1], J.A. Russell[1,2], K.R. Walley[1,2]*. 1) Sirius Genomics, Vancouver, BC, Canada; 2) Department of Medicine, St Paul's Hospital, University of British Columbia, Vancouver, BC, Canada.

Proteins intersecting the coagulation and fibrinolytic pathways are thought to play an integral role in host defense mechanisms. As such, polymorphic patterns in genes encoding these proteins likely mark historical exposure to pathogens acting on these pathways. Sepsis is a complex disease characterized by systemic infection and a hyper-inflammatory response. Interestingly, biomarkers from the coagulation and fibrinolytic pathways have been shown to be associated with differential outcomes in septic individuals. In a sepsis cohort of European ancestry (n=700), we observe that two SNPs from the genes PROC (rs2069912) and SERPINE1 (rs7242) are associated with an increased risk of coagulation dysfunction after developing sepsis. Although we have yet to characterize the explicit mechanisms defining this association, it may be that genotypes promoting a pro-thrombotic/anti-fibrinolytic phenotype are of benefit to the host in thwarting pathogens with virulence factors facilitating increased plasmin utilization. However, following systemic infection, this same phenotype may be detrimental to the host by facilitating impaired hemostasis.

2564/T

Potential Role of RUNX2 in Nonsyndromic Sagittal Craniosynostosis. *C. Nauta[1], Y. Dong[2], H. Drissi[2], S.A. Boyadjiev[1].* 1) Section of Genetics, Department of Pediatrics, University of California Davis, Sacramento, CA; 2) Musculoskeletal Research Center, University of Rochester, Rochester NY.

Nonsyndromic sagittal craniosynostosis (NSC) is the most common type of craniosynostosis, occurring in approximately 1 in 5000 live births. Despite this high prevalence, the genetic etiology of NSC remains unknown. RUNX2 is a transcription factor necessary for regulation of chondrogenesis and osteogenesis in mesenchymal stem cell-derived osteochondroprogenitors. In an attempt to identify genetic factors implicated in NSC we performed SNP-based association studies with 384 SNPs around 60 candidate genes in a total of 89 sagittal NSC case-parent trios. Association to RUNX2 was established (rs2396441; p<0.03) and direct sequencing of RUNX2 in a cohort of 20 patients identified 2 rare familial non-synonomous SNPs, which were not present in 130 control chromosomes - c.709C>T (R237C) and c.1489G>A (G497S). Loss-of-function (LOH) mutations of RUNX2 cause Cleidocranial dysplasia (CCD), resulting in late-closing cranial sutures and decreased skull ossification. We hypothesize that RUNX2 gain-of-function (GOF) mutations may cause the opposite phenotype of increased sutural ossification and synostosis. Analogous situation has been documented for MSX2. Using a multimerized RUNX response element driving the luciferase reporter gene, we examined the transactivating potential of the RUNX2 R237C and G497S expression vectors. A clear GOF effect was observed for R237C but not for G497S in mouse calvarial osteoblasts. Our results indicate that specific mutations in the RUNX2 gene may result in NSC through gain of RUNX2 function. Further studies will delineate the precise molecular mechanisms implicating RUNX2 in the genetic etiology of sagittal NSC.

2565/T

Significant association between *TIM1* promoter polymorphisms and protection against cerebral malaria in Thailand. *P. Nuchnoi[1], J. Ohashi[2], R. Kimura[2], H. Hanantachai[1], I. Naka[2], S. Krudsood[1], S. Looareesuwan[1], K. Tokunaga[2], J. Patarapotikul[1].* 1) Faculty of Tropical Medicine, Mahidol University,Bangkok, Thailand; 2) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

The sequential activation of T helper type 1 (Th1) then Th2 cells is essential for regulating pro-inflammatory Th1 cytokines such as IFN-γ and TNF, which have been implicated in the development of cerebral malaria. The T cell immunoglobulin and mucin domain (TIM) family of proteins are cell surface proteins involved in regulating Th1 and Th2 immune responses. In this study, variation screening was performed for TIM1, TIM3, and TIMD4 genes, and the possible association between the detected polymorphisms and the severity of malaria was then examined in 478 adult Thai patients infected with Plasmodium falciparum malaria. The TIM1 promoter haplotype comprising three derived alleles (-1637A at rs7702919, -1549C at rs41297577 and -1454A at rs41297579), which were in complete linkage disequilibrium in the study population, was significantly associated with protection against cerebral malaria (P = 0.0009, chi-squared test; odds ratio = 0.41; 95% confidence interval = 0.24-0.71). Allele-specific transcription quantification analysis revealed that the level of mRNA transcribed from TIM1 was higher for the protective promoter haplotype than for the other promoter haplotype (P = 0.004, Mann-Whitney U-test). Engagement with TIM1 in combination with T cell receptor stimulation induces Th2 cytokine production. Thus, the present results suggest that the higher TIM1 expression associated with the protective TIM1 promoter haplotype confers protection against cerebral malaria. High TIM1 expression may induce production of Th2 cytokines, which inhibit production of Th1 cytokines.

2566/T

Acetyl CoA carboxylase and malonyl CoA decarboxylase gene promoter SNPs are associated with body weight in a large female cohort. *S.D. O'Dell[1], A.K. Lee[1], T. Kyriakou[1], D. Ge[2], G. Liu[3], H. Snieder[3,4], T.D. Spector[4].* 1) Nutritional Sciences Division, King's College London, UK; 2) Center for Population Genomics and Pharmacogenetics, Duke University, Durham, NC; 3) Department of Epidemiology, University of Groningen, The Netherlands; 4) Twin Research and Genetic Epidemiology Unit, King's College London, London, UK.

Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase (CPT1), which transfers LCFA to the mitochondria for β-oxidation. The formation of malonyl CoA is catalysed by acetyl-CoA carboxylase (ACC) and its degradation by malonyl CoA decarboxylase (MCD). The dual action of ACC and MCD therefore modulates malonyl CoA levels and in turn the cytoplasmic accumulation of esterified FA resulting from CPT1 inhibition. Elevated LCFA in the hypothalamus signals energy surfeit and leads to inhibition of feeding. We proposed that genetic variation influencing the level of expression of ACC gene *ACACB* and MCD gene *MLYCD* could modulate signalling of energy surfeit and thereby influence feeding behaviour and body weight. We investigated whether potential functional SNPs in the promoters of *ACACB* and *MLYCD* were associated with anthropometry, body fat and serum leptin in 2614 healthy Caucasian females from the Twins UK cohort (mean age 47.3±12.6 years). For *ACACB* rs16939972, we found significant associations with weight, total body fat, fasting glucose and HOMA (Ps= 0.002-0.021). For *MLYCD* rs880088 we found significant associations with leptin, BMI, weight, waist circumference, total and central body fat, fasting insulin and HOMA (Ps=0.005-0.03). We then tested the effect of rs16939972 alleles on activity of a luciferase reporter gene in transiently transfected HepG2 cells. The constructs contained a 902bp region (from position -865bp to +37bp relative to the transcriptional start site) carrying either allele. There was no significant difference in luciferase reporter gene activity with respect to the *ACACB* rs16939972 allele. In conclusion, both gene promoter SNPs are associated with anthropometry, body fat and insulin sensitivity, but as yet we have no evidence to suggest that either SNP affects gene expression.

2567/T

A glucocorticoid receptor gene haplotype is associated with increased risk for low birth weight infants among Kenyan mothers. *D. Smelser[1], A. Grant[2], C. Bean[3], G. Satten[3], S. Kariuki[5], L. Zhang[4], A.A. Lal[4], Y.P. Shi[4], L. Slutsker[4], B. Nahlen[4], F. ter Kuile[4], V. Udhayakumar[4].* 1) National Office of Public Health Genomics; 2) National Center on Birth Defects and Developmental Disabilities; 3) Division of Reproductive Health; 4) Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; 5) Kenya Medical Research Institute, Kisumu, Kenya.

Background: Inflammatory pathway components play critical roles in mediating preterm and low birth weight births in response to malaria infection. The glucocorticoid receptor mediates cross-talk between the inflammatory response and endocrine pathways. We selected SNPs based on their previous association with glucocorticoid activity, which may be a factor contributing to low birth weight. **Methods:** We examined if three functional SNPs: 3669A>G (rs6198), *Bcl*I (intron 2) and *Tth111*I within the *NR3C1* (glucocorticoid receptor) gene were associated with delivery of a low birth weight infant among Kenya mothers in a malaria endemic area. A total of 735 mothers were included in the study: 674 delivered a normal weight (≥2500g) infant and 61 delivered a low birth weight (<2500g) infant. **Results:** Among the *NR3C1* polymorphisms analyzed, only the 3669A>G SNP was associated with increased risk for delivering a low birth weight infant (OR 3.3; 95% CI: 1.44-7.54). After adjusting for potential confounders of infant sex, parity, maternal anemia, peripheral and placental malaria parasitemia, the GG genotype of the 3669A>G polymorphism was no longer significantly associated with delivery of a low birth weight infant. Haplotypes were constructed for the polymorphisms and are listed here with their population frequencies: AGG (0.691), ACA (0.143), AGA (0.100), ACG (0.40) and GGA (0.026). Only the GGA haplotype was significantly associated with increased risk for delivering a low birth weight infant in both univariate and multivariate analyses [Adjusted OR=4.58; 95% CI: 1.94-10.82]. **Conclusion:** In this Kenyan maternal population, the 3669A>G (rs6198), *Bcl*I (intron 2) and *Tth111*I GGA haplotype of the *NR3C1* gene was significantly associated with delivery of a low birth weight infant.

2568/T

Evidence that the oxytocin receptor plays a role in preterm labor. *K. Stirling[1], M. Johnson[1], M. Cooper[2], M. Marazita[2], M. Shi[3], J. Dagle[1], J. Murray[1].* 1) Pediatrics, University of Iowa, Iowa City, IA; 2) University of Pittsburgh, Pittsburgh, PA; 3) NIEHS/NIH, Research Triangle Park, NC.

Prematurity as a consequence of preterm labor (PTL) affects more than 500,000 infants each year in the US. Oxytocin and its receptor regulate uterine contractions and may contribute to the initiation of labor. We hypothesized that allelic variations in the genes for oxytocin, the oxytocin receptor (OXTR), and oxytocinase (LNPEP) might play a role in genetic predispositions to PTL. Samples for DNA were collected from preterm infants and parents. TaqMan assays were performed to characterize allelic variations in single nucleotide polymorphisms (SNPs) in the oxytocin, OXTR, and LNPEP genes in 476 preterm infant/parent trios (22-36 weeks). TDT analysis using two week sliding windows of gestational age (GA) was used to look for associations with varying GA. To look for the presence of novel genetic variations as a cause of preterm birth, resequencing was done on the oxytocin gene (3 exons) and OXTR gene (4 exons) in 94 early preterm infants and mothers, 180 late preterm infants, and 94 Caucasian controls. We identified one SNP (rs4686301) in the OXTR gene that is significantly associated with birth at 35-36 weeks gestation (p=0.0011). A 2 degree of freedom test for maternal effect on preterm birth revealed two significant SNPs in the OXTR gene (rs237887 and rs237897). Sequencing of conserved noncoding regions in and near the OXTR gene revealed 3 known SNPs and 8 novel sequence variants. Sequencing of OXTR coding sequence revealed 5 rare missense mutations, 4 novel and 1 named. The novel variants were more common in cases than in controls. Our finding of a SNP in OXTR correlating with delivery at 35-36 weeks is preliminary evidence that genetic variation in OXTR plays a role in late preterm birth. The increased frequency of missense mutations in cases also suggests a role for rare variants in OXTR contributing to PTL. Additional genotyping and sequencing of these high yield areas will further characterize critical gene regions that might be associated with PTL and perhaps help to identify high risk populations.

2569/T

Association of *APOH* Promoter Polymorphisms with Lupus Nephritis and Cardiovascular Disease. *S. Suresh[1], E. Jacobs[1], S. Manzi[2], D.K. Sanghera[1], A. Kao[2], F. Bontempo[3], C. Kammerer[1], F.Y. Demirci[1], M.I. Kamboh[1].* 1) Dept of Human Genetics; 2) Lupus Center of Excellence; 3) Dept of Medicine, Univ. of Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that predominantly affects premenopausal women. Cardiovascular disease and nephritis are major cause of death in these patients. Apolipoprotein H (β_2-glycoprotein I) is necessary for binding of anionic phospholipids to certain antiphospholipid antibodies in SLE and antiphospholipid syndrome. We evaluated the role of 8 *APOH* promoter SNPs for their association with SLE risk and related clinical variables (renal and cardiovascular involvement) in a case-control cohort. DNA from 399 SLE women (350 Caucasians and 49 African Americans) and 496 healthy control women (454 Caucasians and 42 African Americans) were genotyped for 8 *APOH* promoter SNPs using Pyrosequencing. Because of its rare presence (MAF<0.01), rs8178818 SNP was excluded from further analyses. The genotype distributions of rs8178820 and rs3760291 SNPs differed significantly (P<0.001) between Caucasians and African Americans implying a race-specific effect. Due to the small number of African American subjects, association studies were performed only on samples from Caucasian subjects. Haploview analysis of our data revealed strong LD (D'=1, r^2=0.98) between rs8178820 and rs3760291 SNPs, therefore, rs3760291 was excluded from haplotype analysis (6-site analysis was performed) and from multiple regression analyses. The overall haplotype distribution was significantly different (P= 0.004) between cases and controls. When 6 SNPs were included in a multiple regression model, 2 SNPs (rs8178819 and rs3760292) showed association with SLE risk (P=0.015 & P=0.046, respectively) and one SNP (rs8178820) with lupus nephritis (P=0.004). Two SNPs, rs3760292 and rs8178822, showed significant association with carotid plaque (P=0.036 and P=0.007, respectively). Our findings support the hypothesis that *APOH* promoter variants are involved in the etiology of SLE, especially the risk for lupus nephritis and cardiovascular disease and merit further investigation.

2570/T

Mutations in BMP4 are associated with subepithelial, microform, and overt cleft Lip. *S. Suzuki[1,2], M.L. Marazita[3], N. Miwa[2], A. Jugessur[2], N. Natsume[1], K. Shimozato[1], M. Shi[2], N. Ohbayashi[1], Y. Suzuki[1], T. Niimi[1], M. Yamamoto[1], T.J. Altannamar[4], T. Erkhembaatar[4], H. Furukawa[1], S. Daack-Hirsch[2], A. Vieira[3], A.C. Lidral[2], J.F. Martin[5], J.C. Murray[2].* 1) Aichi-Gakuin University, Nagoya 4648651, Japan; 2) University of Iowa, Iowa City, IA 52242, USA; 3) University of Pittsburgh, Pittsburgh, PA 15219, USA; 4) Maternal and Children's Health Research Center Hospital, Ulaanbaatar, Mongolia; 5) Institute of Biosciences and Technology, Texas A&M Health Science Center, 2121 Holcombe Blvd., Houston, TX 77030, USA.

Nonsyndromic cleft lip with or without cleft palate (CL/P), a common birth defect, is a complex trait, arising from the influence of genetic and environmental factors. Evidence is also mounting that the phenotypic spectrum of CL/P includes both microform and subepithelial lip defects. A conditional knockout of the mouse BMP4 gene results in a phenocopy of human microform/subepithelial lip defects. To pursue the role that BMP4 might play in human CL/P we sequenced BMP4 in individuals with subepithelial, microform and overt CL/P defects, plus controls, from several populations. We also assessed association with polymorphic SNP variants in and near BMP4 in a large collection of families from multiple countries in Asia, North America, South America, and Europe. Missense or nonsense mutations were identified in the BMP4 gene in 1 of 30 cases of microform clefts, 2 of 87 cases with subepithelial defects in the orbicularis oris muscle, and in 5 of 968 cases of overt CL/P. No amino acid sequence variants were seen in 529 controls. Differences between case and control groups were assessed with standard chi square tests including Yates' corrections applied due to small cell sizes. The frequency for microform plus OO cases was significantly greater than for controls (p=0.003). Further, the BMP4 mutation frequency in overt CL/P cases was significantly less than the rate in microform plus OO cases (p=0.01).This study supports the role of BMP4 in nonsyndromic CL/P, with mutations in BMP4 associated with microforms and OO, and polymorphic variants increasing the susceptibility to overt CL/P.

2571/T

Novel genetic association of SOCS3 with adiposity measures in Hispanics of the IRASFS. *M.E. Talbert[1,2], C.D. Langefeld[3], J.M. Norris[4], S.M. Haffner[5], D.W. Bowden[1,2].* 1) Depts of Biochemistry,Wake Forest U School of Med,Winston Salem,NC; 2) Cntr for Human Genomics,Wake Forest U School of Med,Winston Salem,NC; 3) Public Health Sciences,Wake Forest U School of Med,Winston Salem,NC; 4) Dept of Preventive Medicine,U of Colorado Health Sciences Center,Denver,CO; 5) Dept of Medicine,UT Health Sciences Cntr,San Antonio,TX.

Our group has reported genetic linkage on chromosome 17q with BMI, visceral adipose tissue (VAT), and waist circumference (WAIST) in Hispanics of the Insulin Resistance Atherosclerosis Family Study (IRASFS) (Sutton,Int J Obes 30:1433-41,2006). Following high density SNP mapping of the linked region, SNP rs9914220 showed evidence of association with BMI, VAT, and WAIST(P=.001-.01). This SNP is ~10 Kb upstream of the Suppressor of Cytokine Signaling 3(SOCS3) gene, which is critical to the feedback inhibition of the leptin receptor response. We hypothesized that SOCS3 genomic variants lead to reduced leptin effectiveness, promoting obesity by altering regulation of appetite and metabolism. Consequently, we genotyped rs9914220 and 15 additional SOCS3 SNPs in 1425 Hispanics from the IRASFS. CEU/YRI HapMap tagSNPs with minor allele frequency(MAF)>5%; and an r2 threshold of 0.8 were supplemented with SNPs from HapMap and dbSNP. Genotypes were tested for association with adiposity measures (BMI; VAT; WAIST; waist to hip ratio, WHR; subcutaneous adipose tissue, SAT) using SOLAR and QPDT analysis. Using SOLAR, 4 highly correlated promoter SNPs (including rs9914220) showed association under 2df and dominant models with BMI, WAIST, WHR, SAT, and VAT (P=.00003-.03). Rs9914220 had a MAF (allele T) of 14% with C/T and C/C genotype subjects having a ~3.3 unit higher BMI than T/T subjects (similar trends observed with other traits). The 3'-UTR SNP rs7221341 also showed association with all 5 adiposity measures, while 2 other SNPs in the 3'-UTR were associated with 2-3 measures of adiposity using SOLAR (P=.001-.04). QPDT analysis supported the 3'-UTR SNP results, identifying haplotypes associated with BMI, VAT, WHR, and SAT (P=.0009-.04). These results suggest a role for genetic variation in SOCS3 in human obesity, and possibly diabetes.

2572/T

Mutation screening of 7 candidate genes within the MYP12 high grade myopia locus. *KN. Tran Viet[1], R. Metlapally PhD[1,2], TR. White[1], D. Kao[1], A. Bulusu[1], YJ. Li PhD[1], TL. Young, MD[1,2].* 1) Duke Center for Human Genetics, Durham, NC; 2) Duke Eye Center, Durham, NC.

Purpose: Myopia, or near-sightedness, is an ocular refractive error of unfocussed image quality in front of the retinal plane. An initial linkage study of a large autosomal dominant high myopia kindred identified a 9.1 cM interval (MYP12) at chromosome 2q37.1. This was contracted by haplotype analysis to a 2.22 cM interval. All genes within the refined interval (5 known and 2 hypothetical genes) were screened for sequence variants associated with the high grade myopia phenotype.

Methods: Utilizing public databases, a compilation of genes in the 2.22 cM region was obtained. All known and hypothetical genes (INPP5D, ATG16L1, NR_003006, NR_003008, SAG, DGKD, and USP40) were screened by direct sequencing. Primers for PCR and sequencing were designed to cover coding and untranslated gene regions, including intron-exon boundaries. Genomic DNA samples of two affected family members with myopic refractive errors of greater than -15 diopters were tested, along with 2 unaffected internal control samples.

Results: In all, 73 polymorphisms were detected by sequencing; 5 were missense, 7 were silent, 34 were intronic, 18 were located in untranslated regions, 8 were deletions, and 1 was an insertion. Fourteen were novel polymorphisms, and will be submitted to appropriate public online databases. No polymorphisms segregated with the myopic affection status in the family.

Conclusion: Within the 2.22 cM region of the MYP12 locus, the screened candidate genes did not exhibit sequence variants associated with the phenotype. Additional studies are currently underway to screen flanking region candidate genes at this locus.

2573/T

Allele Distribution Difference of NOS2 Promoter polymorphisms between Patients With Chronic Rhinosinusitis and Non-sinus Disease Controls. *X. Wang, Y. Di, C. Li.* Lab Medicine and Pathology, University of Minnesota, Minneapolis, MN.

Chronic rhinosinusitis (CRS) is one of the most prevalent of the chronic diseases, affecting about 15% of the U.S population. Its etiology is not well understood. CRS is defined as a condition manifested by an inflammatory response involving the mucous membranes of the nasal cavity and paranasal sinuses. Nitric Oxide (NO) exhibits significant immunoregulatory activity. The enzyme NO synthase 2 (NOS2), often called inducible NOS, plays a central role in the inflammatory reactions that follow infection or tissue damage. Several recent studies indicate that genetic polymorphisms at the NOS2 gene are associated with asthma, atropy, malaria, and parasitic diseases. A study of gene expression profiles in nasal polyps and normal epithelial cells revealed that NOS2 mRNA reduced 5.5 fold in polyp tissue. To evaluate possible role of NOS2 gene in CRS, we have analyzed 261 CRS patients and 147 non-sinus problem controls for three NOS2 promoter polymorphisms. It is a pentanucleotide repeats, $(CCTTT)_n$ sequence located approximately 2.5 kb upstream of the main TATA-directed transcription initiation site, a di-allelic TAAA repeat (4 repeats or 5 repeats) and a single nucleotide substitutions: G>C variation, at position -954 (G-954C). We found that allele distribution in CRS patients is different from controls at $(CCTTT)_n$ (χ^2, p=0.03), but not at TAAA and G-954C polymorphisms. Allele $(CCTTT)_{12}$, the most common allele, has a lower frequency in CRS patients than controls (29% vs 38%, p=0.008). Individual bearing at least one $(CCTTT)_{12}$ allele has a lower risk (OR=0.63, 95%CI 0.42-0.95) for CRS. Allele frequency of $(CCTTT)_{10}$ is higher in the CRS patients than controls (13% vs 7%, p=0.003). Individual bearing at least one $(CCTTT)_{10}$ allele has a higher risk (OR=2.06 95%CI 1.19-3.57) for CRS. These results suggest that NOS2 may be one of the predisposing factors to CRS.

2574/T

Apoptosis in nonsyndromic cleft lip with or without palate. *K.S. Weymouth[1], S. Stal[2], J.B. Mulliken[3], D. Ma[4], S.H. Blanton[4], J.T. Hecht[1].* 1) University of Texas Medical School at Houston; 2) Texas Children's Hospital, Houston, TX; 3) Boston Children's Hospital, MA; 4) University of Miami Miller School of Medicine, Miami, FL.

Nonsyndromic cleft lip with or without palate (NSCLP) is a common birth defect affecting 1/700 livebirths and 4,000/yr in the United States. NSCLP is a complex disease involving multiple genes and environmental factors. Development of the lip and palate is a multifaceted process in which program cell death plays a vital role in craniofacial development. Fusion of the palatine shelves requires apoptosis of the epithelial edge, exposing the basal epithelial cells and allowing the midline seam of the palate to form. Failure of program cell death to occur in the epithelial edge could contribute to the development of NSCLP. Genetic variation in key genes of the mitochondrial-mediated apoptotic pathway may play an etiological role in NSCLP. To test this hypothesis, genes in the apoptotic pathway, CASP3, CASP8, CASP9, CASP10, Apaf-1, Bid (pro-apoptotic genes), Bcl-2 and CFLAR (anti-apoptotic genes), were interrogated. Fifty-four SNPs spanning these apoptotic genes were genotyped in 127 multiplex families and 348 simplex trios of Caucasian and Hispanic ethnicity. All SNPs were in Hardy-Weinberg equilibrium. Analysis with PDT was stratified by population because the allele frequencies differed between Caucasian and Hispanic populations. CASP3 (three SNPs), CFLAR (2 SNPs) and Bcl-2 (1 SNP) were associated with NSCLP in the Caucasian cohort. CASP3 showed association with NSCLP in the Hispanic cohort. One CASP3 SNP (rs4647602) genotyped showed association with NSCLP in both the Caucasian (p=.03) and Hispanic (p=0.006) cohorts. CASP3 is expressed during palatal fusion, thus variation in CASP3 expression could affect the initiation of program cell death. CFLAR and Bcl-2 are both anti-apoptotic genes and variation in these genes would affect their ability to inhibit the apoptotic pathway. Gene-gene interaction studies are underway. These results suggest an association of CASP3, CFLAR and Bcl-2 with NSCLP indicating that variation in apoptotic genes may play a role in NSCLP.

2575/T

Identification of genes that specify human kidney aging. *H.E. Wheeler[1], J. Higgins[2], J.M. Zahn[3], D. Absher[4], J. Li[4], R.M. Myers[1,4], A.B. Owen[5], S.K. Kim[1,3].* 1) Genetics, Stanford University, Stanford, CA; 2) Pathology, Stanford University, Stanford, CA; 3) Developmental Biology, Stanford University, Stanford, CA; 4) Stanford Human Genome Center, Palo Alto, CA; 5) Statistics, Stanford University, Stanford, CA.

Aging is a complex process defined by the gradual decline of a multitude of physiological functions leading to an increasing probability of death and thus best studied using a systems biology approach. We are studying aging of the human kidney, which begins to show functional decline around age 40. Kidneys age at different rates, such that some people show little or no effects of aging whereas others show rapid functional decline of the kidney. We have performed a pilot study to find genes that associate with different rates of kidney aging in humans. We first performed whole-genome transcriptional profiling to find 741 genes that change expression with age in the kidney, and then used these age-regulated genes as candidates in a genetic association study for kidney aging. We genotyped 1041 SNPs in the first set of 346 candidate genes in 261 kidney samples, and found 9 genes that show weak but significant association with kidney aging. We are now screening a new set of 276 candidate genes, which should identify a new set of genes that may be associated with kidney aging. Also, we will test the reproducibility of the pilot screen by performing a second study using ~800 more kidney samples. These studies may provide the first evidence for genes that are associated with kidney aging in humans. Not only will this research uncover basic principles about human kidney aging, but it will also be directly relevant to understanding why some people progress to end stage renal disease whereas others do not. The kidney aging genes could help determine the rate of kidney aging for patients, and mechanistic insight from our studies could eventually lead to treatments that slow down or prevent kidney failure in old age.

2576/T

Positional candidate gene screening within the high grade Myopia-2 locus (MYP2). *T.R. White[1], R. Metlapally[1,2], K.N. Tran-Viet[1], D. Kao[1], J. Ellis[1], A.E. Shay[1], A. Bulusu[1], Y.J. Li[1], S. Zuchner[1], T.L. Young[1,2].* 1) Duke Center for Human Genetics, Durham, NC; 2) Duke Eye Center, Durham, NC.

Purpose: Myopia, or nearsightedness, is a common complex eye disorder that predisposes individuals to ocular morbidities such as retinal detachment, central chorioretinal degeneration, premature cataracts, and glaucoma. Seven families with autosomal dominant high myopia mapped to a 7.6cM genomic interval (MYP2) at chromosome 18p11.31. Previous base pair screening of 9 interval candidate genes revealed no sequence associations with the myopia phenotype. Sequence mutation screening of the remaining known positional candidate genes within the 7.6cM region was performed.

Methods: A physical map of the MYP2 locus was compiled using public databases. Gene expression studies in ocular tissues helped prioritize gene selection for screening. Of the 21 genes screened, 12 genes (CLUL1, TYMS, ENOSF1, YES1, ADCYAP1, C18orf2, METTL4, NDC80, BC006008, KIAA0650, MRCL3, and MRLC2) fall within and 9 genes (USP14, THOC1, COLEC12, CETN1, C18orf18, ZFP161, EPB41L3, TTMA, and L3MBTL4) flank the 7.6cM interval. Coding regions, intron-exon boundaries, and untranslated exons of the genes screened were sequenced by standard techniques using genomic DNA samples from selected affected and unaffected individuals from representative families. Gene sequences were obtained for the 21 genes and compared to known reference sequences from public genomic databases.

Results: In total, 151 polymorphisms were found with sequence analysis; 11 were missense, 12 were silent, 31 were untranslated, 83 were intronic, 1 insertion, and 13 deletions. Twenty polymorphisms were novel. No sequence alterations segregated with the disease phenotype.

Conclusions: Mutation analysis of the 21 positional candidate known genes did not identify sequence variants associated with the MYP2 high myopia phenotype. Efforts are now in place to interrogate several hypothetical genes that fall within and flank the interval.

2577/T

Combined linkage peak fine-mapping strategy identifies locus for muscle strength on chromosome 12. *A. Windelinckx[1], G. De Mars[1], W. Huygens[1], M. Peeters[1], J. Aerssens[2], R. Vlietinck[3], G. Beunen[1], M. Thomis[1].* 1) Dept of Biomedical Kinesiology, K.U.Leuven, Belgium; 2) Dept of Translational Medical Research, Tibotec, Belgium; 3) Dept of Human Genetics, K.U.Leuven, Belgium.

Given the increasing use of genomewide linkage scans for complex traits, fine-mapping of the resulting linkage peaks becomes an important challenge. Fine-mapping techniques mostly focus on refining linkage peaks by genotyping additional markers. However, often no selection regarding e.g. location or functionality is done on the markers, resulting in a less than ideal approach for follow-up association analyses using the same marker data. In an alternative strategy, (association) analyses are restricted to polymorphisms within positional candidate genes. The latter approach does not allow for additional linkage analyses and is only appropriate when focus is on a limited number of candidate genes. To overcome the disadvantages of both strategies, we propose a combined strategy by covering the whole linkage region with additional markers, selected based on their location in or near positional candidate genes. Selection of candidate genes is based on a gene prioritisation procedure based on similarity to genes known to influence the trait of interest using a bioinformatics approach (ENDEAVOUR). TagSNPs and coding SNPs within the candidate genes are determined using CEPH genotypes within Haploview and SNP selection is based on functionality, initial priority ranking of the gene, minor allele frequency and budgetary and genotyping platform technological criteria. Analyses are performed using linkage and association analyses and combined family-based association analysis. Application of this strategy on a previously determined linkage peak (±90cM) for isometric and dynamic knee muscle strength on chromosome 12 resulted in the identification of a new locus for muscle strength. Linkage analyses on selected strength measurements resulted in maximal LOD-scores ranging from 0.30 to 2.05 and follow-up family-based association analyses identified a marker locus with p-values for association between 0.000044 and 0.43. This SNP is located in a gene that presumably has a role in the myostatin signaling pathway.

2578/T

Multiple genes regulating macrophage activation and responses contribute to an immunogenetic phenotype underlying Kawasaki Disease. *V. Wright[1], S. Davila[2], D. Burgner[3], T.W Kuijpers[4], S.B Ng[3], W. Breunis[4], J.C Burns & US KD Genetics Consortium[5], M.L Hibberd[2], UK KD Genetics Consortium[1], M. Levin[1].* 1) Imperial College London, UK; 2) Genome Institute of Singapore, Singapore; 3) School of Paediatrics & Child Health, UWA, Australia; 4) Emma Children's Hospital, Netherlands; 5) Paediatrics, UCSD School of Medicine, La Jolla, CA, USA.

Background: Kawasaki disease (KD) is a common inflammatory disorder of unknown aetiology affecting young children, which may lead to permanent coronary artery damage in a significant proportion of those affected. KD may arise from an excessive or uncontrolled inflammatory response to one or more infectious stimuli occurring in genetically predisposed individuals. We postulated that functional polymorphic variation in any of the genes regulating the IL12/IFNγ pathway of macrophage activation would contribute to the immunological phenotype underlying the disorder. **Methods**: We studied 104 SNPs in 13 genes of the IL12/IFNγ pathway of macrophage activation in 1,903 members of 583 KD families from Australia, UK & US, including 498 trios in a custom Illumina Oligo Pool Assay that successfully typed another 1,391 SNPs in unrelated pathways. We then compared the allelic transmission to affected children of variants in the 13 genes in the IL12/IFNγ activation pathway with a similar number of variants in randomly selected genes not linked within a single biological pathway. **Results**: Significant associations for individual variants within this pathway were identified within IFNγR2, IL12RB1, IL12A and weaker associations for IL12, TGFβ. Using a novel-linked pathway analysis, 13 genes were found to contribute to the overall genetic effect. The combined genetic effect was significantly different from 3 sets of randomly selected SNPs from both known and unknown genes (P<0.0001). **Discussion**: The immunological phenotype underlying the excessive inflammation in KD appears to result from a complex interaction of several genes within the same inflammatory pathway. The method we have used to assess the interaction of genes within a linked functional pathway may be relevant to other complex gene interactions.

2579/T

Genome-wide aCGH analysis in patients with sporadic birth defects. *D. Scott[1], J.F. Felix[4], A.M. Holder[1,6], M. Klaassens[3,4], L. de Jong[4], K.P. Lally[5], C. Fernandes[2], D. Tibboel[4], A. de Klein[3], B. Lee[1,6].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Peds, Baylor Col Med, Houston, TX; 3) Dept of Clin Genet, Erasmus M C, Rotterdam, the Netherlands; 4) Dept of Paed Surg, Erasmus M C, Rotterdam, the Netherlands; 5) Dept of Ped Surg, Univ of Texas Med School, Houston, TX; 6) Howard Hughes Medical Institute.

Congenital diaphragmatic hernia (CDH) and esophageal atresia/tracheoesophageal fistula (EA/TEF) are relatively common sporadic birth defects. Both defects are life threatening and require surgical correction in the newborn period. Although CDH and EA/TEF can occur in isolation, approximately 50% of cases occur with additional anomalies. In the case of EA/TEF, VACTERL (Vertebral, Anal, Cardiac, Tracheo-Esophageal Fistula, Renal, Limb) association is found in approximately 10% of cases. The sporadic nature of these defects makes linkage-based approaches to gene identification impractical. We are using a positional candidate approach based on chromosomal data to localize and identify these genes that cause or predispose to the development of these defects. By reviewing published case reports we have identified 19 recurrently deleted/duplicated chromosomal regions in CDH and 10 recurrently deleted/duplicated chromosomal regions in EA/TEF. We hypothesize that each of these regions harbors a gene(s) related to these birth defects. To identify new regions, and refine those previously reported, we are screening for deletions/duplication in affected individuals using high density genome-wide array comparative genome hybridization (aCGH). With the Agilent 244K platform we typically identify between 20 and 40 copy number variants between subjects and sex match controls. Greater than 90% of these variants have been reported previously in healthy individuals making them less likely to be the cause of these birth defects. We are using qPCR to confirm the novel variants identified in 25 previously unreported patients and to determine their inheritance pattern with families. De novo changes identified in this approach are likely to be disease related and to affect the expression of one or more CDH- or EA/TEF-related genes.

2580/T

Copy number polymorphisms in the type 2 diabetes linked region at 1q22 in diabetic and nondiabetic Caucasian and African American subjects. *S.K. Das, T.A. Gomes, N.K. Sharma, W.S. Chu, S.C. Elbein.* Internal Medicine/Endocrinology, University of Arkansas for Medical Sciences and CAVHS, Little Rock, AR 72205.

Duplication or deletion events involving >1kb of DNA are commonly defined as copy number variants (CNV). Several studies have shown that CNVs are common in the human genome, may be involved in common, complex human diseases, and are present in a ~200 kb region of chromosome 1q22 that is linked to T2DM in eight populations. Furthermore, sequence variation in this region is associated with T2DM in several Caucasian populations, and the region harbors several important candidate genes including PKLR, CLK2, and SCAMP3 among others. We hypothesized that CNV affecting gene dosage might contribute to T2DM and account for the T2DM association in this region. We tested genomic DNA from 128 Caucasians and 128 African Americans, including 64 of each with T2DM and 64 normal control individuals for each group. We tested 4 sets of primers based on known CNV regions (A: chr1:153407866-153407975, B: chr1:153464362-153464455, C: chr1: 153504305-153504420, D: chr1: 153514225-153514331) by quantitative real time PCR (qRT-PCR). Primer sets C and D encompass diabetes candidate genes CLK2 and HCN3. A primer set which amplifies the diploid SPRY3 gene (chrX: 154654501-154654628 and chrY: 57513701-57513828) was used as a reference to normalize and calculate the corrected Ct (KCti) of test primer sets (Weksberg et al., 2005). Additionally, we tested X chromosome gene TEX11 (chrX: 69761280-69761379) as a technical validation of our qRT-PCR technique. Mean Delta KCti value for male and female genomic DNA samples for TEX11 was 0.95. Only 2/128 Caucasian subjects, both controls, showed loss of copy number for primer set A . Gain of one copy number was observed in 11/63 T2DM and 6/56 control subjects successfully evaluated for primer set B in our African American cohort (p=0.309). These observations were validated by analyzing 3 additional DNA aliquots for the same samples. CNVs found in our study were centromeric to known T2DM candidate genes. Our data suggests that CNVs in chromosome 1q22 possibly do not confer susceptibility to T2DM in African American or Caucasian population.

2581/T

Association of the PREX1 gene in 20q13 with type 2 diabetes in European Americans. *J.P. Lewis, N.D. Palmer, J. Bento, B.I. Freedman, D.W. Bowden.* Wake Forest University School of Medicine, Winston-Salem, NC 27157.

We carried out a dense SNP map analysis of the 20q12-13.13 type 2 diabetes mellitus (T2DM)-linked region on chromosome 20 in a European American (EA) diabetic cohort enriched for end-stage renal disease (ESRD). In the initial survey three genes nuclear receptor coactivator 5 (*NCOA5*), cadherin-like 22 (*CDH22*), and phosphatidylinositol 3,4,5-triphosphate-dependent RAC exchanger 1 (*PREX1*) showed evidence of association with diabetes. Suggestive evidence of association in *PREX1* was observed within and 3' to the gene. To more thoroughly understand this 316 kb region, 31 additional SNPs were genotyped in the same cohort consisting of 300 Caucasian T2DM patients and 310 controls for a total of 59 markers genotyped across *PREX1*. Twelve of these SNPs were significantly associated with T2DM with P-values ranging from 0.002-0.033. In an effort to confirm these associations a subset of SNPs were genotyped in a replicate cohort recruited with the exclusion of ESRD consisting of 469 diabetic cases and 442 controls. In this replication population 6 SNPs located approximately 150 kb 3' of *PREX1* were associated with T2DM with P-values ranging from 0.021-0.042. Three of these SNPs showed significant association in the first cohort (rs7263053, rs1321006, and rs926692). In total, 769 cases and 752 controls were genotyped. The combined analysis resulted in 10 SNPs associated with T2DM and led to generally stronger evidence of association (P= 0.001-0.049) with odds ratios in the 1.17-1.30 range. Haplotype analysis was performed to assess whether a combination of alleles of these SNPs led to an enhanced risk or protective effect. Within the region of replicated association this analysis identified statistically significant differences in haplotype frequencies between cases and controls in multiple 2 and 3 marker haplotypes (P=0.001-0.030). These results suggest that the *PREX1* gene may contribute to diabetes susceptibility in European Americans.

2582/T

The possible role of UCP2 -866 G/A polymorphism in Type 2 Diabetes in two population groups of North India. *E. Rai[1,2], S. Sharma[1,2], A. Koul[1], A.K. Bhat[1], A.J.S. Bhanwer[2], R.N.K. Bamezai[1].* 1) NCAHG, SLS, Jawaharlal Nehru University, New Delhi, India; 2) Department of Human Genetics, Guru Nanak Dev University, Amritsar, India.

A common UCP2 promoter -866G/A polymorphism (rs659366) is suggested as an important link between obesity, beta cell dysfunction and type 2 diabetes mellitus (T2DM). Indians show low BMI but higher central obesity and more insulin-resistance than Europeans.

We have explored the association of UCP2 -866G/A polymorphism with the development of T2DM in 868 T2DM patients and 930 healthy controls belonging to two diverse population groups of North India (Punjab and Kashmir) in a replicate study. Further, we explored mtDNA 10398 G/A polymorphism proposed to be involved in ROS modulation, independently as well as in interaction with UCP2 -866G/A polymorphism. The UCP2 -866G/A polymorphism showed a significant association with T2DM in Population 1 and 2. The population risk attributable (PAR) to the UCP2 -866 GG genotype was approximately 15% for Population 1 and 20% for Population 2. Interestingly, independently mtDNA 10398 A allele was observed to be significantly associated with increased risk of T2DM (Bhat et al. 2007) in Population 1 and Population 2. And, the PAR (population attributable risk) for UCP2 -866 GG genotype increased to approximately 23% in Population 1 and 34% in Population 2 in mtDNA 10398A background, which was higher than the risk provided independently by UCP2 -866 GG genotype. Further, in interaction analyses between mitochondrial 10398 A background and genotype status (XA versus GG) of UCP2 gene, subjects carrying UCP2 -866 XA genotype were at reduced risk to develop T2DM which was not the case in mtDNA 10398 G background. The effect of UCP2 -866 XA genotype towards a reduced risk for T2DM could be explained by its role in protection from mitochondrial ROS production and pancreatic beta cell apoptosis.

Reference: Hum Genet; 120(6): 821-6.

2583/T

A candidate gene association study of refractive error in the 1958 British Birth Cohort. *C.L. Simpson[1,2], P. Hysi[1], S.S. Bhattacharya[2], C.J. Hammond[3], A.R. Webster[2], C.S. Peckham[1], P.C. Sham[4], J.S. Rahi[1,2].* 1) Ctr Pediatric Epid & Biostat, Inst Child Health, London, WC1N 1EH, United Kingdom; 2) Institute of Ophthalmology, University College London, London EC1V 9EL, UK; 3) Twin Research and Epidemiology Unit, Kings College London, London UK; 4) Genome Research Center, University of Hong Kong, Hong Kong SAR China.

Refractive error (RE) is a common complex quantitative trait, with myopia affecting up to 60% of some populations. Development is influenced by multiple genes and environmental factors. Many genetic studies focus on rare extreme RE phenotypes such as high myopia, which is inherited in a Mendelian fashion. However most commonly occurring RE is not extreme, has complex inheritance and may have different underlying causes. We aimed to identify variants which affect common RE in a well characterised national population, the 1958 British Birth Cohort. The distribution of RE is leptokurtic and skewed towards myopia. 1196 individuals were selected at random from the two outer tertiles of the cohort RE distribution. Candidate genes were chosen based on recently published linkage peaks and further selected by biological relevance. 1536 tagSNPs were selected across 111 candidate genes and genotyped on the Illumina GoldenGate platform. This experiment had 80% power to exclude any candidate gene contributing >10% of the variance of RE in this cohort, which is reasonable given the assumption of the common disease, common variant hypothesis. All SNPs were in Hardy Weinberg equilibrium and genotyping failure rate was <5%. Using single SNP and moving-window haplotype-based analyses, interim findings provide statistically significant association (p-values range from 0.0007 to 0.01) between multiple SNPs and RE in multiple genes. Two strong candidates based on biological function, PAX6 and SOX2, have already been definitively excluded as being associated with RE. A replication study of the most promising results, consisting of 292 SNPs in 4 novel candidate genes, is currently close to completion. We will present our novel findings and discuss the implication of these associations and the involvement of these candidate genes in the pathogenesis of RE.

2584/T

Analysis of candidate loci in primary open angle glaucoma families. *J.P.C. Vasconcellos[1], A. Tavares[2], I. Lopes-Cendes[2], C.V. Maurer-Morelli[2], R. Secolin[2], M.R.B. Moraes Silva[3], F.F. Costa[4], V.P. Costa[1], M.B. Melo[5].* 1) Ophthalmology, University of Campinas, Campinas, São Paulo, Brazil; 2) Medical Genetics, University of Campinas, Campinas, São Paulo, Brazil; 3) Ophthalmology, UNESP, Botucatu, São Paulo, Brazil; 4) Hemocentro, University of Campinas, Campinas, São Paulo, Brazil; 5) CBMEG, University of Campinas, Campinas, São Paulo, Brazil.

Purpose: Glaucoma is one of the major causes of irreversible blindness worldwide, characterized by progressive loss of optic nerve ganglion cells, associated with correspondent visual field damage. There are at least 13 loci (GLC1A - GLC1M) associated with POAG identified from genetic mapping studies, most of them involving families that follow a Mendelian inheritance pattern but only three genes were identified: myocilin (MYOC - GLC1A), optineurin (OPTN - GLC1E) and WD Repeat-Containing Protein 36 (WDR36 - GLC1G) genes. The goal of this study was to evaluate nine candidate loci associated with POAG in Brazilian families through linkage analysis. Methods: Seven families with POAG (121 individuals - 47 affected) were enrolled in this study. Thirty three microsatellite markers were used to genotype nine candidate regions linked to POAG (GLC1A - GLC1I). Two-point linkage analysis was performed using the MLINK program of the LINKAGE package. Results: Among the seven families, two (28.6%) presented mutations (Cys433Arg) in the MYOC gene (GLC1A) segregating with POAG. The remaining 5 families did not show evidence of linkage in GLC1A (MYOC gene), GLC1C, GLC1E (OPTN gene), GLC1F, and GLC1G (WDR36 gene) loci, with LOD scores < -2.00. The data related to loci GLC1B, GLC1D, GLC1H and GLC1I were inconclusive (LOD scores between +3.00 and -2.00) being necessary the genotyping of additional markers to narrow the candidate regions. Conclusions: MYOC gene alterations seem to contribute to the development of POAG among Brazilian families with autosomal dominant pattern of inheritance. However, the analysis of the remaining families support the heterogeneity of POAG and stresses the importance of the evaluation of additional families in order to search for different loci and predisposing genes to better understand the genetic basis of glaucoma.

2585/T

A Candidate Gene Association Study Identifies a New Susceptibility Gene for Crohn's Disease Involved in IL-1 Processing. *A.C. Villani[1], M. Lemire[2], E. Louis[3], M.S. Silverberg[4], C. Collette[1], G. Fortin[1], C. Libioulle[3], A. Bitton[1], D. Gaudet[5], A. Cohen[1], D. Langelier[6], J.D. Rioux[5], P. Rutgeerts[7], S. Vermeire[7], T.J. Hudson[2, 8], D. Franchimont[1].* 1) McGill University Health Center, Canada; 2) McGill University & Genome Quebec Innovation Centre, Canada; 3) CHU of Liège, Belgium; 4) Mount Sinai Hospital IBD Center, Canada; 5) Université de Montréal, Canada; 6) Centre Hospitalier de Sherbrooke, Canada; 7) University Hospital Gasthuisberg, Belgium; 8) Ontario Institute for Cancer Research, Canada.

We used a candidate association approach to identify novel Crohn's disease (CD) susceptibility loci, and to complement the list of CD candidates recently reported from WGA studies. We focussed on genes involved in IL-1 processing and signalling, a cytokine known to play a pivotal role in inflammation. Methods: 738 CD trios, 239 CD cases and 107 controls were assembled. 55 tagging SNPs were selected within a 72kb interval and genotyped in all samples. Association testing was done using FBAT, UNPHASED and Chi-Square tests. Results: The major allele of three SNPs was significantly associated with CD (p as low as 0.0147) in the Leuven exploratory cohort (356 CD trios). We replicated two of these signals in three independent cohorts: Liege trios (156 CD trios) (p=0.0130), Liege case-controls (239 CD and 107 controls) (p=0.00330) and Canadian trios (226 CD trios) (p=0.0310). Combined analysis of 4 cohorts revealed significant associations as low as p=6.694E-5 (OR:1.497; CI:1.223-1.833). Two haplotypes within our region were also significantly associated with CD and replicated across all 4 cohorts (combined p=3.45E-4), supporting the region as a potential CD risk factor. We selected 24 individuals based on carrier status of risk alleles and sequenced the 9kb associated region. No coding SNP could explain the signals, yet three SNPs in LD with the associated markers are located on putative functional sites, which we are currently evaluating. Conclusion: We have uncovered a novel CD susceptibility gene using a candidate gene approach, emphasizing the importance of IL-1 in the pathogenesis of CD. These results highlight the utility of a hypothesis driven methodology to complement WGA studies.

2586/T

Folate metabolism genes and their associations with oral facial clefts. *A.L. Boyles[1], A.J. Wilcox[1], J.A. Taylor[1], M. Shi[2], C.R. Weinberg[2], K. Meyer[3], A. Fredriksen[3], P.M. Ueland[3], C.A. Drevon[4], K. Solvoll[4], J.C. Murray[5], A. Jugessur[6], R.T. Lie[6].* 1) Epidemiology Branch, NIEHS/NIH, Durham, NC; 2) Biostatistics Branch, NIEHS/NIH, Durham, NC; 3) Dept Pharmacology, Univ Bergen, Norway; 4) Dept Nutrition, Inst Basic Med Sciences, Univ Oslo, Norway; 5) Dept Pediatrics, Univ Iowa, Iowa City, IA; 6) Dept Public Health and Primary Health Care, Sect Epidemiology and Med Statistics, Univ Bergen, Norway.

Prenatal folic acid supplementation reduces the risk of neural tube defects and probably oral facial clefts as well. Folate pathway gene polymorphisms have been inconsistently associated with cleft risk in previous studies. In a Norwegian population-based study, 377 cleft lip with or without cleft palate (CL/P) families and 196 families with cleft palate only (CPO) were genotyped for 13 polymorphisms in 9 folate pathway genes using a MALDI-TOF MS multiplex method. We looked for associations of clefting with fetal polymorphisms, maternal polymorphisms, as well as parent-of-origin effects, using combined likelihood-ratio tests (LRT). We also stratified by maternal periconceptional intake of folic acid (400+μg) to explore gene-exposure interactions.

There was a reduced risk of CL/P with mothers who carried the *CBS* C699T variant (rs234706); relative risk was 0.94 with one copy (95% CI 0.63-1.4) and 0.50 (95% CI 0.26-0.96) with two copies. The LRT had a p-value of 0.008. We found no evidence of interaction of this variant with folic acid status. There was no evidence of risk from the *MTHFR* C677T SNP (rs1801133) either overall or stratified by maternal folic acid. No associations were found between any of the folate polymorphisms and CPO. In a preliminary assessment of a large set of additional 331 gene assays, a subset of 86 haplotype-tagging SNPs in 26 folate metabolism genes showed no strong evidence of risk for combined orofacial clefts in unstratified haplotype-based analyses. Further exploration of these genes and their interaction with folate supplementation is needed.

2587/T

Sequence Evaluation of FGF and FGFR Gene Conserved Non-Coding Elements in Nonsyndromic Cleft Lip and Palate Cases. *B.M. Riley, J.C. Murray.* Pediatrics, University of Iowa, Iowa City, IA.

Nonsyndromic cleft lip and palate (NS CLP) is a complex birth defect resulting from multiple genetic and environmental factors. We have previously reported the sequencing of the coding region of genes in the fibroblast growth factor (FGF) signaling pathway, in which missense and nonsense mutations contribute to approximately 5-6% NS CLP cases. Mutation searches in human disease should include both coding regions of genes and neighboring non-coding elements to comprehensively examine the functional elements within each FGF or FGFR locus. We report the sequencing of conserved non-coding elements (CNE) in and around 11 of the FGF and FGFR genes, which identified 55 novel variants. Seven of the novel variants are highly conserved among ≥8 species and 31 variants alter transcription factor binding sites, 8 of which are important for craniofacial development. In addition, 33% of individuals sequenced have a novel CNE variant that was conserved across ≥6 species or are located in craniofacial transcription factor binding sites. There were combinations of two or more coding and CNE variants in 15 NS CLP cases, suggesting that an accumulation of variants in the FGF signaling pathway may contribute to clefting. In the aggregate, adding CNE variants to the mix of more traditional mutations that can contribute to CLP affords opportunities for improved genetic counseling and understanding of complex gene/gene interactions.

2588/T

Polymorphisms in the genes of interleukin 12 and its receptors in association with protection against severe malarial anemia in children residing in western Kenya. *L. Zhang[1], D. Prather[1], E. Jodi Vanden[1], S. Crawford[1], S. Kariuki[2], F.O ter Kuile[3], B. Nahlen[1], A.A Lal[1], V. Udhayakumar[1], Y.P. Shi[1].* 1) National Center for Zoonotic, Vector-Borne & Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA; 2) Kenya Medical Research Institute, Kisumu, Kenya; 3) Liverpool School of Tropical Medicine, Liverpool, UK.

Of the more than 1 million Africans who die from Plasmodium infection each year, most are under the age of 5 and die from severe malarial anemia (SMA). Plasmodium falciparum has been shown to drive selection of human genetic variants for conferring protection against severe forms of malaria such as SMA, which is characterized by the destruction of red blood cells infected by malaria and suppression of erythropoiesis. Interleukin 12 (IL12) significantly boosts erythropoietic responses in murine models and its production is suppressed in African children with SMA. For these reasons the genes encoding the two IL12 subunits, IL12A and IL12B, and its receptors, IL12RB1 and IL12RB2, are attractive candidate genes for studying SMA. In this study, a total of 75 tagging single nucleotide polymorphisms (tagSNPs) covering these four genes were examined. Genotyping was performed with the iPlex MassARRAY technology in a cohort of 940 children from the Asembo Bay region of western Kenya, an area with intense malaria transmission. Individuals possessing two copies of IL12A common allele (rs2243140) at 3'UTR showed increased susceptibility to SMA (Hb < 6g/dl and the presence of P. falciparum > 10,000/uL) (p = 0.006, RR 3.63, 95%CI 1.27-10.38). Individuals possessing two copies of a rare variant in IL12RB1 (rs429774) appeared to be strongly protected against SMA (p = 0.00005, RR 0.18, 95%CI 0.05-0.69). Identification of genetic polymorphisms that influence human host susceptibility to malaria infection and severe disease outcomes may help us to better understand the immune response to malaria and design novel treatments against severe malarial anemia.

2589/T

Regulatory polymorphisms in IL18 are associated with hepatitis C virus clearance. *P. An[1], C.L. Thio[2], G.D. Kirk[3], S. Donfield[4], J.J. Goedert[5], S.J. O'Brien[6], C.A. Winkler[1].* 1) Laboratory of Genomic Diversity, SAIC-Frederick Inc, Frederick, MD; 2) Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD; 3) Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 4) Rho, Inc., Chapel Hill, NC; 5) Epidemiology Branch, National Cancer Institute, Bethesda, MD; 6) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD.

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease. People with chronic HCV infection are at risk of developing liver cirrhosis and cancer. After acute HCV infection, the majority of individuals become persistently infected and only about 20% achieve clearance. The precise host and viral factors responsible for differential outcomes of HCV infection have not yet been defined. The recover from acute HCV infection was generally associated with vigorous HCV-specific T-cell responses. Thus, genetic variation in host factors involved in immune response may influence HCV outcome. Interleukin (IL)-18 is a pivotal mediator of Th1-driven immune response, and a high level of expression of IL18 has been reported to correlate with HCV persistence and hepatic injury. We hypothesized that IL-18 polymorphisms may play a role in HCV clearance. Two functional promoter SNPs was genotyped in a matched case-control sample consisting of 91 African Americans who were infected with HCV parenterally but subsequently cleared the virus and 182 matched controls with persistent infection. The case and controls were matched on ethnicity, gender and HIV-1 status. The SNP and haplotype associations were analyzed using a conditional logistic regression. Both SNPs were associated with viral clearance (OR=2.21 to 2.90; p<0.01). Among three haplotypes formed by these two SNPs, two haplotypes were also significantly associated with viral clearance compared to the most common haplotype, which has been associated with high gene express level. These results suggest a plausible role of IL18 in resolving HCV infection. [Funded by NCI contract NO1-CO-12400].

2590/T

Population stratification in a case-control study of brain arteriovenous malformation (BAVM) among Hispanics. *H. Kim, P.G. Hysi, L. Pawlikowska, C.E. McCulloch, S. Choudhry, P.-Y. Kwok, E.G. Burchard, W.L. Young.* University of California-San Francisco, San Francisco, CA.

Genetic association studies conducted in recently admixed populations, such as Hispanics, may be confounded by population stratification. Two major statistical approaches have been developed (genomic control and structured association), both of which use unlinked genetic markers to identify and correct for population stratification. We genotyped 83 ancestry informative markers (AIMs) in a case-control study of 79 BAVM cases and 215 healthy controls of self-reported Hispanic race/ethnicity. Individual ancestry estimates (IAE) were obtained using Structure, assuming three underlying populations. Average group admixture estimates were 47% Native American, 45% Caucasian, and 8% African ancestry, with dramatic heterogeneity observed between individuals. The summary χ^2 test comparing genotype frequency of AIMs between Hispanic cases and controls was significant (χ^2 = 204.40, df=163, P=0.015), suggesting population stratification. We further investigated the effect of stratification on the association between BAVM and a promoter variant in the IL6 gene (-174G>C), which was previously associated with hemorrhagic presentation in BAVM patients. IL6-174 G and C alleles are equally common in Caucasians, whereas the C allele is rare (<5% frequency) among Africans and Asians genotyped in HapMap. Among Hispanics, IAE were associated with IL6-174G>C genotypes; the GG genotype was associated with ~6% higher Native American ancestry (P=0.023). The age and sex-adjusted risk of BAVM associated with the IL6-174GG genotype was 1.85 (95% CI=0.99-3.48, P=0.055); further adjustments for IAE yielded an OR of 1.96 (95% CI=1.03-3.72, P=0.039). The increased OR after accounting for genetic ancestry differences suggests subtle but negative confounding and illustrates the importance of addressing population stratification in case-control studies conducted in admixed populations.

2591/T

Genome-Wide Association Study of Major Depression. *S.P. Hamilton[1], J.B. Kraft[1], E.J. Peters[1], H.A. Garriock[1], G.D. Jenkins[2], M.S. Reinalda[2], P.J. McGrath[3], S.L. Slager[2].* 1) Department of Psychiatry and Institute for Human Genetics, University of California, San Francisco, CA; 2) Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 3) New York State Psychiatric Institute and Columbia University, New York City, NY.

Background: Major Depressive Disorder (MDD) is a common and disabling psychiatric illness determined to be influenced by genetic factors. Candidate gene approaches to identifying risk genes for depression have not provided meaningful findings. We have thus undertaken a genome-wide association study to look for novel genetic determinants of susceptibility to MDD. Methods: We used a subset of 780 MDD cases who are enrolled in the antidepressant trial Sequenced Treatment Alternatives to Relieve Depression (STAR*D). We used 895 controls from the NIMH Center for Collaborative Genetic Studies on Mental Disorders who did not meet lifetime criteria for MDD. All subjects were genotyped on 500K Affymetrix mapping arrays. Filtering of SNP genotype data was carried out by call rate and Hardy-Weinberg equilibrium. Single locus association tests were performed using the Armitage trend test. Results: Preliminary analyses indicate a greater than expected number of associations meeting genome-wide levels of statistical significance. We are determining the empirical significance of our association findings. Assessments of population structure differences between the STAR*D cases and NIMH Controls demonstrates that the populations are largely similar. Conclusions: Our initial results suggest promising novel genes and chromosomal regions that are associated with MDD. However, these results from our first stage of the study will need to be confirmed in our validation stage.

2592/T

Association of Single Nucleotide Polymorphism in the *Interferon Gamma Receptor 1* gene with Japanese Cedar Pollinosis. *M. Sakashita[1,2], T. Hirota[1], M. Harada[1], M. Tamari[1], S. Fujieda[2], Y. Nakamura[3].* 1) Lab Genetics Allergy, RIKEN SNP Research Ctr, Yokohama, Japan; 2) Department of Otorhinolaryngology, Fukui University, Matsuoka, Fukui, Japan; 3) The institute of Medical Science, University of Tokyo, Tokyo, Japan.

The marked increase in the incidence of Japanese cedar (Cryptomeria japonica; JC) pollinosis is a social problem in Japan. The prevalence is considered more than 20% in Japanese adult people. JC pollinosis (JCPsis) is a complex disorder caused by combination of genetic and environmental factors. Clinical and experimental evidence indicates barrier effect of respiratory epithelial cells play crucial role of developing allergic diseases. To clarify the genetic factor implicated in the etiology of JCPsis, we have conducted case-control study using SNPs of genes related infection and innate immunity. We recruited 331 cases with nasal allergy symptoms on JC pollen season whose CAP RAST score to JC pollen was above 2. We also recruited 183 controls with negative CAP RAST score to seven inhaled antigens who have no complications of other allergic disease including seasonal or perennial allergic rhinitis. We have found a significant association between JCPsis and promoter SNP of *Interferon Gamma Receptor 1* (*IFNGR1*) (p=0.013). This promoter SNP also revealed positive association with adult bronchial asthma (case 371, control 743). These findings suggested this promoter SNP might have important role in development of airway allergic inflammation. Functional analysis of the SNP using real-time quantitative RT-PCR, transient transfection reporter gene assays, EMSA are on going.

2593/T

Polymorphisms of *RIG-I* are associated with adult bronchial asthma. *M. Tamari[1], T. Hirota[1], M. Harada[1], M. Sakashita[1], S. Doi[2], A. Miyatake[3], Y. Nakamura[4].* 1) Lab Genetics Allergy, RIKEN SNP Research Center, Yokohama, Japan; 2) Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan; 3) Miyatake Asthma Clinic, Osaka, Japan; 4) The institute of Medical Science,University of Tokyo,Tokyo, Japan.

Bronchial asthma is a complex disorder caused by combination of genetic and environmental factors, and clinical and experimental evidence suggest an important role for respiratory viral infections in the development of asthma. An epidemiologic study showed that ~50% of adult asthma attacks ware associated with viral upper respiratory infections. Recent study has shown that retinoic-acid inducible gene-I (RIG-I) is a helicase-domain-containing protein and plays a crucial role in the host response to viral infection. These findings implicated *RIG-I* as a candidate gene for involvement in asthma. To assess genetic functional variants of *RIG-I* related to susceptibility and clinical phenotypes in adult asthma in a Japanese population, we screened for polymorphisms in *RIG-I* and conducted association studies of 465 subjects with adult asthma and 744 controls. We identified a total of 25 variants, and two non-synonymous substitutions, 19C/T (Arg7Cys) and 33636C/T (Ser144Phe), were found in *RIG-I* with minor allele frequencies of 4%. We characterized the linkage disequilibrium (LD) mapping of the gene by using the Haploview 3.2 program and three variants were selected for genotyping with regard to the LD pattern. We found significant associations between two 3'UTR polymorphisms (69438T/C and 70483T/del) and adult asthma susceptibility (P = 0.026 and P = 0.015, respectively). These findings suggest that the *RIG-I* gene might be involved in the development of adult asthma and the genetic polymorphisms might affect the sensitivity to viral infections. Further investigations off the connection between genotypes and the functional role of RIG-I will be helpful to clarify the etiology of asthma.

2594/T

***WDR36*: a Potential Modifier Gene Altering Glaucoma Severity in a Huge French-Canadian *Myocilin* Family.** *P. Belleau[1], K. Lebel[1], R. Arseneault[1], J.L. Anctil[2], A. Duchesne[1], M.A. Rodrigue[1], G. Côté[2], M. Amyot[3], V. Raymond[1,2], The Québec Glaucoma Network.* 1) Ocular Genetics & Genomics, CREMO, Laval University Hospital (CHUL) Res Ctr, Québec City, PQ, Canada; 2) Ophthalmology, Laval Univ, Québec City; 3) Ophthalmology, Univ of Montréal, Montréal, PQ, Canada.

Primary open-angle glaucoma (POAG), characterized by optic nerve degeneration and blindness, is genetically heterogenous. Three POAG genes are known: *myocilin* (*MYOC*), *optineurin* and *WDR36*. *MYOC* accounts for about 4 % of cases while contributions of *WDR36* are not well understood. In the French-Canadian CA family, the $MYOC^{K423E}$ mutation causes wide phenotypic variability of autosomal dominant POAG. In the pedigree, we observed distinct clusters for age-at-onset (AAO) of glaucoma supporting the presence of ≥ 1 modifier gene. To assess if *WDR36* was 1 of these modifiers, we studied genotype/phenotype correlations in double variants who simultaneously carried $MYOC^{K423E}$ and WDR36 variations. The family comprises 749 members with 156 $MYOC^{K423E}$ heterozygotes. Ophthalmologic records, some going back to the 1950s, were examined for 142 carriers; all of them were screened for *WDR36* variations by sequencing. AAO, defined as age at which ocular hypertension (OHT) or POAG was first detected, varied from 7 to 63 years old. 95 carriers were diagnosed POAG or OHT with *Rx*, 19 were OHT, 28 were still asymptomatic. Penetrance was 78% in carriers aged ≥ 40. Six WDR36 amino acid (AA) changes were detected in our 142 carriers. When we excluded the I264V polymorphism, 24 double variants were found to harbor $MYOC^{K423E}$ and one WDR36 AA change. To assess for an effect of these WDR36 variations on AAO, we compared AAO of glaucoma in these double variants to the median of AAO of the $MYOC^{K423E}$ heterozygotes who were $WDR36^{wild\text{-}type}$ and who shared a kinship coefficient ≥ 0,0625 (neighborhood). 18 double variants remained for analysis. Of these 18 individuals, 10 showed a younger AAO and 5 were below, but within, the range of the median AAO of their neighborhood. Interestingly, 4 $WDR36^{D658G}$ double variants were diagnosed > 10 years younger than their median AAO. In conclusion, *WDR36* may contribute to the glaucoma phenotype as a disease-modifier gene.

2595/T

LDLR polymorphisms, cholesterol and Alzheimers disease. *S. Estus[1], H. Zhu[1], F. Zou[2], J. Lok[3], S. Younkin[2], A.K. Manning[3], K.E. Grear[1], I.F. Ling[1], H.M. Tucker[1], J.F. Simpson[1], J. Kelly[4], D. Bennett[4], L.A. Cupples[3], S.G. Younkin[2].* 1) Physiology, University of Kentucky, Lexington, KY; 2) Neurosciences, Mayo Clinic, Jacksonville, FL; 3) Biostatistics, University of Boston, Boston, MA; 4) ADC, Rush University Medical Center, Chicago, IL.

Functional SNPs within the low-density lipoprotein receptor (LDLR) represent powerful investigative tools. Here, we focus on cholesterol homeostasis because LDLR mutations cause familial hypercholesterolemia and on Alzheimers disease (AD) because LDLR is a receptor for apoE, alleles of which modulate AD risk. Our first SNP of interest, rs688, neutralizes a putative LDLR exon 12 splicing enhancer and associates with decreased exon inclusion in vivo in a gender-dependent fashion; in vitro minigene studies establish rs688 as a functional SNP. We hypothesize the rs688 minor allele decreases LDLR function because the LDLR isoform lacking exon 12 encodes a truncated, non-functional receptor. The second SNP of interest is rs2738464 within the LDLR 3'UTR, the minor allele of which we associated with increased LDLR mRNA by comparing LDLR allelic expression in heterozygous individuals; we hypothesize the rs2738464 minor allele enhances LDLR function. We evaluated these SNPs for association with cholesterol homeostasis in the Framingham Offspring Study and with AD in several case-control series. The minor allele of rs688 associates with modest but significant increases in LDL (7 mg/dl) and total cholesterol (5 mg/dl) in women (adjusted values, $p \leq 0.05$) but not men; this gender difference reflects rs688 association with splicing efficiency in the female but not male liver. Preliminary analyses indicate that the rs688 minor allele associates with increased AD odds in men (OR of 1.48, 95% CI of 1.13-1.96, p=0.005, recessive model, n=1,535), but not women, reflecting its association with decreased splicing in the human male brain. Preliminary analyses indicate that rs2738464 associates with lower LDL and total cholesterol in men and women. Evaluation of rs2738464 with AD is on-going. In summary, these studies suggest that LDLR variants may contribute to cholesterol homeostasis and risk for AD in a gender-dependent fashion.

2596/T

Mitochondrial variation does not affect age at onset of neurologic symptoms of Huntington's disease. *S. Kishikawa[1], R. Saxena[1, 2], P.I.W. de Bakker[1, 2], R.H. Myers[3], M.E. MacDonald[1], D.M. Altshuler[1, 2], J.F. Gusella[1].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Neurology, Boston University School of Medicine, Boston, MA.

Huntington's disease (HD) is inherited as an autosomal dominant neurodegenerative disorder caused by an expanded polyglutamine tract in the huntingtin protein. The expanded polyglutamine tract accounts for up to 70 % of variance in age at onset, but remaining variation is strongly heritable, which indicates that genetic modifiers affect the pathogenic process. Defective energy metabolism and deficiency of respiratory chain complex activity in mitochondria, especially complex II/III and complex IV have been suggested in HD. We tested six hundred and thirty one (279 unrelated singletons and 352 siblings) HD affected individuals with younger or older than expected onset ages (±0.5 S.D. from expected repeat adjusted onset age) using tag SNPs in the human mitochondrial genome to test for an effect of mitochondrial genome variation on age at onset of HD. The 64 genotyped SNPs can predict all 114 SNPs that are over 1% frequency in Europeans based on a reference panel of over 900 publicly available European mtDNA sequences. In this genetic test of association, mitochondrial genome common variation captured by tag SNPs failed to explain residual variance in age at onset of neurologic symptoms of HD. The effect of mutant huntingtin on energy metabolism is likely to be indirect rather than a direct inhibition of mitochondrial function.

2597/T

Candidate system genes, SLC1A3, and the risk for substance use disorder. *M.M. Vanyukov[1,2,3], B.S. Maher[1,4], B. Devlin[1,2,3], R.E. Ferrell[1,2], G.P. Kirillova[1], H. Chilcoat[6], L. Murrelle[5], R.E. Tarter[1,3].* 1) Center for Education and Drug Abuse Research (CEDAR), Dept. of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Dept. of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Dept. of Psychiatry, University of Pittsburgh, Pittsburgh, PA; 4) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; 5) GSK, RTP, NC.

Liability to substance use disorder (SUD) is highly heritable. Candidate systems of genes can be identified based on the neurobiology of SUD and related traits, directing the search for loci accounting for SUD heritability. A custom Illumina panel of 1,536 SNPs covering 106 neurobiological system genes was selected using an iterative approach. Candidate system genes were selected based on current neurobiological knowledge and prioritized via consensus conference, substantially overlapping with the NIDA Genetics Consortium gene list. The next steps focused on inclusion of functional SNPs and LD-coverage of the top ranking genes. All HapMap SNPs were selected in each of the candidate genes and submitted to Illumina for quality scoring. SNPs returning a QS < 1 were deleted from the candidate list. The list of QS=1 SNPs for the top ranking candidate genes was submitted to the H-Clust algorithm for SNP selection. H-Clust identified 1,500 SNPs that provided an average coverage of r^2=.615 (based on HapMap) of the 106 highest-ranking candidate genes. In addition, all known non-synonymous common SNPs in each of the genes was selected for genotyping. This SNP panel was analyzed in 566 case and 195 control European-American males. Several genes representing different systems yielded multiple significant hits for SUD and related traits, after FDR correction for multiple testing. In particular, associations were detected with three SNPs in the glial high affinity glutamate transporter gene (SLC1A3) (rs10512660: p = .0003; rs891189: p=.0008; rs7734056: p=.0013). The data suggest that SLC1A3 may contribute to variation in common (non-drug-specific) liability to SUD.

2598/T

A protective variant of the PTPN22 locus in rheumatoid arthritis in the New Zealand Caucasian population. *W. Wan Taib[1], K. Gendall[1], P. Chapman[2], N. Dalbeth[3], P. Gow[3], A. Harrison[2], J. Highton[2], P. Jones[4], L. Stamp[2], J. O'Donnell[2], T.R. Merriman[1].* 1) Biochemistry Department, University of Otago, Dunedin, NZ; 2) School of Medicine, University of Otago, NZ; 3) Middlemore Hospital, Auckland, NZ; 4) QE Hospital, Rotorua, NZ.

Rheumatoid arthritis (RA) is a complex autoimmune disease with a strong genetic contribution to its pathogenesis. The first non-major histocompatibility complex (MHC) gene discovered to be reproducibly associated with RA is the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene in the Caucasian population. The PTPN22 functional variant, R620W, has been associated with RA in many studies in different populations. We have previously published a genome-wide association scan that identified MHC and PTPN22 as major loci in RA. Within the *PTPN22* haplotype block (365kb), there were disease-associated SNPs (rs3789600 and rs3789598) that suggested an association of *PTPN22* independent of *R620W*, possibly related to the disease-protective 'haplotype 5' previously identified by Carlton et al. (OR=0.65; frequency=0.35; *AJHG*, 77:567, 2005). The aim of the study reported here was to extend the previous data on 'haplotype 5', in particular studying variation in other genes mapping in the *PTPN22* haplotype block. The rs3789600 and rs3789598 SNPs were genotyped over 863 NZ Caucasian RA cases and 564 NZ controls. Both were associated with disease (*P*=0.0006 and *P*=0.0003, respectively) and clearly defined a protective haplotype, related to 'haplotype 5'. Subsequent typing of 'haplotype 5' tagging SNPs in the extended *PTPN22* haplotype block identified one SNP (rs1539438) in *C1orf178* that refined the protective haplotype to OR=0.53, $P=1x10^{-5}$, frequency=0.11). These data confirm the presence of a protective RA effect at *PTPN22* independent of *R620W* and possibly mapping outside of *PTPN22*.

2599/T

Comprehensive analysis of 331 candidate genes for orofacial clefting in a population-based infant-parent case-control study from Norway. *A. Jugessur[1], M. Shi[2], H. Gjessing[3], AJ. Wilcox[2], RT. Lie[1], CR. Weinberg[2], T. Nguyen Trung[1], AC. Lidral[4], AL. Boyles[2], K. Christensen[5], JC. Murray[4].* 1) University of Bergen, Norway; 2) NIEHS, Durham, NC; 3) Norwegian Institute of Public Health, Norway; 4) University of Iowa, IA; 5) University of Southern Denmark, Denmark.

Orofacial clefts rank among the most common birth defects in humans (1-2/1000 live births). The relative risk (RR) for recurrence in families is about 40, suggesting a partly genetic etiology. Identifying causative gene variants with adequate statistical power requires large and well-characterized datasets. The treatment of clefts is centralized in Norway, enabling a large population-based study of clefting with a high proportion of case-ascertainment. Moreover, Norway has an ethnically homogeneous population and one of the highest prevalence of cleft lip in the world (2/1000). The Norwegian dataset comprised 425 isolated case and 562 control infant-parent triads. We selected candidate genes based on linkage and association studies, studies of chromosomal rearrangements, and expression analyses in animal models. After CIDR genotyped a complete panel of 1536 SNPs in 357 genes, the genotypes for 1218 SNPs in 331 of the genes could be unambiguously assigned. We conducted single-marker and haplotype-based analyses using the program "HAPLIN" (Pubmed ID: 16674560) to estimate RRs when the infants carry one or two copies of a designated "risk" allele (or haplotype). We also identified risk-related haplotypes using "TRIMM" (Shi et al. AJHG, in press), which is a test that uses only the genotypes of affected members and their parents, does not require HWE, and circumvents the need to know or assign haplotypes and their phases. HAPLIN identified 24 genes for which the overall p-value was lower than 0.05, and TRIMM identified 26. The genes identified by both methods include ADH1B, APOA5, FOXE1, FZD2, HOXB6, IRF6, MSX1, RYK, UGT1A7, and VCL. Reassuringly, this list contains IRF6, FOXE1 and MSX1 that have repeatedly shown strong statistical associations with clefts in multiple diverse populations. Analyses on an independent set of 235 case-parent triads from a Danish cleft population is currently underway to confirm these findings.

2600/T

Family-based study of ten immunity-related genes and tuberculosis: association with TLR2, TLR9, SLC11A1, and NOS2A. *W.K. Scott[1], W.F. Hulme[1], J.B. Rimmler[2], M.E. Stryjewski[3], E.H. Abbate[4], R. Estevan[4], J.R. Gilbert[1], C.D. Hamilton[2].* 1) University of Miami, Miami, FL; 2) Duke University, Durham, NC; 3) CEMIC, Buenos Aires, Argentina; 4) F.J. Muñiz Hospital, Buenos Aires, Argentina.

Tuberculosis (TB) is a significant cause of premature mortality worldwide. Ten percent of exposed individuals develop pulmonary TB, suggesting that host factors, partly under genetic control, determine development of active TB. We collected samples from 233 African-American and 165 white families with at least one case of pulmonary TB. People older than 14 years with culture-confirmed pulmonary TB and children younger than 14 years with culture- or clinically-confirmed TB were recruited from the North and South Carolina TB control programs and the outpatient clinics of F.J. Muñiz Hospital, Buenos Aires, Argentina. Unaffected siblings, parents, and spouses/partners were enrolled as controls. We examined 167 haplotype-tagging SNPs (tagSNPs) in ten immunity-related genes (TLR2, TLR4, TLR9, SLC11A1, NOS2A, TNFA, INFG, INFGR1, VDR, PARK2) for association with TB. tagSNPs were selected from HapMap Phase II data and captured the common variation (minor allele frequency (MAF) > 5 %) in each gene with r^2>0.8. Other coding SNPs with MAF > 1% were genotyped as well. Genotypes were determined using TaqMan assays. Analyses using the association in the presence of linkage (APL) test were conducted stratified by self-reported race to limit confounding. In African-American families, TB was associated with SNPs in TLR2 (rs653939, p=0.04), TLR9 (rs352139, p=0.03; rs187084, p=0.049), SLC11A1 (rs3731865, p=0.03), and NOS2A (rs2255929, p=0.03; rs2314809, p=0.004; rs2779248, p=0.04). Only TLR2 (rs3804100, p= 0.04) was associated with TB in white families. None of these SNPs has known functional significance, but may be in linkage disequilibrium with a functionally relevant variant. While associations in TLR2, TLR9 and SLC11A1 have been previously detected in subsets of these families, the findings with intronic SNPs near the 3' end of NOS2A (rs2255929, rs2314809) are novel. These results suggest that innate immunity genes are potential risk factors for development of TB, particularly in individuals of African descent.

2601/T

Fine-mapping of an Asthma Susceptibility Locus on Chromosome 3p14. *T.D. Howard[1], E.R. Bleecker[1], E.A. Ampleford[1], G.H. Koppelman[2], D.S. Postma[3], D.A. Meyers[1].* 1) Ctr Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Dept of Pediatric Pulmonology, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, The Netherlands; 3) Dept of Pulmonology, University Medical Center Groningen, University of Groningen, The Netherlands.

Asthma is an increasingly common disease caused by bronchial inflammation and characterized by bronchial hyperresponsiveness (BHR) and intermittent airways obstruction. In an effort to delineate the genetic susceptibility to asthma, we have previously performed a genome-wide screen in a Dutch population of 200 families ascertained by a proband with asthma. The two genomic regions with the strongest evidence for linkage with BHR and "asthma" (determined by an algorithm incorporating BHR and other components) were located on chromosomes 3p14-p21 and 5q31. On chromosome 3p, the peak lod score after fine-mapping with 36 additional microsatellite markers was 3.4 for asthma and 3.9 for BHR, between the markers D3S1514 and D3S2452. Using BHR, the lod - 1 support interval extends from 102cM to 105.5cM, between markers D3S3588 and D3S1547. We have recently constructed a high-density SNP map of this ~4Mb region in the Dutch population, utilizing LD data available from the International HapMap Project. A total of 776 SNPs were genotyped with minor allele frequencies of >0.01 in this Dutch population. Two of these significantly deviated (<0.001) from Hardy-Weinberg Equilibrium, while an additional 27 SNPs showed modest deviation (<0.05). Both FBAT and parenTDT (as implemented in HaploView) analyses were performed with BHR (defined as a 20% drop in forced expiratory volume in 1 second with <32mg/ml histamine) and "asthma." Forty-six SNPs were significant with either BHR or asthma with either analytical method (p = 0.0001 - 0.01). Several SNPs are located in potential candidate genes, including CAST and IL17RD, while others are in intergenic regions. Additional work is in progress to replicate and confirm these findings in independent multi-ethnic populations and to determine the overall relevance of these SNPs in asthma susceptibility.

2602/T

BDNF gene polymorphisms are associated with stroke recovery at 3 months. *I. Albergaria[1], H. Manso[1,2], T. Krug[2], B. Nunes[1], G. Gaspar[1], L. Gouveia[3], I. Matos[4], M.V. Baptista[5], G. Lopes[6], R. Taipa[6], J.P. Gabriel[7], M.R. Silva[8], C. Dias[1], F. Gonçalves[9], M. Correia[6], J.M. Ferro[3], S. Oliveira[2], A.M. Vicente[1,2].* 1) Instituto Nacional Saúde Dr. Ricardo Jorge, Portugal; 2) Instituto Gulbenkian de Ciência, Portugal; 3) H. Sta. Maria, Portugal; 4) H.Distrital Mirandela; 5) H.Garcia de Orta; 6) H.Geral Sto. António, Portugal; 7) H. S. Pedro; 8) H.Fernando Fonseca; 9) H.Universidade de Coimbra, Portugal.

Stroke is a major cause for morbidity in developed countries. After a stroke episode 50-70% of patients regain functional independence, while 15-30% are permanently disabled and 20% require institutional care. Family history of stroke is associated with poor functional outcome but not stroke severity, age at onset or 90-day mortality. In spite of this evidence, few studies have investigated the impact of genetic factors in stroke outcome and recovery. In the present work, we analysed the role of a compelling candidate gene, Brain-derived Neurotrophic Factor (*BDNF*), in stroke functional outcome. BDNF is implicated in neuronal regeneration and proliferation, and has been shown to induce antiapoptotic mechanisms after stroke and to reduce infarct size and secondary neuronal cell death after induced stroke in animal models. Functional outcome in 403 stroke patients under 65 was assessed 3 months after a stroke episode using the modified Rankin Scale (mRS). 14 *tag* SNPs covering the *BDNF* coding and flanking sequences were tested for association with Rankin scores at 3 months using the Kruskal-Wallis non-parametric test. SNP rs10835210 was nominally associated with recovery (χ^2=6.75, 2 df, *P*=0.034). When only ischemic stroke patients were considered (N=304), three SNPs were significantly associated with mRS scores, rs10835210 (χ^2= 9.873, 2 df, *P*=0.007), rs6265 (χ^2=6.398, 2 df,*P*=0.041), rs4923460 (χ^2=6.225, 2 df,*P*=0.044). A case/control association study in a population sample of 533 stroke patients and 507 controls in the same age range provided no evidence for the involvement of *BDNF* in stroke susceptibility. The results suggest a contribution of *BDNF* gene variants to the process of neuronal recovery after ischemic stroke that is independent of stroke susceptibility.

2603/T

Association analysis of growth factor genes FGF2 and VEGF with stroke susceptibility. *B.V. Fonseca[1], H. Manso[1,2], T. Krug[1], B. Nunes[2], I. Albergaria[2], G. Gaspar[2], L. Gouveia[3], I. Matos[4], M.V. Baptista[5], G. Lopes[6], R. Taipa[6], J.P. Gabriel[7], M.R. Silva[8], C. Dias[2], F. Gonçalves[9], M. Correia[6], J.M. Ferro[3], S.A. Oliveira[1], A.M. Vicente[1,2].* 1) Inst. Gulbenkian de Ciencia, Portugal; 2) Inst. Nacional Saúde Dr. Ricardo Jorge, Portugal; 3) H. Sta. Maria, Portugal; 4) H. Distrital Mirandela, Portugal; 5) H. Garcia de Orta, Portugal; 6) H. Geral Sto. António, Portugal; 7) H.S. Pedro, Portugal; 8) H. Fernando Fonseca, Portugal; 9) H.Universidade Coimbra, Portugal.

Growth factors like basic fibroblast growth factor (FGF2) and vascular endothelial growth factor (VEGF) are key regulators of neuronal regeneration and proliferation previously implicated in stroke pathophysiology and functional outcome. FGF2 is known to protect against ischemic injury in rat brain, while improving sensorimotor function and reducing infarct size after focal ischemia. VEGF is a secreted mitogen associated with angiogenesis enhancement in the ischemic brain and with reduced neurological deficits during stroke recovery. We therefore evaluated the role of the genes encoding FGF2 and VEGF in stroke susceptibility and recovery. We tested 14 tag SNPs in the FGF2 gene and 8 tag SNPs in the VEGF gene for association with stroke risk in a population of 533 stroke patients and 507 unrelated controls. A weak association of one FGF2 SNP (rs1960669: χ2=4.342, 2df, P=.037) with stroke susceptibility was found. One specific haplotype was associated with increased stroke risk (rs6899540-rs6900017-rs6905288, χ2=7.564, P=.006). Two VEGF SNPs were associated with stroke risk (rs3025010, χ2=8.139, 2df, P=.017; rs3025033, χ2=6.916, 2df, P=.032). Haplotype analysis further showed an association of one specific haplotype with stroke protection (rs11938826-rs308441-rs308442, χ2=4.625, P=.0315). The impact of these genes in functional outcome was tested in 403 stroke patients, assessed 3 months after a stroke episode using the modified Rankin Scale, but no evidence for association was found for any of the tested markers. Overall these results indicate that FGF2 and VEGF are not major stroke risk factors and do not contribute to variation in stroke recovery in this population.

2604/T

Contribution of complement factor H Y402H polymorphism to stroke risk. *L. Gouveia[1], T. Krug[2], H. Manso[2,3], I. Albergaria[3], G. Gaspar[3], R. Taipa[4], M.R. Silva[5], M. Correia[4], M.V. Baptista[6], A. Pinto[7], R. Silva[7], C. Ferreira[8], J.P. Gabriel[8], I. Matos[9], G. Lopes[4], A.M. Vicente[2,3], S.A. Oliveira[2], J.M. Ferro[1].* 1) H. de Santa Maria, Portugal; 2) Instituto Gulbenkian de Ciência, Portugal; 3) Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal; 4) H. Geral de Santo António, Portugal; 5) H. de São Pedro, Portugal; 6) H. Garcia de Orta, Portugal; 7) H. Fernando Fonseca, Portugal; 8) H. São Marcos, Portugal; 9) Distrital de Mirandela, Portugal.

The evidence that inflammation is an important mechanism in atherogenesis and stroke is growing with recent data from human and animal studies, and both complement factors and complement regulatory factors have been linked to cardiovascular diseases. Complement inhibitor factor H (CFH) is a plasma protein essential in the regulation of the alternative complement pathway and has been suggested to play a part in complement inhibition in atherosclerotic lesions. The Y402H (rs1061170) polymorphism in the CFH gene has been firmly established as a risk factor for age-related macular degeneration. This exonic polymorphism seems to alter the ability of CFH to suppress excess complement activation, ultimately leading to complement-related damage to arterial walls and vessel injury. There are inconsistent findings regarding the CFH role in susceptibility to myocardial infarction. To investigate the role of CFH in stroke susceptibility we assessed the association of the Y402H genetic variant in a Portuguese dataset of 533 stroke patients (82% with ischemic stroke) and 507 unrelated controls. We found a weak allelic association of this polymorphism with ischemic stroke (OR=1.23, 95%CI:1.00-1.50, p=0.043), but no association when ischemic and hemorrhagic stroke were combined (OR=1.21, 95%CI:0.99-1.46, p=0.054). These results suggest that the polymorphism Y402H in CFH is, at best, a minor stroke risk factor.

2605/T

Leptin Gene Polymorphisms: Association with Obstructive Sleep Apnea in Children. *M. Kalra[1], P. Pal[3], S. Guha[3], L. Dolan[2], R. Deka[3], R. Chakraborty[3].* 1) Division of Pulmonary Medicine, Cincinnati Children's Hosp, Cincinnati, OH; 2) Division of Endocrinology, Cincinnati Children's Hosp, Cincinnati, OH; 3) Center for Genome Information, University of Cincinnati, Cincinnati, OH.

Obstructive sleep apnea (OSA)is a complex disorder with an interplay between factors related to airway anatomy, airway neuromuscular control, and ventilatory control implicated in its pathogenesis. Although genetic predisposition for this disorder has been demonstrated, the exact genetic underpinning is not yet clear. Reports on the effect of plasma Leptin levels on ventilatory drive support the role of *Leptin* in mediating genetic susceptibility to OSA. The objective of this study was thus to test the association of *Leptin* polymorphisms and OSA status. All Caucasian children diagnosed with OSA at Cincinnati Children's Sleep Center between January and June 2006 were recruited as cases and ethnicity matched controls were selected from the population-based "Princeton School District Study". Three SNPs (rs 7795794, rs 3828942 and rs 2060715) that tag the Leptin gene were selected using SNP browser ver. 3.5. Genotyping was performed using the SNPlex (ABI) high-throughput genotyping platform. OSA was defined as apnea hypopnea index >1 on polysomnogram; cases were then compared to population-based controls. The mean age of the 74 OSA cases was 13.6 years (S.D. 4.3), 60% were males, and mean BMI was 31.9 (S.D. 11.0). The mean age of the 92 controls was 14.3 years (S.D. 2.9), 60 % were males, and mean BMI was 25.7 (S.D. 2.9). The genotype frequencies of all SNPs were in Hardy Weinberg Equilibrium. Structure analysis revealed absence of significant population stratification between cases and controls. Comparison of genotype frequencies between cases and controls revealed significant differences for all 3 SNPs (rs 779574,p=<0.0001; rs 3828942, p= 0.03; and rs 2060715, p= 0.04). Multivariate logistic regression analysis with age and BMI as covariates revealed significant association between OSA status and SNP rs779574 (P=0.0002). This is the first report of association of *Leptin* polymorphisms with OSA in children, supporting the role of Leptin in the pathogenesis of pediatric OSA independent of obesity.

2606/T

A variant form of the signal transducer and activator of transcription gene (*STAT4*) increases genetic susceptibility to rheumatoid arthritis and systemic lupus erythematosus. *E.F. Remmers[1], R.M. Plenge[2], A.T. Lee[3], R.R. Graham[2], G. Hom[4], T.W. Behrens[4], P.I.W. de Bakker[2], J.M. Le[1], H.-S. Lee[3], J.P. Carulli[5], L. Padyukov[6], L. Alfredsson[6], L. Klareskog[6], W.V. Chen[7], C.I. Amos[7], L.A. Criswell[8], M.F. Seldin[9], D.L. Kastner[1], P.K. Gregersen[3].* 1) NIAMS, Bethesda, Md; 2) Broad Institute of Harvard and MIT, Cambridge, Mass; 3) Feinstein Institute for Medical Research, North Shore L.I.J. Health System, Manhasset, N.Y; 4) Genentech, Inc., South San Francisco, Ca; 5) Biogen Idec, Inc., Cambridge, Mass; 6) Karolinska Institutet, Stockholm, Sweden; 7) University of Texas, M.D. Anderson Cancer Center, Houston, Tx; 8) University of California San Francisco, San Francisco, Ca; 9) University of California Davis, Davis, Ca.

Rheumatoid arthritis (RA) is a chronic inflammatory disease with a significant genetic component. Susceptibility to disease has been linked with a region on chromosome 2q. We performed association studies using tag SNPs for 13 candidate genes within this region and fine mapped the *STAT1/STAT4* region with 63 SNPs in a total of 1620 established RA cases and 2635 controls. One of the disease-associated SNPs was also genotyped in 1529 recent onset RA cases and 881 controls from Sweden and three systemic lupus erythematosus (SLE) case-control series totaling 1,036 cases and 1188 independent controls. Four SNPs located in the third intron of *STAT4* were strongly associated with susceptibility to RA, minor allele frequency (MAF)=0.27 in established RA cases compared with 0.22 in controls (for rs7574865, $P=3x10^{-7}$; OR=1.32, 95% CI=1.19-1.46). This association was also seen in the Swedish recent onset RA cohort (P=0.02). The haplotype marked by rs7574865 was even more strongly associated with SLE (MAF=0.31 versus 0.22 in the combined SLE cases and controls, $P=2x10^{-9}$; OR= 1.55, 95% CI=1.34-1.79). STAT4 transmits signals from cytokines such as IL-12 and type 1 interferon, thereby regulating gene expression programs that are required for T-cell differentiation and for activation of mature dendritic cells. These data emphasize an important role for these pathways in the pathogenesis of both RA and SLE.

2607/T

Association of proopiomelanocortin gene variation with cocaine or opioid dependence: evidence from both family and population-based studies. *H. Zhang[1,2], H.R. Kranzler[3], R.D. Weiss[4], K.T. Brady[5], R.F. Anton[5], L.A. Farrer[6], J. Gelernter[1,2].* 1) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 2) VA Connecticut Healthcare System, West Haven, CT; 3) Department of Psychiatry, University of Connecticut School of Medicine, Farmington, CT; 4) Alcohol and Drug Abuse Treatment Program, McLean Hospital, Belmont, MA; 5) Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC; 6) Department of Medicine (Genetics Program), Boston University Schools of Medicine and Public Health, Boston, MA.

The proopiomelanocortin gene (*POMC*) encodes several biologically active peptides including the adrenocorticotropic hormone and the β-endorphin. In this study, we examined the association of *POMC* variants with cocaine dependence (CD) or opioid dependence (OD). Four *POMC* tag single nucleotide polymorphisms (tag SNPs) (SNP1 and SNP2 in the promoter region, SNP3 in intron 1, and SNP4 in intron 2) were genotyped by Illumina array methodology in 1,344 subjects in 612 families [363 African-American (AA) and 249 European-American (EA)] with sibling pairs affected with CD or OD. Family-based association tests demonstrated an association of SNP1 and SNP4 with OD in AA families ($P = 0.002$ and 0.012, respectively) and SNP4 with CD in EA families ($P = 0.039$). Moreover, haplotype-based association tests showed a risk effect of a specific haplotype on CD ($P = 0.021$) in AA families. To confirm the findings, we performed a replication study using two sets of case-control samples (196 AA and 492 EA controls, and 432 AA and 304 EA cases, also affected with CD or OD). A marginally significant association between two *POMC* SNPs (SNP1 and SNP2) and OD was found in AAs. Additionally, a strong association between SNP4 and CD was observed in EAs (allele-wise $P = 0.007$, genotype-wise $P = 0.032$). Interestingly, the risk haplotype identified in AA families was significantly more frequent in CD cases than in controls in both AA and EA populations (AAs: $P = 0.025$; EAs: $P = 0.031$). In summary, our data from both family and population-based studies indicate that genetic variation in *POMC* may confer vulnerability to CD or OD.

2608/T

Creation of maternally inherited mouse models of mitochondrial cardiomyopathy and directional mitochondrial DNA (mtDNA) segregation by introduction of a homoplasmic mtDNA COI missense mutation and a linked heteroplasmic ND6 frameshift mutation into the female mouse germ line. *W. Fan[1], K. Waymire[1], P. Li[2], N. Narula[3], P.E. Coskun[1], M.A. Vannan[2], C. Rocher[1,4], J. Narula[2], G. MacGregor[1], D.C. Wallace[1].* 1) MAMMAG, University of California, Irvine, Irvine, CA; 2) Medicine, University of California, Irvine, Irvine, CA; 3) Pathology, University of California, Irvine, Irvine, CA; 4) INSERM, U. Bordeaux 2, Bordeaux, France.

We have created the first mouse models of maternally inherited mitochondrial myopathy and cardiomyopathy caused by a missense mutation and of directional segregation of a heteroplasmic mtDNA frameshift mutation. A mtDNA was isolated from cultured mouse cells that was homoplasmic for a COI T6589C (V421A) missense mutation and an ND6 13885insC frameshift mutation. This mtDNA was introduced into the mouse germ line via cybrid transfer into rhodamine 6G-treated female embryonic stem (mES) cells generating an ES cell homoplasmic for the COI mutation in which 4% of the mtDNAs contained a 13885insCdelT ND6 reversion. One chimeric mother produced a female offspring whose mtDNA was homoplasmic for the COI mutation and heteroplasmic for the ND6 mutation (47% ND6 13885insC + 53% 13885insCdelT). This mouse developed mild myopathy and cardiomyopathy and had a partial complex I + IV defect. The frameshift mtDNA was directionally lost within the first three generations, decreasing successively from 47 to 14 to 6 to 0% ND6 13885insC. The resulting homoplasmic COI T6589C + 13885insCdelT mutant mice had a 37%-48% complex IV deficiency in brain, heart, liver, and skeletal muscle and increased heart mitochondrial proliferation, disordered mitochondrial distribution, and cristae-lysis. Echo cardiograms revealed that these animals developed a hypertrophic cardiomyopathy associated with a 26% increase in left ventricular wall thickness, a 30% decrease in left ventricular diastolic internal dimension, a 39% decrease in circumferential strain, and a 74% decrease in radial strain. This stable maternally inherited mouse model now opens new avenues for studying the pathophysiology and therapeutic of mitochondrial disease.

2609/T

Assessing the transcription factor gene grainy-head like 3 (*GRHL3*) as a candidate for causation of spina bifida meningomyelocele. *K.S. Au[1], M.R. Dewhurst[1], C.C-D. Tsai[1], J.M. Fletcher[2], G.H. Tyerman[3], T.M. King[1], H. Northrup[1,4].* 1) Dept Pediatrics, Univ Texas Medical Sch, Houston, TX; 2) Dept of Psychology, Univ of Houston, Houston, TX; 3) Shriners Hospital for Children, Los Angeles, CA; 4) Shriners Hospital for Children, Houston, TX.

Spina bifida meningomyelocele (SBMM) is a common birth defect with an overall incidence of 7/10,000 live births in the United States. Among known mouse models of neural tube defects (NTDs), the *ct* (*curly-tail*) mouse represents one of the best models for non-syndromic SBMM in humans. Recent findings suggested the ct mice are hypomorphs of the grainy-head like 3 gene (*Grhl3*). *Grhl3* knock-out mice showed thoraco-lumbar-sacral SBMM and curly tail. *Grhl3* is mapped to the same locus as the ct mouse gene and the *Grhl3* knock-out allele failed to rescue the ct phenotype. *Grhl3* mRNA is expressed in non-neural ectoderm adjacent to the neural fold. Globally, *Grhl3* protein is a transcription regulator and functions as a "master" protein in many aspects of epidermal movement and fusion. In humans the *GRHL3* gene spans a region of 35.9 Kbp on chromosome 1p36.11 and is highly conserved among species. We have selected single nucleotide polymorphic (SNP) sites with known minor allele frequencies greater than 0.1 spanning the *GRHL3* region with a density of 2-3 Kb per SNP to examine the association of this gene with approximately 600 SBMM families and 200 controls. Analyses of the *GRHL3* SNP genotypes of 200 controls using Haploview 3.32 showed two distinct Linkage Disequilibrium (LD) blocks with one 50 Kb block spanning the upstream region from the promoter and the other 12Kb block spanning exons 5 to 15. Analyses of SBMM patient genotypes showed similar patterns except the block at the promoter region is less defined. A lower frequency of the TTG haplotypes (rs3887581, rs4648859, rs1203005) spanning exons 11-13 is observed among SBMM patients. This finding appears to be consistent with our discovery of several synonymous and nonsynonymous variants in exons 11 and 12 among SBMM patients pointing to possible functional defect of these variants may contribute to SBMM risk.

2610/T

VEGF gene increases susceptibility to endometriosis. *W.Y Lin[1], S.H Juo[1,3], R. Wu[1], C.Y Long[4], E.M Tsai[2,4].* 1) Institutes of Medical Genetics, Kaohsiung Medical University , Kaohsiung, Kaohsiung, Taiwan; 2) Graduate Institute of Medicine; 3) Department of Medical Research; 4) Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background: It is believed that angiogenesis is essential for the development of endometriosis. Vascular endothelial growth factor (VEGF) is believed to be a pivotal angiogenic factor with endothelial cell-specific mitogenic and vascular permeability activities. We conducted a case-control study to test whether the polymorphisms of the VEGF gene increase susceptibility to endometriosis. Methods: Three common functional polymorphisms were chosen, which were -2578C/A (rs699947), -634G/C (rs2010963), and +936C/T at the 3'UTR (rs3025039). We recruited 120 patients with endometriosis and 400 women free of the disease. The diagnosis of endometriosis was based on both clinical and pathology evidence. The data were analyzed by multivariate regression analysis with adjustment for age, parity and body mass index. Haplotype analyses with adjustment for covariates were performed using the Hap-Clustering program. Results: All the 3 SNPs were in Hardy-Weinberg equilibrium. The genotypic distributions of promoter SNP rs2010963 were significantly different between cases (40% GG, 52.5% GC and 7.5% CC) and controls (36.8% GG, 46.8% GC and 16.4% CC). We found that the C allele carriers of this SNP had a lower risk for endometriosis (CC vs GG adjusted OR=0.34; p =0.02, and CG vs GG adjusted OR=0.77; p=0.35). Since the C allele appeared to have a dose effect, we further calculated the C allele genetic effect assuming an additive model. The result demonstrated an adjusted OR for C allele of 0.64 (p=0.028). The other two SNPs did not yielded any significant results. The haplotype composed by the two promoter SNPs (rs2010963 and rs699947) did not yield a better result (global p=0.08) than the result from the significant SNP rs2010963. Similarly, the haplotype analysis based on all three SNPs did not show significant results. Conclusions: The present study showed that the promoter SNP at VEGF may confer risk for endometriosis.

2611/T

AKT2 gene variants associate with muscle phenotypes in young men and women. *F.E Orkunoglu-Suer, H. Gordish-Dressman, EP. Hoffman, JM. Devaney.* Research Center for Genetic Medicine,Children's National Medical Center,Washington,DC.

PURPOSE:AKT2 is a key signaling intermediate for insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle and is activated by exercise and muscle contraction in both rodents and humans.This makes AKT2 an attractive candidate gene for examining human skeletal muscle size and function following resistance training. We hypothesized that variants in AKT2(rs2304186,rs969531,rs892118)would be associated with muscle strength and size phenotypes.METHODS:524 European Americans(23±5y)were enrolled by eight different exercise physiology sites.Phenotype measures included muscle volume/strength,bone and fat volume and exercise induced changes. SNP/phenotype associations were tested using a general linear model with Sidak post-hoc tests and logistic regression;covariates included baseline body weight and age.RESULTS:AKT2 rs2304186 was significantly associated with muscle strength,whole muscle volume and baseline bone volume in males($p<0.05$).In males TT homozygotes had higher baseline one repetition max(1RM) values($p=0.0009$,4.1%) but did not show any training effect.For rs2304186, females homozygous for the T allele had lower baseline subcutaneous fat volume($p=0.04$).For rs96953,females with two copies of the A allele had higher change in isometric strength after exercise($p=0.014$).For rs892118, males with a copy of the T allele showed a gain in muscle volume with exercise that explained 3.6% of population variance.CONCLUSIONS:These data suggest a sex-specific effect of AKT2 genotypes on exercise training induced strength changes in young white adults.AKT2 SNP rs969531 is associated with responses to resistance training in females.In addition, rs2304186 genotype associated with higher baseline muscle strength values but did not show any training effect in males.Additionally, males with two copies of the T allele for rs892118 gained muscle volume with resistance training.The genetic variation in AKT2 may affect the phosporylation and it's regulation of creatine kinase in muscle cells, thus skeletal muscle differentiation and predisposition to rehabilitation medicine and metabolic syndrome.ACKNOWLEDGEMENT: This study was supported by NINDS-1R01NS040606.

2612/T

Association between vitamin D receptor gene (VDR)polymorphisms and tuberculosis in Mexican Mestizo Patients. *A. Tomasena-Glennie[1,3], J.M. Oliva-Ortiz[1,2], A.L. Corona-Nakamura[4], G. Amaya-Tapia[5], C. Morán-Moguel[1], J. Sanchez-Corona[1], L. Sandoval-Ramirez[1,2].* 1) División de Genética, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara,Guadalajara, Jalisco, México; 3) CUCEI, Universidad de Guadalajara, Guadalajara, Jalisco, México; 4) 3 Servicio de Infectología, Hospital de Especialidades, UMAE, Hospital de Especialidades,CMNO, IMSS, Guadalajara, Jalisco, México; 5) Hospital General de Occidente, SS, Guadalajara, Jalisco, México.

Introduction. Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis, that along with Malaria and HIV is responsible for approximately six million deaths per year1 and has been described as one of the five pandemics of the 21st century by the World Health Organization (WHO). Polymorphisms in certain genes, like NRAMP1 and VDR, have been associated with susceptibility to TB. In addition to that, epidemiological studies show a relation between vitamin D deficiency and susceptibility to TB and some in vitro studies suggest that there's a close effect of the active metabolite of vitamin D, 1,25D3, on the mycobacterial growth 2. Our objective is to determine whether there exists any association between VDR gene polymorphisms (BsmI, FokI, ApaI and TaqI) and tuberculosis in Mexican mestizo patients. Method: A total of 63 patients with TB were included along with 77 controls reported as healthy. The genotyping of the four polymorphisms was made by PCR/RFLP method. Results:. The genotype frequencies found among patients for BsmI were BB 33.3%, Bb 65% and bb 1.6%; for FokI: FF 41.3%, Ff 46% and ff 12.7 %; for TaqI: TT 54%, Tt 39.7% and tt 6.3% and for ApaI: AA 60.3%, Aa 30.2% and aa 9.5%. Controls genotype frequencies were: For BsmI BB 31.9%, Bb 57.1% and bb 11%; for FokI: FF 44%, Ff 46.2 % and ff 9.9%; for TaqI: TT 63.7 %, Tt 29.7% and tt 6.6% and for ApaI: AA 54.9%, Aa 28.6% and aa 16.5%. Conclusions: We didn't find any association between VDR gene polymorphisms (BsmI, FokI, ApaI y TaqI) and TB although it has been found in other populations. A larger study with a bigger number of samples would be recommended.

2613/T

Paternal histoincompatibility effects of the *HLA-G* gene and increased risk for pre-eclampsia. *S.S. Chong[1], C.Y. Tan[1], J.F.V. Ho[1], Y.S. Chong[2], A. Loganath[2], Y.H. Chan[3], J. Ravichandran[3], C.G. Lee[4].* 1) Pediatrics, National Univ Singapore, Singapore; 2) ObGyn, National Univ Singapore, Singapore; 3) Biostatistics, School of Medicine, National Univ Singapore, Singapore; 4) Biochemistry, National Univ Singapore, Singapore; 5) Sultanah Aminah Hospital, Johor, Malaysia.

Hypothesis: Pre-eclampsia (PE) is a leading cause of maternal and fetal mortality and morbidity which occurs only during pregnancy. Alterations in HLA-G function at the maternal-fetal interface have been postulated to affect placental vascular remodeling and predisposition to PE. We postulated that paternal alleles of *HLA-G* may increase risk for PE in susceptible mothers.

Methods: Association between *HLA-G* and PE was tested in a case-control study of 86 PE and 245 normotensive Malay women. Superimposed PE cases were excluded. The *HLA-G* gene was amplified in a single-tube multiplex PCR reaction and genotyped for 18 single nucleotide polymorphisms (SNPs) or variations using a multiplex minisequencing strategy. Haplotype/haplogroup analyses and case-control comparisons were performed statistically using Fisher's exact test, and associations with disease were expressed as odds ratios with 95% confidence intervals.

Results: Risk for PE was not associated with maternal *HLA-G* haplotype, but was strongly associated with presence of fetal haplotype G*0106 (p=0.014; OR=3.299, 95% CI 1.317-8.262). The CT heterozygote frequency at the codon 258 SNP, which defines haplotype G*0106, also differed strongly only between case and control babies (p=0.012; OR=3.450, 95% CI 1.316-8.806). Furthermore, the frequency of fetal-maternal genotype mismatch at codon 258, where maternal genotype was CC and fetal genotype was CT, was significantly higher in PE pregnancies compared to normal pregnancies (p=0.016, OR=4.253, 95% CI 1.313-13.779).

Conclusion: Paternal contribution of *HLA-G* G*0106 in the fetus significantly increases risk for PE in mothers who do not carry this haplotype. Maternal immune response to variant paternal HLA-G antigens present in the fetus may be a risk factor for PE in certain pregnancies.

2614/T

Association of a Common Haplotype in the Annexin A5 (ANXA5) Gene Promoter with Recurrent Pregnancy Loss. *J. Horst[1], N. Bogdanova[1], M. Chlystun[2], P.J.P. Croucher[3], A. Nebel[4], A. Bohring[1], A. Todorova[5], S. Schreiber[4], V. Gerke[2], M. Krawczak[3], A. Markoff[2].* 1) Institut für Humangenetik der WWU Münster, Münster, Germany; 2) Institut für Medizinische Biochemie, ZMBE, WWU Münster, Germany; 3) Institut für Medizinische Informatik und Statistik, Christian-Albrechts-University, Schleswig-Holstein, Kiel, Germany; 4) Institut für Klinische Molekulare Biologie, Christian-Albrechts-University, Schleswig-Holstein, Kiel, Germany; 5) Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynecology, Medical University of Sofia, Sofia, Bulgaria.

Annexin A5 is a typical member of the chordate annexin family and is one of the few annexins that can be found extracellularly (12). Annexin A5 is thought to function as an inhibitor of coagulation owing to its ability to bind to anionic phospholipids exposed on the surface of, for example, platelets, thereby inhibiting aggregation. In this work we sought to verify whether variation in the promoter of the placental anticoagulant protein annexin A5 (ANXA5) gene represents a risk factor for recurrent pregnancy loss (RPL). Sequence analysis of 70 German RPL patients revealed four consecutive nucleotide substitutions in the ANXA5 promoter that were transmitted as a joint haplotype (M2). Reporter gene assays revealed that M2 reduces the in vitro activity of the ANXA5 promoter to 37-42% of the normal level. Carriers of M2 were found to exhibit a more than two-fold higher RPL risk than non-carriers (odds ratio = 2.42, 95% confidence interval: 1.27 - 4.58) when using unselected controls (PopGen), and an almost four-fold higher risk when using the Münster 'super-controls', i.e. women with successful pregnancies and no previous history of pregnancy losses (odds ratio = 3.88, 95% confidence intervall: 1.98 - 7.54). This statistically significant association should facilitate the development of improved prognostic algorithms for RPL, involving a more precise assessment of individual disease risks, and provide a guide to offering adequate therapies where relevant.

2615/T

Large scale transcriptomic analysis of *ACVR2A*, a pre-eclampsia positional candidate gene. *M.P. Johnson[1], J.E. Curran[1], J. Kent Jr.[1], H.H.H. Göring[1], T.D. Dyer[1], R. Austgulen[2], S.A. Cole[1], J.W. MacCluer[1], S.P. Brennecke[3], J. Blangero[1], E.K. Moses[1].* 1) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA; 2) Faculty of Medicine, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; 3) Department of Perinatal Medicine and the University of Melbourne Department of Obstetrics & Gynaecology, Royal Women's Hospital, Carlton, Australia.

We have previously identified three putative pre-eclampsia/eclampsia (PE/E) susceptibility quantitative trait loci (QTLs) on chromosomes 2q, 5q and 13q in a cohort of Australian/New Zealand (Aus/NZ) families. Comprehensive interrogation of the chromosome 2q QTL has implicated the *ACVR2A* gene as a plausible PE/E susceptibility candidate. In this study we now report a novel integrative genomic approach to assist us further with the genetic dissection of these PE/E susceptibility QTLs that utilizes a unique dataset of whole-genome lymphocyte transcriptional profiles from 1,240 individuals in large extended Mexican American families of the San Antonio Family Heart Study (SAFHS). A linkage-based genome-wide scan of *ACVR2A* transcript levels in this dataset identified a putative *trans*-acting QTL on chromosome 5q (LOD 3.7 at 123cM). This putative *trans*-acting QTL in Mexican Americans lies in very close proximity to our PE/E susceptibility QTL on chromosome 5q at 121cM in Aus/NZ families. In an attempt to identify the putative *trans*-acting gene at the 5q QTL we examined the genetic correlations of expression levels between the *ACVR2A* transcript and all transcripts residing under our 5q 1-LOD support interval (~117cM - 133cM). There were 12 transcripts significantly genetically correlated with *ACVR2A* (FDR 0.05). Members of the SAFHS are now being genotyped using Illumina's humanhap550 beadchip for which the gene centric SNP data under the putative 5q *trans*-acting QTL will be interrogated against *ACVR2A* expression levels. This integrative genomic approach may provide a valuable means to genetically dissect complex human disorders such as PE/E.

2616/T

Relationship of apolipoprotein H (*APOH*) polymorphisms with susceptibility to systemic lupus erythematosus. *L.S. Corthell[1], F.Y. Demirci[1], A.H. Kao[2], E.Y. Rhew[3], F. Bontempo[4], C. Kammerer[1], R. Ramsey-Goldman[3], S. Manzi[2], M.I. Kamboh[1].* 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Lupus Center of Excellence, Univ. of Pittsburgh, Pittsburgh, PA; 3) Div. of Rheumatology, Northwestern Univ., Chicago, IL; 4) Dept. of Medicine, Univ. of Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is an autoimmune disease of primarily unknown etiology. SLE is 3-4 times more common in African Americans than Caucasians and predominantly affects women of child-bearing age. SLE causes a variable amount of morbidity, shortened life expectancy, and substantial total health expenditures, due to complications such as thrombosis, atherosclerosis, renal disease, and antiphospholipid syndrome (APS). Apolipoprotein H (*APOH*), also known as β2-glycoprotein I, is thought to have anti-atherogenic properties and has been shown to be a major autoantigen for antiphospholipid antibodies present in patients with APS. The purpose of this study was to investigate the role of *APOH* genetic variation in the pathogenesis of SLE. We have genotyped 12 *APOH* SNPs (9 tag SNPs that include 1 promoter SNP and 1 coding SNP and 3 additional coding SNPs) in 399 SLE women (350 Caucasians and 49 African Americans) and 496 healthy control women (454 Caucasians and 42 African Americans) using Pyrosequencing or TaqMan allelic discrimination methods. The allele and genotype association of these SNPs with race, SLE, and lupus nephritis were analyzed. Significant allelic distribution differences were observed between Caucasians and African Americans for 9 SNPs, indicating a race-dependent variation. Due to the small number of African American subjects, association studies were performed only for Caucasian subjects. Haplotype analysis of 9 tag SNPs revealed 6 haplotypes that significantly differed in frequency between SLE cases and controls ($P < 0.001$ for overall haplotype distribution). The haplotype with the most striking distribution was present in 15% of cases vs. 1% of controls, suggesting a potential risk factor for SLE. In conclusion, our data suggests that combined effects of *APOH* SNPs may be implicated in modifying the risk of SLE.

2617/T

Association between genetic variation in *Toll-like receptor 1 (TLR1)* gene and adult bronchial asthma. *T. Hirota[1], M. Harada[1], S. Doi[2], A. Miyatake[3], K. Fujita[4], Y. Nakamura[5], M. Tamari[1].* 1) Lab Genetics Allergy, RIKEN SNP Research Center, Yokohama, kanagawa, Japan; 2) Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan; 3) Miyatake Asthma Clinic, Osaka, Japan; 4) School of Human Nursing, The University of Shiga Prefecture, Shiga, Japan; 5) The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Bronchial asthma is defined as a chronic inflammatory lung disease characterized by airway hyperreactivity and mucus hypersecretion that results in intermittent airway obstruction. TLRs play an essential role in activation of the in innate immune system, which in turn activates adaptive immunity. Recent study has shown that immunization with an antigen in the context of TLR2 ligands can result in experimental asthma and genetic variation in *TLR2* is a major factor in the susceptibility to asthma in children of farmers. Because TLR2 cooperates with TLR1 in the recognition of Pathogen-associated molecular patterns (PAMPs), TLR1 is also likely to be associated with the development of asthma, but the genetic influences of *TLR1* are unclear. To investigate whether variants of *TLR1* were related to adult bronchial asthma in a Japanese population, a case control association study was conducted. We resequenced the *TLR1* gene and carried out linkage disequilibrium (LD) mapping. We identified a total of 27 variants including 5 non-synonymous substitutions (Arg31Gly, Ser44Pro, Leu144Pro, Ser248Asn, Thr685Asn). We used the Tagger (Haploview 3.32) for tagSNP selection and three SNPs, -6375 C/T (5'-flanking region), -5755 T/G (5'-flanking region), 743 G/A (exon 4, Ser248Asn), were selected for genotyping. We conducted an association study with 467 asthmatic subjects and 747 controls, and found a significant association between -6375 C/T and adult asthma susceptibility (OR=1.42, 95% CI=1.09-1.84, P=0.0086). These results suggest that the *TLR1* gene play important roles in the pathogenesis of adult asthma. Further genetic and functional studies are needed to elucidate the role of TLR1 in the molecular mechanisms underlying adult asthma.

2618/T

A new mouse model for Proliferative Diabetic Retinopathy. *J.T. Tosi, J.M. Kasanuki, K.M. Janisch, S.H. Tsang.* Bernard & Shirlee Brown Glaucoma Laboratory, Edward S. Harkness Eye Institute, and Dept. of Pathology, Columbia Univ. College of Physicians & Surgeons, New York, NY.

PURPOSE: Patients with proliferative diabetic retinopathy suffer a high incidence of ocular complications leading to diminished vision and activities of daily living. Chronic hypoxia induces vascular endothelial growth factor (VEGF) expression, causes damage to blood vessels in the eye and results in widespread leakiness and focal areas of closure of the vessels. We hypothesize that diabetic retinopathy phenotypes can be simulated in the mouse by upregulation of hypoxia inducible factor (HIF) signaling, which is known to directly regulate VEGF expression. METHODS: Using a transgenic mouse system, HIF-1a was expressed in the retina, using a bipolar cell-specific driver. Dynamic fluorescein angiographies were performed after single FA dye injections (20 mg) into tail veins of mice. A scanning laser ophthalmoscope was used for video angiography. Quantitative immunoblots were performed to assess levels of HIF-1a expression. RESULTS: We observed angiographic evidence of wide-spread microaneurysms, capillary closure, vitreous hemorrhage and neovasularization of the optic discs, due to sustained expression of HIF-1 and VEGF. Mice also developed iris neovascularization and cataracts, similar to patients with proliferative diabetic retinopathy. CONCLUSIONS: Retinopathy phenotypes result from overexpression of HIF-1a and VEGF. This is the first appropriate animal model that can be used for the development of novel therapies for proliferative diabetic retinopathy. Treatments that interrupt the HIF-1a response may prevent proliferative retinopathy. Dissection of the HIF-1a induced pathways will be relevant to the treatment of diabetic vascular diseases in which hypoxia or ischemia plays an important pathophysiologic role.

2619/F

A computational system for integrative analysis of cancer genomes and epigenomes. *W.L. Lam, B.P. Coe, W.W. Lockwood, R. Chari.* British Columbia Cancer Research Centre, Vancouver, BC, Canada.

Advances in array based technologies have enabled high throughput genome wide measurement of genetic polymorphism, gene dosage, epigenetic status, and gene expression pattern. The integration and parallel analysis of multi-dimensional datasets requires specialized bioinformatics tools to facilitate the combination of complementary data to be analyzed in a unified environment. The objective of our work is to develop a software platform to organize, visualize and analyze multi-dimensional datasets that enables the application of molecular systems approaches to analyzing clinical cancer specimens.

We have established a software package in Java which uses a MySQL database for storage of data and results, and employs the statistical package R for analysis. This new software platform is called SIGMA2 for System for Integrated Genomic Microarray Analysis Version 2. The program is developed in Java to facilitate use across all operating systems. A secure, searchable database has been established to facilitate the storage and optional sharing of genomic data. SIGMA2 is highly versatile, having the ability to view data from a variety of commercial and custom microarray platforms. For array based gene dosage analysis (comparative genomic hybridization), multiple visualizations, including signal ratio value and frequency plots, are available at different magnifications, algorithms for automated data segmentation/analysis, and linkage to other datasets. For example, we have incorporated displays for loss of heterozygosity and gene expression data, as well as analysis tools for correlation of gene dosage and gene expression data. SIGMA2 is also designed for ease of extension so that new data types can be handled effortlessly and additional algorithms are simple to incorporate. In conclusion, we have developed a system to perform integrative genomic, epigenomic and gene expression analysis. Such tools will be necessary for the analysis and interpretation of high-throughput multi-dimensional datasets.

This work is supported by funds from Genome Canada, NIDCR and Genome Canada.

2620/F

Whole-genome Resequencing with Short Reads: Accurate Mutation Discovery with Mate Pairs and Quality Values. *S.F. McLaughlin[1], H.E. Peckham[1], Z.H. Zhang[2], J.A. Malek[1], J.M. Sorenson[2].* 1) Applied Biosystems, 500 Cummings Center, Beverly, MA 01915; 2) Applied Biosystems, 850 Lincoln Centre Dr, Foster City, CA 94404.

The next generation of DNA sequencing platforms produces sequencing reads with different qualities from the familiar data characteristics of Sanger-based automated DNA sequencing. Reduced read lengths and lower per-base accuracy have been compensated by significant increases in the available depth of coverage. The use of such reads for whole-genome resequencing requires a re-examination of previously solved algorithmic issues such as optimal alignment, consensus calling and the incorporation of quality metrics into raw and finished results. The Applied Biosystems SOLiD system (a massively parallel sequencing technology based on ligation of oligonucleotides) is the only next-generation system capable of utilizing 2-base encoding to significantly reduce the raw error rate. We have developed algorithms for the SOLiD system that utilize 2-base encoding as well as quality values and systematic error to improve upon the raw resequencing ability of short unpaired (<50bp) reads. By incorporating per-base quality values into the consensus calling we are able to successfully discriminate between false positives and true polymorphisms and provide predictions for where false negatives might occur. These algorithms have been tested on several bacterial genomes using a variety of data sets from the SOLiD system. In addition, we show that the availability of highly parallel mate-paired reads allows increased mappability resulting in more accurate characterization of single-base changes as well as the detection of large-scale rearrangements and indels.

2621/F

Building Workflows for the Quality Control of Genome Wide Association Data. *R. Munro[1], E. Pugh[2], C. Zhang[1], W. Newell[1], L. Watkins, Jr[2], Y. Sun[2], B. Craig[2], D. Kalaitzopoulos[1].* 1) InforSense, London, United Kingdom; 2) Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research is exploring the addition of Affymetrix GWA arrays to an existing Illumina Infinium GWA service. CIDR has developed a series of informatics tools to monitor quality and produce a variety of reports and data files for Infinium. Faced with the desire for a new informatics pipeline at a time when programming resources were committed to other projects, CIDR collaborated with InforSense to explore if InforSense could be used to quickly develop similar tools for Affymetrix SNP chips while minimizing the need for programmer involvement. During this process, the InforSense team customized the InforSense GenSense platform for building the pipeline in response to CIDR feedback.

Nodes within GenSense calculate Mendelian inconsistencies, duplicate errors, completion rate by sample and locus, and test for Hardy Weinberg Equilibrium. CIDR used these nodes to quickly build a workflow that described a small dataset of 88 Affymetrix 5.0 arrays and build a list of problematic SNPs. Nodes within InforSense then filtered the data, and GenSense recalculated statistics on the filtered data.

This initial successful proof of concept has led to a continued collaboration as CIDR is expanding the size of the datasets attempted and the range of problems to address with InforSense workflows.

2622/F

Celsius: A Community Resource for Genomic Data. *B.D. O'Connor, A. Day, M.R.J. Carlson, J. Dong, S. Nelson.* Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA.

The Celsius project is a data warehousing effort designed to import, store, query, and export large amounts of primary Affymetrix microarray data in a format appropriate for meta-analysis along with associated annotations. Currently over 82,000 CEL files, representing 15 billion assay measurements, are available in the system and a pipeline for automated quantification using best practices has been established. Celsius is the largest public repository of CEL file level microarray data and enables sophisticated, novel questions to be asked about the transcriptome. For instance over 20,000 human hybridizations on U133A, U133B and U133_2.0 exist, and data can be retrieved within Bioconductor. Recently, Celsius has been expanded to include support for Solexa's next generation sequencing technology. This adaptation includes the representation of Solexa experimental meta data along with the encapsulation of the base calling process into the Celsius workflow and the storage and retrieval of approximately 1 billion base pairs of sequences generated per run. The capability to process both Affymetrix CEL file data as well as Solexa sequence data demonstrates the systems inherit flexibility to represent and process data from a variety of high-throughput genomic methods. As new technologies develop, Celsius will continue to provide a natural point of integration.

2623/F

Research Collaboration Database (ReCo): A Multi-User Database to Improve Genetic Data Quality and Facilitate Online Collaboration. *J.C. Papp, R. Sripracha, E.M. Sobel.* Human Genetics, University of California, Los Angeles, CA.

The Research Collaboration database (ReCo) provides an improved method for management, security, and quality control of genetic data generated in large multi-center research studies. ReCo is designed for large genetic datasets, including dense SNP data from genome-wide association studies, with features for improving quality and management of phenotype data. The major attributes of the database are 1) data cleaning and integrity tools; 2) streamlined and flexible project administration; 3) easy collaboration across sites.

Data cleaning and integrity is achieved through a variety of controls. Clinical measures and patient histories can be stored as forms that can be customized to reproduce existing forms, or created from within ReCo. The forms can be filled out on-line, or distributed in paper or electronic copies. ReCo is designed with the goal of minimizing the entry of data errors into the database. ReCo allows creation of one-time, limited guest accounts for study participants. This allows direct online data entry, facilitates phenotypic data collection in large case/control studies, and avoids error-prone transcription from paper records. Data entered by guest users must be approved before data can be processed. To achieve the lowest possible error rate in data entry from paper forms, an easy-to-use n-tuple data entry system provides multiple levels of data checking. A robust interface allows detection and resolution of conflicting entries. ReCo supports a variety of formats for importing and exporting data.

ReCo is built on an enterprise-level database in a multi-user environment, allowing scalability for large projects. Although ReCo supports complex study designs and permission sets, an innovative architecture with an intuitive interface allows users at each site to set up and administer their own studies and accounts, and selectively share forms and data within or across sites. This architecture allows administration to be distributed for more efficiency and autonomy, while maintaining high security and easy collaboration. ReCo can be viewed online at http://reco.genetics.ucla.edu.

2624/F

SOLiD™ Sequencing and 2-Base Encoding. *H.E. Peckham[1], S.F. McLaughlin[1], M.D. Rhodes[2], J.A. Malek[1], K.J. McKernan[1], A.P. Blanchard[1].* 1) Applied Biosystems, Beverly, MA; 2) Applied Biosystems, Foster City, CA.

The next generation of DNA sequencing platforms produces sequencing reads with increased depth of coverage but reduced read length and lower per-base accuracy than data from Sanger-based DNA sequencing. New approaches are needed to overcome these issues and provide accurate mutation discovery and consensus sequences. 2-Base encoding is uniquely enabled by the ligation-based sequencing protocol used in the SOLiD™ system (a massively parallel sequencing technology based on ligation of oligonucleotides). Sequencing is carried out via sequential rounds of ligation with high fidelity and high read quality. In this system there are 16 dinucleotide combinations with 4 fluorescent dyes, each dye corresponding to a probe pool of 4 dinucleotides per pool. Using this dinucleotide, 4-dye encoding scheme in conjunction with a sequencing assay that samples every base, each base is effectively probed in two different reactions. The double interrogation of each base causes a SNP to result in a two-color change while a measurement error results in a single color change. In addition, only one-third of all possible two-color combinations are considered valid and result in a base change. 2-Base encoding rules (a single mismatch is a measurement error, only one-third of adjacent mismatches are valid) significantly reduce the raw error rate (30 bp reads have a 45x reduction in raw measurement errors) and this benefit increases 3/2 as the read length is increased. The reduction in raw error rate enabled by 2-base encoding translates into more accurate alignment of short reads, polymorphism discovery and consensus calling.

2625/F

Relational Networks of Differentially Expressed Candidate Genes for Glaucoma in Human Retina and Ciliary Body. *V. Raymond[1,2], P. Belleau[1], E. Deilhes[1], N. Boivin[1], R. Arseneault[1], E. Calvo[2].* 1) Ocular Genetics & Genomics, CREMO, Laval University Hospital (CHUL) Res Ctr, Quebec City, PQ, Canada; 2) Molecular Endocrinology & Oncology (CREMO), CHUL Res Ctr.

Primary open-angle glaucoma (POAG) is characterized by an optic neuropathy and blindness. As of January 2007, 13 POAG loci, named *GLC1A* to *GLC1L*, have been mapped for the disorder. Only 3 of these POAG genes have been characterized: *myocilin* (*MYOC*), *optineurin* (*OPTN*) and *WDR36*. To prioritize the screening of candidate genes for glaucoma within *GLC1* loci, we built relational networks of differentially expressed genes using data obtained from genomic convergence, a multistep approach that combines gene expression with genetic linkage. Affymetrix microarrays (HG U133 plus 2.0) were used to probe the retina and ciliary body obtained from 2 asymptomatic individuals (a 67 year old male & a 75 year old female) and one 76 year old POAG female. Candidate genes to inherited diseases (G2D) was used to identify Gene Ontology (GO) terms associated with glaucoma. Keywords were used with Entrez Programming Utilities to search differentially expressed genes on pubmed. Relational networks, that associated differentially expressed genes with the GO, terms *GLC1* loci and keywords, were drawn using GUESS. In our microarray experiments, 530 and 265 differentially expressed transcripts were identified, respectively, in the retina and ciliary body of the patient versus the controls. These transcripts corresponded to 74 genes mapping to 1 of the *GLC1* loci for unidentified glaucoma genes. Using these data, 795 graphs were generated to overview the global implication of each group of genes. These networks were highly related to apoptosis GO terms. This classification of information on genes highlighted the best candidates for glaucoma. For instance, *BCL2L1* was found to interconnect with 6 keywords and 20 other differentially expressed genes, 4 of them mapping to 1 of the *GLC1* loci. By focusing on pathways and genes cited together, networks of genes differentially expressed in glaucoma were defined to identify the best candidate genes for POAG. These networks will be available at www.sequences.crchul.ulaval.ca.

2626/F

RECOVERING CHALLENGING ASSAYS USING NOVEL METHODS ON THE ILLUMINA GOLDENGATE GENOTYPING PLATFORM. *Y. Renaud[1], A.M.K. Brown[1], C. Taylor-Lawlew[2], C. Lin[2], R. Shen[2], C. Harris[2], M.S. Phillips[1].* 1) Pharmacogenomics, GQ MHI PGx Center, Montreal, Quebec, Canada; 2) Illumina Inc, San Diego, California 92121.

The Illumina BeadArray platforms can genotype from 384 to 1536 SNPs simultaneously using GoldenGate technology. This technology has been shown to both sensitive and reproducible. To support several clinical pharmacogenomics studies, we have developed a set of broad-based drug metabolism (ADME) genotyping panels that screen for both functional and HapMap SNPs found in ~260 ADME genes. Despite the consistent results generated for the majority of the genotyping assays contained on these panels,, analysis has identified several failure modes explaining why specific markers did not convert. The failure modes that we identified were due to: 1) limitations in the chemistry of the technology; 2) known and unknown underlying polymorphisms found under the oligo sequences; 3) areas of high SNP density; and 4) regions of homology (including CNV and low complexity DNA). Therefore, we have been working in collaboration with Illumina to successfully convert these SNPs to working assays using several novel modifications to the standard GoldenGate workflow and marker design. We have developed a novel bioinformatics cluster prediction tool which helps recognize failure modes such as underlying SNPs and regions of homology. In order to adjust our assays for underlying SNPs, we have developed strategies to incorporate degenerate bases into the oligos when underlying SNPs have been identified. In order to adjust for regions of high SNP density that generate assays that fail due to close proximity, a method to split/isolate SNPs into sub-panels to avoid interference was developed. In order to adjust for regions of homology, we have developed a method to spike in PCR products into various stages of the standard GoldenGate workflow. These novel approaches have been merged together into one OPA that can be read on a single Sentrix array. Use of these methods in future assay designs will significantly improve our ability to convert difficult assays using the GoldenGate technology.

2627/F

Mouse SNPbrowser Software: SNP Selection for Genetic Mapping and Monitoring in Laboratory Mice Strains. *S. Tang, F.C.L. Hyland, F.M. De La Vega.* Applied Biosystems, Foster City, CA.

Single-nucleotide polymorphisms are widely used in mouse genetics, including genome-wide phenotype-genotype association studies and genetic monitoring of laboratory mice strains. Genome-wide mapping of QTL in the mouse is performed via genetic crosses of phenotypically distinct or mutagenized inbred mouse strains in order to infer the phenotype associated variant in the genome. By genotyping an informative panel of SNPs in backcrosses or F2 progeny, phenotypic traits can be linked with chromosomal blocks that are represented by selected SNPs. SNPs are also useful for genetic monitoring, or strain QC, which detects genetic contamination by genotyping a specific panel of SNPs and using allelic distribution to differentiate between the diverse strains. We developed the Mouse SNPbrowser Software to aid researchers in selecting informative panels of SNPs for genetic mapping and for genetic monitoring in common mouse strains. For genetic mapping, the user specifies the strain pair, the mapping resolution, and global (genome-wide) vs. local selection. An evenly-spaced set of SNPs that specifically distinguishes between the two strains is selected. For genetic monitoring, the user specifies the list of strains to differentiate between, SNPs to exclude, and SNPs to preferentially include in the selection algorithm (to facilitate re-use of assays). A minimal set of SNPs to distinguish among the strains is selected across the genome or per chromosome. SNPs are visualized on the main chromosomal display to display distance relationships, highlight uncovered regions of the genome, and contrast SNP sets with different properties. The display also links to SNP annotations on the NCBI dbSNP database. Currently, SNPs from various published data sets are consolidated and over 10,000 SNPs genotyped on 44 strains are included. In addition, a shopping cart enables direct ordering of corresponding TaqMan® SNP Genotyping Assays from the AB website, thus expediting the set-up of mouse genetic studies with an increased probability of success. This free software application can be downloaded at www.allsnps.com/mousesnpbrowser.

2628/F

Design issues related to the generation of a 50,000 SNP array for studying heart, lung, blood and sleep candidate genes. *S. Tischfield[1,2], B. Keating[6], P.I. De Bakker[3], T.R. Bhangale[3], M. Fornage[4], G. Papanicolao[5], S. Gabriel[3], D.A. Nickerson[3], J.N. Hirschhorn[1,2].* 1) Genetics and Endocrinology, Children's Hosp Boston & Harvard Med. School., Boston, MA; 2) Broad Inst of MIT and Harvard, Cambridge, MA; 3) Genome Sciences, U of Washington Seattle; 4) Candidate-gene Association REsource SNP Comm; 5) NHLBI/DPPS,Bethesda, MD; 6) ITMAT, Univ. of Penn., Phil. PA.

Commercial whole genome products are valuable for association studies and cover most common variation in the genome, but coverage varies across genes. To complement these products, we designed an array of 50,000 SNPs to uniformly capture common variation in nearly 2,100 candidate genes related to heart, lung, blood, and sleep phenotypes, in multiple ethnicities. We used the Tagger software package to choose SNPs to capture common variation, individually or in multimarker combinations. We used a cosmopolitan tagging approach to capture common variants in each of the HapMap populations. Some genes were "tag hogs" (the top 5% required 33% of the tags), only the most compelling candidates among this set were retained. The 400 genes of greatest interest to the group were tagged more intensively. For these genes, we tagged all variants between 5 kb upstream and the 3' end of the gene that had MAF >2% in HapMap, using an r2 threshold of 0.8, requiring an average of 29 SNPS/gene. We supplemented these genes with additional SNPs identified through resequencing efforts (SeattleSNPs) when available. For the remaining genes, variants with MAF >5% were tagged at an r2 threshold of 0.5, requiring an average of 17 SNPs per gene. Several additional strategies were not adopted because of the cost of extra tags. For example, requiring a nonredundant set of variants from commercial genomewide genotyping products required an additional 9 SNPS/gene and tagging a larger flanking region (20 kb 5'/10 kb 3') required 10 additional tags per gene. To complete the array of SNPs, we added missense SNPs and SNPs in highly conserved noncoding regions, a set of ancestry informative SNPs and SNPs with prior evidence of association to a phenotype of interest.

2629/F

Integrative genomics to dissect the age-at-onset heterogeneity in type 1 diabetes. *X. Wang, S. Gao, J. Schiller, J. Basken, E. Luczkowski, M. Klinker, V. Magnuson, T. Valle, T. Wang, S. Ghosh.* Medical College Wisconsin, Milwaukee, WI.

Identifying all the genetic factors contributing to the risk of a complex disease remains a challenge. Type 1 diabetes is a complex human disease with a rapid rise in incidence in the recent decades. The exact disease etiology or the genetic mechanism is still not fully understood. Contributing to the difficulty is the extensive phenotypic and genetic heterogeneity differentiated by the age at onset. Here we describe a multilevel integrative genomics approach to this problem. It starts with identifying candidate disease pathways by integrating information from mathematical modeling of disease dynamics and pathogenesis, disease-related quantitative traits analysis, and gene expression and genomic data mining. Subsequently a comprehensive candidate gene list is compiled that includes genes within the candidate disease pathways, and genes that are related to known genes in the pathways either functionally or by being in the same genetic networks. Lastly, the network structures of all candidate genes are examined and they are prioritized according to: (1) membership of disease pathways; (2) importance to the disease pathway (such as its topological position, cluster coefficients, etc); (3) being a transcription factor with enhanced promoter binding sites of the pathway genes; (4) co-expression with key genes of the disease pathways; and (5) being positional candidates. We have recently genotyped one tag SNP each for four top candidate genes that we have identified, ATF2, STAT1, GLP1R, and MAPK8. None of these genes has been associated with T1D previously. A T1D cohort collected from Finland and Wisconsin with both young- (<15y) and adult-onset T1D (>17y) singleton families in each category, were genotyped. To date we have typed over 200 families of the young-onset cohort (Wisconsin), and over 300 families of the adult-onset cohort (Finland). Three of the 4 markers typed have yielded highly suggestive p-values of $p<0.05$. We are currently in the process of typing more families, and additional SNPs of these genes and other candidate genes.

2630/F

Detecting Loss-of-Heterogeneity and Amplification events from Illumina SNP genotyping data in the presence of stromal contamination and intra-tumor heterogeneity. *C. Yau[1], S. Colella[3], D. Peiffer[3], J. Ragoussis[2], C.C. Holmes[1,4].* 1) Department of Statistics, University of Oxford, Oxford, UK; 2) Genomics Laboratory, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 3) Illumina, Inc. San Diego, CA, USA; 4) MRC Mammalian Genetics Unit, Medical Research Council, Harwell, Oxford, UK.

Existing approaches for the detection of copy number alterations from SNP genotyping data do not take into consideration the effect of tissue heterogeneity which is common within tumor samples. Normal tissue contamination and intra-tumor heterogeneity cause severe problems for copy number calling algorithms as the data, in the form of probe intensities, is derived from a population of cells that typically have differing copy number alterations. This produces unusual artefacts in the data and leads to regions of loss-of-heterozygosity (LOH) or amplification being missed. We have developed a novel Hidden Markov model-based approach for analyzing Illumina SNP genotyping data that allows for the identification of regions of LOH and amplification in tumors - even in the presence of tissue heterogeneity. Central to our approach is a generative probability model of genotyping data under copy number alterations and tissue heterogeneity. The model incorporates special "mixture" states (such as mixtures of LOH or amplifications with normal copies) and, using Bayesian inference, we show we are able to detect heterogeneous chromosomal regions and de-convolve these to identify the alterations in the constituent sub-populations. We demonstrate our method using data from samples containing known mixtures of normal and tumor cell line DNA and paired tumor samples from the Illumina Hap300 and Hap550 Genotyping BeadChips. We show that our method can detect LOH and duplication events under normal tissue contamination and heterogeneous conditions. For example, 32/34 LOH events in a pure tumor sample were detectable in 50:50 mixtures of normal and tumor DNA that are all missed using a conventional approach. Our method leads to the improved genomic profiling of tumor samples using Illumina SNP genotyping BeadArray data.

2631/F

Quantitative chimerism detection technology. *D. Merrill.* Applied Biosystems, Foster City, CA.

Here we present a highly sensitive assay technology to detect and quantitate the presence of two different genomes in a chimeric or mixture sample derived from varied source. For this, quantitative allele specific real time PCR assays were developed and validated for a fixed set of markers; each assay can confidently identify the presence of 0.1% of a minor allele or genome in a potentially chimeric DNA sample. Highlighting the utility of the technology is the versatility to use the same panel of markers and assays for a wide array of applications, including but not limited to research in transplants of bone marrow and stem cells, stem-cell line quality control, forensic identification and rare variant discovery and validation. The concept involves an initial screening of pre-selected genomic insertion deletion markers to provide a genotypic profile for one or both genomes to be detected using a panel of validated genotyping assays. The markers were selected to be able to distinguish any two given genomes with a statistical probability of 99.9% or greater. Using the resulting genotypic profiles, an informative marker is chosen based on criteria specified for the given application. An allele specific PCR assay for the chosen marker is run on the unknown or chimeric sample, quantitatively detecting the presence of both the minor component genome as well as the majority component genome. Results from the initial test site presented here, regarding post bone marrow transplant monitoring has demonstrated the utility, sensitivity and ease of workflow that will enable researchers to quickly and accurately go from sample to result. For this test site, the application involved assaying post bone marrow transplant samples to detect the possible regeneration of the patients' original genome. Key to this application is the detection of this regeneration early on, requiring the identification of as little as 1 copy of the original recipient genome in a majority of 1000 copies of transplanted donor genome. The quantitative chimerism detection technology described within demonstrated with high confidence the ability to detect at this level of sensitivity, beyond the capabilities of current technologies such as FISH and STR assays.

2632/F

A replication-based mechanism may mediate complex genomic rearrangements causing Pelizaeus-Merzbacher disease. *J.A. Lee[1], C.M.B. Carvalho[1], J.R. Lupski[1,2,3].* 1) Dept Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

The prevailing mechanism for rearrangements causing genomic disorders is non-allelic homologous recombination (NAHR) between region-specific low-copy repeats (LCRs) for recurrent events that have breakpoints which cluster and for some non-recurrent alterations. For rearrangements that are non-recurrent, with junctions scattered instead of clustered, non-homologous end joining (NHEJ) has been implicated as the recombinational repair mechanism. Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive dysmyelinating disorder caused most frequently by non-recurrent duplication including the dosage-sensitive proteolipid protein 1 (*PLP1*) gene, but also by non-recurrent deletion and point mutations. Whereas the DNA sequence analysis of breakpoint junctions for deletions and duplications of *PLP1* have thus far been reported to be consistent with NHEJ repair, the majority of *PLP1* duplication junctions are apparently refractory to breakpoint sequence analysis. Upon analysis of junction sequences in PMD patients with different-sized (~200 kb to ~7 Mb) genomic duplications and deletions, we have both confirmed the occurrence of simple *PLP1* tandem duplications and also have found evidence for sequence complexity at some recombinant junctions representing a composite from more than two discreet genomic locations. Our data are suggestive of "complex" *PLP1* duplications and deletions occurring via a replication-based mechanism, which we term FoSTeS for replication Fork Stalling and Template Switching. We propose that some of the more "complex" duplication and deletion rearrangements associated with PMD that have been reported in the literature, and potentially other non-recurrent complex genomic rearrangements, may be explained by this replication-based mechanism.

2633/F

Towards a pathway definition of Parkinson's disease. *L.B. Moran, M.B. Graeber.* University Department of Neuropathology, Imperial College London and Hammersmith Hospitals Trust, London, UK.

We have used brain tissue from well characterised cases of sporadic Parkinson's disease (PD) and established a first whole genome transcriptomic profile of the medial and lateral substantia nigra as well as frontal cortex (1). 570 highly significantly ($p<0.001$) deregulated sequences were initially identified. By focusing on the most comparable PD cases and controls in our cohort we have refined our analysis and established an extended list of 892 deregulated genes which we expect to form the core of the 'disease pathway'; underlying PD. In addition, our dataset was reanalysed using a new software package and a database of eukaryotic molecular interactions, Resnet 5.0 (PathwayStudio, Ariadne). Back-mapping to brain tissue of mRNAs of interest has already resulted in two new Lewy body markers (2). The validated dataset (1) will be deposited in the GEO database. The complete gene regulatory network now under scrutiny contains more than 100 genes whose association with PD is known from the literature. Of those more than 40 genes belong to the highly significantly deregulated group. Further tissue back-mapping of all deregulated gene products by means of antibodies and in situ hybridisation is necessary to 'stratify'; the current tentative pathway signature for the different cell types present in the human substantia nigra, i.e. neuronal subtypes, astrocytes, microglia, oligodendrocytes and vascular and perivascular cellular elements. SNCA appears to have a central role in PD pathogenesis as it forms part of several of the deregulated regulatory networks so far investigated (3). However, a number of heat shock proteins including HSPA1A, metallothioneins and various synaptic proteins also figure prominently. A list of all components of the current PD pathway definition will be presented at the meeting. New genes not previously associated with PD include FGF13, NRXN1, RELN, SDC1 and SYT1. 1. Moran et al. Neurogenetics 2006;7:1-11; 2. Moran et al., ABN meeting abstract, Cambridge, UK, 12 Apr 2007, J Neurol Neurosurg Psychiatr, in press; 3. Moran et al. Acta Neuropathol 2007;113:253-63. Funding from the UK Parkinson's Disease Society is gratefully acknowledged.

2634/F

Development of a diagnostic gene chip for detection of Mycobacterium tuberculosis. *C.S. Sun[1], S.R. Lin[2], C.C. Chen[1], I.W. Chong[1,3].* 1) Graduate Institute of Medicine; 2) Graduate Institute of Medical Genetics; 3) Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background and purpose: Tuberculosis (TB) is an old-age, widespread disease which is one of the major causes of death throughout the world. Therefore, it is a critical issue to alleviate the growing worldwide TB epidemic. Recent biotechnological advances have fuelled a revolution in the diagnosis of infectious disease but is still limited that each genetic marker must be detected separately. Thus, our goal is to develop a sensitive, time-saving, and high-throughput active TB chip for detection of Mycobacterium tuberculosis. Materials and Methods: Total of 48 sputum samples were collected from Kaohsiung Medical University Hospital. We have applied L-J medium culturing and auramine-rhodamine staining in primary sputum smears. In order to characterize each of the isolates, we applied PCR and RFLP to identify each of its genotypes. Furthermore, the PCR amplicons were sequenced and confirmed by the BLAST analysis that the amplified products represent the specific region of interest. The diagnostic value of the candidate genes of active TB chip was evaluated with 30 M. tuberculosis DNA samples by membrane array method. Results: All of the 48 isolates were L-J medium culture-positive and acid-fast stain positive. We further analyzed the distribution of each genotype of the isolates in Taiwan by PCR-RFLP. As a result, 52.1%(n=25)have been identified as M. tuberculsosis, 22.91%(n=11)have been identified as NTM and 22%(n=12)did not belongs to Mycobacterium species. In this study, we selected 11 candidate genes for active TB chip and constructed the prototype of the chip. Overall, the sensitivity of the active TB chip was 80% and the specificity was 85%. Conclusion: With the establishment of the active TB chip, it can speed up the whole process of diagnosing tuberculosis patients and differentiate the various species of Mycobacterium tuberculosis complex. Hence, we suppose the active TB chip may have a great potential for clinical applications.

2635/F

Molecular Characterization of Deletion Breakpoints in Xp22-p21 Chromosomal Rearrangements. *Y.H. Zhang[1], B.L. Huang[1], L.L. McCabe[1], E.R.B. McCabe[1,2].* 1) Pediatrics, UCLA, Los Angeles, CA, USA; 2) Human Genetics, UCLA, Los Angeles, CA, USA.

Chromosomal rearrangements in Xp22-p21 typically involve the adrenal hypoplasia congenital (AHC), glycerol kinase (GK) and/or Duchenne muscular dystrophy (DMD) loci. The deletion sizes and breakpoints are unique within each family, and the genomic mechanism(s) for the submicroscopic chromosomal rearrangements remains unknown. The purpose of our investigation was to find patients' deletion breakpoints and identify potential mechanism(s) responsible for the recombination events. To define precisely each breakpoint, primers were designed across the expected telemetric and centomeric breakpoint. PCR products were purified by the Qiagen Gel Extraction kit and were directly sequenced with an ABI 3700 automated sequencer and the BigDyeTM terminator cycle sequencing kit (Perkin-Elmer). After identifying the exact breakpoints, we used junction-specific PCR and STS-content PCR to confirm the breakpoints in carriers and normal samples, respectively. The sequence analysis of the breakpoints in the affected four males (A-D) have shown: patient A-4486 kb interstitial deletion involving IL1RAPL1, DAX1, GK and DMD; patient B-401 kb interstitial deletion involving IL1RAPL1 and DAX1; patient D-126 kb interstitial deletion also involving the DAX1 gene only; and patient D-7 kb interstitial deletion involving the DAX1 gene only. The patients' phenotypes were consistent with the observed deletions in all the cases. Of the four patients, three of them inherited their mutations from carrier mothers and the fourth remains unknown. Patient A appeared to have the exact same breakpoints as another patient (AW) defined previously; however, mitochondria D-loop analysis suggested that the two were unrelated. The recombination breakpoints in these patients suggest the same mechanisim: non-homologous end joining (NHEJ) events with 2-3bp micro-homologies. Although NHEJ appears to be less precise, it is believed that NHEJ is the major pathway for double strand break (DSB) repair. Our data are consistent with the previous observations involving rearrangements in other chromosomes in the human genome.

2636/F

Characterizing satellite I and satellite III sequences on human chromosome 21. *R. Patel[1], K. Gregori[1], P. Chennamaneni[1], S. Xavier[1], R. Hettinger[1], J. Bavarian[1], R. Ennesser[1], M. Cummings[2], J. Doering[1].* 1) Loyola Univ Chicago; 2) Univ. of Illinois, Chicago.

The human genome sequence does not include the heterochromatic regions, although these sequences comprise 10-15% of the genome. We are constructing a detailed physical map of the HC21 centromere and short arm as a model for the organization of these regions. Our previous work identified two subfamilies of satellite I (sat I) on HC21 which share 80% sequence identity. The N6 subfamily is located solely on the p arm distal to the rDNA cluster, while pTRI-6 sequences are present both in the p arm proximal to the rDNA and in the centromere. The HC21 centromeric sat I cluster has been physically mapped, and portions of it have been now been sequenced. It is 0.3 Mb long and flanked on its p arm end by 760bp of a "Y chromosome-specific" sat I subfamily and 1kb of a TA simple repeat. Its q arm end is located within 76kb of the major alphoid cluster, D21Z1. Internally, the sat I cluster consists of tandemly repeated pTRI-6 sequences frequently interrupted by other sat I sequences that have only 80% identity to pTRI-6. There is a high degree of sequence heterogeneity between monomers in the cluster and no obvious higher order repeat. These newly-identified sat I sequences are quite distinct from the N6 subfamily or any other sat I sequences in the database, indicating they represent a new subfamily. This centromeric sat I cluster is not found on other chromosomes, and is thus a strong candidate for an HC21-specific centromeric marker. Satellite III (sat III) sequences are found on the p arm of HC21 both proximal and distal to the rDNA. We have identified at least five distinct sat III clusters on HC21p, three proximal to the rDNA and two distal. They are all less than 90kb in length. Sequencing reveals that all these clusters are members of sat III subfamily I and have highly heterogeneous organizations with no obvious higher order repeats. BLAST comparisons showed that these sat III sequences have no more than 80% identity to each other or with any other currently known sat III sequence in the human genome. Thus, they are candidates for HC21p-specific probes.

2637/F

Comparison of high density genotyping results from saliva and blood samples on Affymetrix GeneChip® GenomeWide 6.0 arrays. *J.D. Reynolds, I.K. Kuramoto, W.H. Biggs III, C.K. French.* Affymetrix Clinical Services Laboratory, Affymetrix, Inc., West Sacramento, CA.

INTRODUCTION: Currently, EDTA-stabilized whole blood is the most common sample type used for high density genotyping in a clinical environment. This experiment involves extracting DNA from paired blood and saliva samples, comparing not only the DNA quality and quantity, but also the microarray call rates (CR) in order to demonstrate saliva's suitability for genetic association studies. Initial feasibility tests on saliva and blood samples have shown comparable results. Saliva samples, as an alternative DNA source for high density genotyping, are easier to collect in remote sites, less invasive, and have more robust storage conditions. METHODS: Collection of paired EDTA anti-coagulated whole blood and DNAGenotek Oragene saliva samples from 90 IRB-approved volunteer donors will be compared using the Affymetrix GeneChip® GenomeWide 6.0 arrays. DNA is extracted from both of the sample sets using Agencourt chemistry on a Beckman-Coulter NX^P platform- an automated extraction system using magnetic bead particles for isolation of Nucleic Acids. All samples are analyzed for purity and yield on a NanoDrop ND-1000 spectrophotometer as well as for integrity on a 1% agarose gel. GW6.0 testing will be performed according to the manufacturer's instructions. RESULTS: The Beckman-Coulter NX^P can purify DNA from 96 samples in approximately 3 hours. The blood samples, on average, have an A260/A280 ratio of 1.78 ±0.05 and a concentration of 90 ±50.0 ng/µl. These averages fall within the tolerance limits defined by Affymetrix for its high density genotyping arrays. Initial feasibility testing on saliva samples demonstrated an average A260/A280 ratio of 1.94 ± 0.05 and a concentration of 90.2 ± 8.0 ng/µl. GW6.0 testing on these initial saliva samples indicated that DNA extracted from blood and saliva have comparable results. All samples successfully hybridized to the arrays and produced call rates sufficient for genetic association studies (CR>96%). GW6.0 testing of both blood and saliva samples will proceed after the completion of the DNA extraction from the 90 paired donor samples.

2638/F

High-throughput parallel re-sequencing of conserved genomic elements on chromosome 9 in Alzheimer disease. *P. Whitehead[1], J. Gilbert[1], E. Martin[1], J. Haines[2], M. Pericak-Vance[1], S. Züchner[1].* 1) Miami Inst. for Human Genomics, University of Miami, Miami, FL; 2) CHR, Vanderbilt University, Nashville, TN.

Alzheimer's disease (AD) is the most common form of dementia in the elderly and is characterized by an irreversible loss of neurons. AD is a complex disorder with genetic and environmental risk factors contributing to the onset of disease. We have previously identified a 19Mb (18.6-37.8 Mb) region on chromosome 9p21.3 with a peak heterogeneity LOD of 4.95 at D9S741 (24.5 Mb) in a dominant model. Analyses of a dense array of SNPs across the region showed strong evidence for linkage, but only limited support for association in a subset of 199 families with at least one autopsy-confirmed AD case. We hypothesized that the LOD score was driven by a number of independent and possibly rare sequence variations in a subset of our families in the vicinity of a transcriptional unit or in a conserved genomic element at 9p21.3. We thus set out to explore whether massive parallel re-sequencing is feasable and could yield further insight into the molecular genetic basis of this AD locus. We selected 12 individuals from the families with the highest LOD scores for re-sequencing. By in-silico analyses we ranked genomic elements under the linkage peak by their conservation across 17 different species. The 92 most conserved regions were subjected to a novel high-throughput sequencing platform, 454 Life Sciences. We sequenced a total of 391,284 fragments with an average size of 252 bp amounting to 98.7 Mb with an average coverage of the targeted conserved sequences of 82%. We identified a total of 1420 sequence variations including substitutions, insertions, and deletions. 251 of those variations fulfilled our quality requirements with 112 being novel. Some of those variations clustered around conserved sequence elements; however, further analysis is currently under way to clarify their potential functional role in the context of this AD study. We conclude that massive high-throughput parallel re-sequencing presents a powerful and potentially effective tool, but the sheer amount of data challenges traditional methods of genetic data-mining.

2639/F

Galaxy: bridging the gap between experimental and computational biology. *J. Taylor[1], D. Blankenberg[2], I. Schenck[2], N. Coraor[2], G. Von Kuster[2], R. Lazarus[3], A. Nekrutenko[2].* 1) New York University, New York, NY; 2) Penn State University, University Park, PA; 3) Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

High-throughput data production technologies are revolutionizing modern biology. Translating this experimental data into discoveries of relevance to human health increasingly relies on sophisticated computational tools that can handle large-scale data. Many such tools exist or are currently being developed; however, making computational tools easy-to-use requires significant effort, which tool developers frequently cannot afford. Thus, for an average experimental biologists with limited computer expertise, there is a substantial barrier to taking advantage of these tools. Galaxy (http://g2.bx.psu.edu) removes this barrier by providing easy-to-use interfaces to existing computational tools. Galaxy is unique in two ways. First, in the ease with which it allows complex large-scale analyses to be performed with nothing more than a web browser. Second, in how simple it is for existing computational tools to be integrated into Galaxy, gaining a modern user interface, and substantial added value through connections with other tools and databases. Here we will show how Galaxy bridges the gap between experimental and computational biology, and highlight the exciting new developments in Galaxy including:

1) A new and unique suite of tools for working with large scale multiple genomic alignments in Galaxy, along with powerful phylogenetic tools, together a substantial advance in the accessibility of high-end comparative genomic analyses.

2) The availability of a set of statistical genetics tools, leveraging the Rgenetics (http://rgenetics.org) project, that bring the power, efficiency, and ease-of-use Galaxy has achieved in genomics, to bear challenges such as the analysis of whole-genome SNP chip association data.

3) Advances in the collaborative and workflow features that make Galaxy an ideal platform for sharing reproducible analyses.

2640/F

Development of strategies for efficient use of revolutionary sequencing technology for detecting human sequence variation. *W. McCombie, G. Hannon, R. Lucito, E. Hodges, M. Kramer, V. Balija.* Genome Research Ctr, Cold Spring Harbor Lab, Cold Spring Harbor, NY.

The combination of a human reference sequence and the availability of a new generation of DNA sequencers are revolutionizing our ability to detect the sequence variation that causes a wide range of human disorders. We are using the Illumina sequencing platform as a base to study human sequencing variation associated with several disorders. As part of this effort we are exploring ways to target selected large sub-regions of the genome for resequencing. We are also developing methods such as the use of molecular barcodes to pool multiple samples from different individuals. These tools are enabling strategies to be developed to sequence crucial genome regions from hundreds or even thousand of samples. The resulting information from studies such as these will radically change our knowledge of the association of sequence variation and sickness or health.

2641/F

A population-based WGAS approach to identify genes associated with plasma levels of GGT levels, as a marker for liver disease in Metabolic Syndrome. *H.A. Stirnadel[1], X. Yuan[2], P. Vollenweider[3], D. Waterworth[2], K.S. Song[2], B. Koshy[2], G. Waeber[3], V. Mooser[2].* 1) GlaxoSmithKline, R&D, London UK; 2) GlaxoSmithKline, R&D, King of Prussia PA, RTP NC; 3) CHUV University Hospital Lausanne, Switzerland.

BACKGROUND : Non-alcoholic fatty liver disease, a condition associated with metabolic syndrome (MS), is usually accompanied by an elevation in plasma levels of Gamma Glutamyl Transferase (GGT). Susceptibility to this condition may have an underlying genetic component. We hypothesized that genes determining GGT levels have a more profound effect on this trait in the presence of stressors like MS, alcohol and certain drugs. The primary goal of the present study was to identify such susceptibility genes for liver diseases, using plasma GGT levels as proxy. METHOD : We performed a four-step WGAS on the Lausanne CoLaus population-based study with 5641 participants, 35-75 years of age, genotyped with the Affymetrix 500K SNP chip. Linear regression analysis was performed on GGT levels as quantitative trait. RESULTS : In the overall study population 797 subjects were classified with having MS according to the ATP-III criteria, and 1764 subjects reported to drink at least 10 alcoholic beverages per week (alcohol group). The serum GGT levels (Mean±SD) were significantly higher in the MS (52 ± 66 IU/L; p<0.0001) and alcohol group (42 ± 53 IU/L; p<0.0001) compared to the general population (32±40 IU/L). Out of 21,522 SNPs associated with GGT levels in the general population, 2900 were specific to MS patients. From this pool, 495 SNPs (130 genes), were also associated with GGT levels in alcohol drinkers. Eighty-eight of these genes are expressed in human liver. CONCLUSION : Our preliminary results indicate that 88 liver-related genes are associated with plasma GGT levels. Replication of these genetic associations may help identify genes that predispose susceptibility to metabolic syndrome-associated and/or drug-induced liver disease.

2642/F

Quantitative Microsphere Hybridization (QMH): A High-throughput Assay for Multiplexed Detection of Genomic Disorders. *H.L. Newkirk[1], L.D. Cooley[2], D.C. Bittel[3], M.G. Butler[3].* 1) Genomics, Children's Mercy Hospital and Clinics, Kansas City, MO; 2) Cytogenetics Laboratory; 3) Section of Medical Genetics and Molecular Medicine.

We developed a novel multiplexed quantitative microsphere suspension hybridization (QMH) assay for determination of genomic rearrangements involving copy number variation as well as balanced rearrangements. Unique sequence genomic fragments are conjugated to spectrally-distinct microspheres and used in multiplex hybridization to detect homologous sequences in biotin-labeled genomic DNA. Hybridization is detected with phycoerythrin-labeled streptavidin and analyzed by flow cytometry. Copy number differences are made by comparing mean fluorescence intensities (MFI) of test probes with a disomic reference probe (eg, ACTB). QMH is an attractive option for clinical diagnostic tests with its high-throughput platform using a flow cytometer and minimal amounts of DNA are required. The resolution of QMH is 3 bp, which is significantly greater than conventional clinical tests. We developed a multiplexed QMH assay for 8 common genomic disorders including Down, Klinefelter, Turner, Prader-Willi syndromes, trisomy 13 and trisomy 18, cystic fibrosis and Duchene muscular dystrophy. Test probes specific for all disorders were hybridized with the reference probe to patient's DNA. The relative average MFI ratios were 0.54±0.07 in subjects with cytogenetic deletions, 1.45 ± 0.03 in subjects with duplications or aneuploidy and 0.99±0.08 for normal loci. To detect balanced reciprocal translocations, we used a modified QMH method involving a two-step hybridization procedure. For example, we analyzed a subject with PWS and a balanced reciprocal translocation, t(15:19)(q12;q13.41), and narrowed the breakpoint to a 207bp region. These studies illustrate the utility of QMH for high-throughput multiplexed detection of genomic disorders and should be a valuable tool for a precise recognition of subtle chromosome rearrangements and breakpoint assignments.

2643/F

DNA resequencing microarrays identify mutations causing severe combined immunodeficiency (SCID). *E.S. Mansfield[1], T. Lebel[2], S. Venkatapathy[1], R. Chiles[1], J.A. Warrington[1], J.M. Puck[2].* 1) Advanced Applications and Standards R&D, Affymetrix, Santa Clara, CA; 2) Pediatrics Dept., UCSF, San Francisco, CA.

Early onset of recurrent infections is the hallmark of severe combined immunodeficiency (SCID), which is generally fatal in the first year of life unless treated by bone marrow transplantation, enzyme replacement or gene therapy. Recognizing SCID early is essential successful treatment, but immunologic confirmation and DNA sequencing are expensive, laborious and available only in specialized laboratories. There are 12 presently known disease genes for SCID, with many possible mutations in each gene. To test whether microarray technology can identify mutations efficiently from genomic DNA samples, we produced a custom GeneChip microarray representing the full coding and splice regions of 20 genes underlying SCID and other primary immunodeficiencies. Long range PCR was used to make enriched genomic DNA templates, which were fragmented, labeled and hybridized to arrays. After washing and scanning, data was analyzed by GCOS software. In a pilot with IL2RG, JAK3 and IL7R genes, we tested DNAs from 61 SCID previously genotyped patients and 55 obligate carriers. Overall nucleotide correct call rates were 99.5% for X-linked haploid (XSCID) and 98% for diploid sequence. Of 35 known XSCID samples, mutations were recognized in 34 (97%) with the precise mutation defined in 33 (94%). In heterozygous maternal carrier samples, 21 of 22 mutations were flagged (95%) with the exact mutation defined in 19 (86%). Known autosomal mutations were also found. Importantly, 7 of 7 DNAs from SCID patients not previously genotyped had mutations identified by the array and later confirmed by conventional sequencing, including 3 with previously unreported XSCID defects and a compound heterozygous JAK3 case. Certain nucleotides in regions of high GC content could not be called automatically by the software. However, with further development and experience several primary immunodeficiency diseases might ultimately be diagnosed using microarray-based methodology.

2644/F

Human Genotyping Using Next Generation Sequencing Technology. *N. Xiao[1,2], B. Desany[4], P. Bouffard[4], L.A. Burdett[1,2], R. Welch[1,2], M. Yeager[1,2], T.P. Jarvie[4], T.T. Harkins[5], L. Qi[1,2], J. Lu[1,2], S.J. Chanock[2,3].* 1) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services; 3) Pediatric Oncology Branch, Center for Cancer Research, NCI, NIH, DHHS; 4) 454 Life Sciences, Branford, CT; 5) Roche Applied Science, Indianapolis, IN.

A next generation sequencer was used to sequence a 136 kb region of human chromosome 8 that has been implicated in prostate cancer by whole genome association studies. Using the GS Reference Mapper, included with the GS-FLX, we were able to accurately genotype this region in 4 samples from the HapMap - CEPH panel and demonstrate concordance. For four HapMap samples, 32 PCR products spanning the 136 kb region were generated, nebulized, pooled and sequenced. Here, we used the software to map the reads to the reference sequence in flow space and detect HapMap variations in both homozygous and heterozygous states. For the four HapMap DNA samples that have been sequenced, average read length was 250 bp, and average coverage depth was 160 reads (including forward and reverse directions). We examined the distribution of variant proportion in multiple sequence reads, and developed heuristics to refer heterozygous and homozygous genotypes. In conjunction with additional examination of alignment between sequence reads and reference sequence, we were able to make accurate genotype calls. Comparison between the resulted genotypes with HapMap data of the corresponding individuals for 181 SNPs within the 136 kb region reveals almost perfect concordance. In the rare cases where the genotype calls are discordant with the HapMap data, violations of Mendelian inheritance in the HapMap data among the trio have been observed. Taken together, our result demonstrates the utility of this approach for targeted genotyping applications. Funded by NCI-Contract N01-CO-12400.

2645/F

Finding indel using short sequencing reads. *Z. Zhang, J. Sorenson, J. Malek.* Applied Biosystems, Foster City, CA.

Introduction: In next-generation shotgun resequencing, many short reads (~30bps long) are produced. One challenge is to find indels in the reference genome. Mapping reads to a large genome with indels will result in many false alignments. Some new sequencing technologies include the ability to generate paired reads whose approximate distance can be obtained. For large size indels, the deviation from the average distance can be used to derive the existence and size of an indel. In this work, we address the problem of finding small to medium indels using paired reads. Algorithm: The approach is a two-step algorithm: (1) Map all reads requiring very high similarity (allow at most 1 mismatch); (2) For each pair of reads in which only one tag maps uniquely, we align the other tag allowing 1 indel and/or more mismatches in the small region that is the right distance away from the mapped tag. This distance is determined by the library insert size and its variation. Analysis: For the second step of the algorithm we analyzed the probability that a random sequence can achieve the same alignment. With an independent random sequence model, we find FP is very low so that we can find one deletion of up to 100 bps and insertion of up to 10 bps. For an alignment with an indel, there can be two possible hypotheses: (1) the deletion is real; (2) the read is the result of sequencing errors. We initiated another analysis to test the two hypotheses. Briefly, using a Bayesian approach we estimate the probabilities the read supports either hypotheses, and decide when to accept an indel as real. The analysis tells us that the indel must be relatively in the middle of the read for us to be confident that it is real: e.g., for a deletion, the read must have at least 6 alignable bps at both ends of the deletion. Combining these analysis with other well known results, for a given genome size, coverage rate, read length, and sequencing error rate, we can estimate the chance we find any real indel. Simulation: We did simulation to estimate FP and FN, and the result is similar to the statistical analysis. And in conclusion, under some reasonable assumption, we can find deletion of up to 50 bps long, and deletion up to 8 bps long using only short reads.

2646/F

Genome-wide association scans and replication studies identify multiple loci that influence height. *G. Lettre[1,2], MN. Weedon[3], RM. Freathy[3], CM. Lindgren[3], B. Voight[1], C. Gieger[4], I. Heid[4], T. Tuomi[5], U. Lindblad[5], L. Peltonen[1,6], V. Salomaa[6], G. Davey-Smith[3], AT. Hatterskey[3], MI. McCarthy[3], HE. Wichmann[4], L. Groop[5], TM. Frayling[3], JN. Hirschhorn[1,2], Diabetes Genetics Initiative.* 1) The Broad Institute; 2) Children's Hospital Boston, Harvard Medical School; 3) WTCCC Height Team UK; 4) KORA Germany; 5) Botnia/Skara Studies; 6) KTL Finland.

Adult height is a classic complex polygenic trait, as initially proposed by Fisher in 1918. Despite high heritability (70-90%), the genetic factors influencing stature in the general population remain unidentified.

The Diabetes Genetics Initiative (DGI) recently completed a genome-wide association study (GWAS) of type 2 diabetes (T2D) in 3025 cases and controls from Scandinavia. We also analyzed adult height as a quantitative trait in this sample. There was a slight enrichment of SNPs above the null expectations at the tail of the distribution, but no SNPs reached unequivocal levels of significance in the DGI dataset alone. To increase our power, we followed up our top results from the DGI data in additional samples and combined the DGI and WTCCC T2D GWAS height results (N=4921). A SNP in the 3'UTR of the *HMGA2* gene achieved a P=8X10^{-8} in the combined data and was strongly replicated in adults (N=20876; P=2X10^{-13}) and children (N=6067; P=1X10^{-6}). A SNP near the *SH3GL3*/*ADAMTSL3* genes also showed association in both DGI (P=0.0001) and FINRISK97 (N=6498, P=0.0004). Finally, we combined the DGI height dataset with GWAS data from the KORA study (N=4669), and found 8 loci with P<1X10^{-5} (vs. 3 expected by chance). Replication results for these findings and additional meta-analyses will be presented.

We identified the first robust association to height variation: a common SNP in the 3'UTR of the *HMGA2* gene. Meta-analyses and replication of GWAS studies also identified other promising loci, demonstrating the power of large cohorts to identify loci for complex traits. We expect that the identification of novel height genes using GWAS will shed light on the biology of growth and on the architecture of complex traits in humans.

2647/F

The GLUT9 gene is associated with serum uric acid levels in Sardinia and Chianti cohorts. *S. Li[1], S. Sanna[2, 3], M. Dei[2], S. Lai[2], G. Usala[2], A. Maschio[2], F. Busonero[2], A. Mulas[2], M. Orrù[2], G. Albai[2], S. Bandinelli[4], D. Schlessinger[1], A. Scuteri[1,5], S. Najjar[1], A. Cao[2], G. Abecasis[3], L. Ferrucci[1], M. Uda[2], WM. Chen[3], R. Nagaraja[1].* 1) Gerontology Research Center, National Institute on Aging, Baltimore, MD; 2) Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cagliari, Italy; 3) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, United States of America; 4) Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy; 5) Unita Operativa Geriatria, Istituto Nazionale Ricovero e Cura Anziani, Rome, Italy.

High serum uric acid levels are associated with higher risk of cardiovascular events and metabolic syndrome; they are also likely accompanied by activation of inflammation cascade. We executed a genome-wide association scan in the genetically isolated population of Sardinia to identify genetic variants associated with levels of uric acid as a quantitative trait. Specifically, 3329 individuals were genotyped with the Affymetrix 10K SNP Mapping Array and 1,412 individuals with the Affymetrix 500K Mapping Array set. With the latter data, and using modified Lander-Green algorithm, full genotype on the 2,893 individuals typed with 10K panel was derived. Using the 362,129 SNPs that passed quality control checks, we found associated SNPs on chromosome 4 in the GLUT9 gene, a class II glucose transporter predominantly expressed in liver and kidney. Within the gene, rs6855911 showed the strongest association with uric acid levels (p = 1.84*10-16) along with 8 other SNPs (p-values 7.75*10-16 to 6.05*10-11), all in the 5' portion of the gene. In Sardinia, homozygotes for the rare allele of this SNP (minor allele frequency = 0.26) had 0.9 mg/dl less uric acid than homozygotes for the common allele; the results were replicated in an unrelated cohort from the Tuscany region (InCHIANTI study). GLUT9 polymorphisms could lead to differential glucose assimilation and indirectly, affect uric acid levels.

2648/F

Kidney Transplantation Genomics: Whole Genome Association to Rejection. *S.L. Musone[1], J. Chen[1], C. Ha[1], S. Horvath[2], D. Salomon[3], P.Y. Kwok[1].* 1) CVRI, University of California, San Francisco, San Francisco, CA; 2) Dept. of Biostatistics, University of California, Los Angeles Los Angeles, CA; 3) The Scripps Research Institute La Jolla, CA.

Approximately 17,000 kidney transplants are performed in the United States each year due to various disease states that include type II diabetes and hypertension. Despite donor-recipient matching techniques, acute rejection and chronic allograft nephropathy remain obstacles to post transplant health. We hypothesize that these two forms of organ rejection are complex traits with genetic signatures that can be identified through a whole genome association approach using single nucleotide polymorphisms (SNPs). We are scanning 2400 matched donor-recipient pairs on Affymetrix Gene Chips for SNP genotyping and expression analysis, in addition to running high throughput LC-MS/MS proteomics. Analysis and patient enrollment are ongoing.

2649/F

A Robust, Scaleable Solution for High Throughput Data Generation Using Affymetrix Genome-wide 5.0 and 6.0 SNP Arrays. *M. Parkin, C. Gates, B. Blumenstiel, M. DeFelice, D. Gage, W. Winslow, P. Lin, F. Kuruvilla, J. Korn, M. Nizzari, M. Daly, D. Altshuler, S. Gabriel.* Broad Institute of MIT and Harvard, Cambridge, MA.

Resources and technologies required to systematically and efficiently scan the human genome for association between common genetic variations and disease have become available over the past year. To conduct Genome-Wide Association Scans (GWAS), it is necessary to create laboratory capabilities with appropriate scale, quality control and integration to advanced bioinformatics capability. To meet this need, we have developed an automated lab process for target prep, data management, and custom tracking systems to support GWAS using Affymetrix SNP arrays. The process has been used for early versions of the SNP arrays (the 100K and 500K), with which we generated genome scan data for over 16,000 samples. A collaborative effort between our group and Affymetrix has resulted in the development of new single-chip products, the SNP 5.0 (470,000 SNPs) and more recently the SNP 6.0 array which interrogates 906,600 SNPs and 920,000 copy number sites. Initially at a scale of 384 samples per week in January 2007, the pipeline scaled to 1152 samples within 4 weeks and reached full scale in June 2007 generating 1536 samples per week. In total over 29,904 arrays have been scanned, with quality and accuracy of data maintained throughout the scale up. Overall call rates using the Birdseed algorithm across five different datasets average 99.6% and accuracy as assessed by segregation tests in family samples and comparison to the Hap Map is 99.7%. To ensure sample integrity we have implemented a fingerprinting panel using the Sequenom Iplex technology in conjunction with the FQC SNPs on these arrays. We also present these and other quality control tests implemented to take advantage of the product content and the dense genotype data.

2650/F

Genome-Wide Germline Variation and Treatment Response in Acute Lymphoblastic Leukemia (ALL). *J. Yang[1], C. Cheng[1], W. Yang[1], L. Trevino[1], Y. Fan[1], S. Pounds[1], D. French[1], N. Shimasaki[1], D. Campana[1], J. Downing[1], W. Evans[1], C. Pui[1], M. Devidas[2], W. Bowman[3], B. Camitta[4], C. Willman[5], M. Borowitz[6], W. Carroll[7], S. Hunger[2], M. Relling[1].* 1) St Jude Children's Res Hosp, Memphis, TN; 2) Univ. of Florida, Gainesville, FL; 3) Cook Children's Med. Ctr, Ft. Worth, TX; 4) Med. College of Wisconsin, Milwaukee, WI; 5) Univ. of New Mexico, Albuquerque, NM; 6) Johns Hopkins Med. Inst, Baltimore, MD; 7) NYU Med. Ctr, NY, NY.

Although cure rates for pediatric ALL exceed 80%, the contribution of germline genetic variability to therapy response remains largely unknown. We performed a genome-wide study to identify single nucleotide polymorphisms (SNPs) that predict minimal residual disease (MRD) after remission chemotherapy in 2 independent cohorts. Using Affymetrix platforms, we genotyped ~588,000 SNPs in germline DNA from 318 children on St. Jude (SJ) trials Total XIIIB & XV and 185 patients on the Children's Oncology Group (COG) P9906 trial. MRD status was categorized as <0.01%, 0.01-1%, or >1% residual leukemic lymphoblasts. Associations (P<0.01) by Spearman rank correlation of genotypes with MRD were noted for 6995 SNPs in SJ and 6433 SNPs in COG cohorts. The number of significant associations exceeded that expected: 115 (SJ) and 90 (COG) SNPs with P<1e-4 (45 expected), and 12 (SJ) and 22 SNPs (COG) with P<1e-5 (~5 expected). Among SNPs with P<0.01, 69 predicted MRD in both SJ and COG. We explored mechanisms by which these 69 SNPs may affect therapy outcome. Of the 69 SNPs, 17 were associated with anticancer drug pharmacokinetics (6 with methotrexate (MTX) clearance, 4 with MTX accumulation in lymphoblasts, and 7 with etoposide clearance), all plausibly linked to MRD eradication corresponding to greater drug exposure. Moreover, 43 of the 69 SNPs predicted levels of 38 genes whose expression in the leukemic blasts differentiated MRD+ from MRD- patients. We conclude that host genetic variability affects treatment response for childhood ALL, and that germline variants may exert their effects on MRD by affecting host metabolism of anticancer drugs and by affecting gene expression in target leukemic blasts.

2651/F

Genome-wide association study identified a locus on 3p21 for cross-sectional geometry at the femoral neck. *L.J. Zhao[1], X.G Liu[1,3], L. Wang[1,3], J.F. Liu[1], Y.F. Pei[1,3], H. Yan[1,3], D.H. Xiong[4], F. Yang[2], H.W. Deng[1,2,3].* 1) Departments of Orthopedic Surgery and Basic Medical Sciences, University of Missouri - Kansas City, Kansas City, MO 64108,USA; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P R China; 3) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P R China; 4) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, NE 68131, USA.

Bone geometry is an important determinant of bone strength and osteoporotic fractures. Femoral neck cross-sectional (FNCS) geometry is significantly associated with the risk of hip fracture. We first conducted a genome-wide linkage study in 3,998 individuals from 434 pedigrees for FNCS phenotypes CT (cortical thickness) and BR (buckling ratio) with 410 microsatellite markers. Strong evidence of linkage was found for CT at 3p21 (LOD=2.19, P= 0.0006). This locus is established in previous independent linkage studies and plausible causative genes (CCR2, PTHR1 COL7A1) are located on this region. To further replicate the finding and identify susceptible variants for FNCS, we conducted a genome-wide association (WGA) study for CT and BR. We examined ~500,000 markers in 1000 unrelated white subjects. Helixtree was used for the data analysis. The WGA study indicated that one SNP (rs7430431) in the receptor-transporting protein 3 located at 3p21.31 was associated with BR (P=4.8 X 10-7) and CT (P=9.8 X 10-5). Additional test using Engisoft confirmed the association (P= 7.6 X 10-8 for BR and P=3.8 X 10-6 for CT). The results for BR remained significant after conservative Bonferroni correction. Genotype analysis revealed that the subjects with genotype CC at the rs7430431 had, on average, 8.6% lower BR than TT (P=4.01 X 10-5). This is the first GWA study for FNCS. The observations, combined with compelling evidence from other groups and our functional genomic study, strongly indicate that the locus at 3p21.31 is important for FNCS.

2652/F

Module eigengene networks and their applications to understanding human disease. *P. Langfelder, S. Horvath.* Dept. of Human Genetics, UCLA, Los Angeles, CA.

One of the challenges in gene expression analysis is the dichotomy between the large number of variables (typically on the order of 20000 genes) and the much smaller number of samples (typically below or around 100). Several data reduction methods have been proposed to capture the relevant information using a smaller set of variables. Here we study a network-based microarray data reduction method relying on module eigengenes as representatives of whole gene modules. We present a set of methods for construction and analysis of eigengene networks. Eigengenes represent the characteristic expressions of modules, while the weighted links represent the relationships between the modules. When augmented by clinical traits such as disease status, eigengene networks provide a natural framework for studying relationships among gene modules and clinical traits. In applications to cancer and a complex disease, we illustrate the use of eigengene networks to (1) identify array outliers, (2) cluster microarray samples (unsupervised learning), and (3) to classify array samples (supervised learning). Our applications indicate that eigengene networks are highly preserved across datasets and that they are a biologically meaningful data reduction scheme.

2653/F

Development of a Low Cost SNP Barcode Panel. *B. Marosy, J.M. Romm, K.N. Hetrick, K.F. Doheny, E.W. Pugh, Y. Tsai.* Center for Inherited Disease Research (CIDR) and Genetic Resources Core Facility (GRCF), IGM, JHUSOM, Baltimore, MD.

Advances in SNP genotyping technology have made it possible to produce up to 1 million genotypes for a given sample. These assays are costly; therefore, it is imperative to establish a validation process to pre-test and track samples through the lab and analysis process. CIDR is developing a SNP barcode panel that will uniquely identify individuals and provide pre-testing data to confirm gender, detect Mendelian inconsistencies, cryptic relatedness, mixed/contaminated DNA samples and low concentration samples prior to production genotyping. In order to utilize a SNP barcode, a more cost effective SNP assay is required. The Illumina® VeraCode offers low-plex SNP genotyping at a reasonable cost.

Markers were chosen from the 384 "Ancestry Informative Markers" within all Illumina GWA products. We selected a subset of SNPs based on assay qualities including: location, clustering in both HapMap and experimental samples, allele frequencies in the 3 HapMap populations that minimized the probability that two samples will have identical genotypes even if the two samples are related.

To determine sample performance using SNP pre-testing, without inflating false-pos/neg poor performance rates, we utilized call rates from previous SNP data generated on 5,204 samples from one study that was pre-tested using STRPs. This data was then compared to the actual performance of the samples in production. False pos/neg rates were then calculated based on these comparisons, STRP 0.48%, 3.2% and SNP 0.15% and 2.5%, respectively. To evaluate the VeraCode assay, we performed an Illumina GoldenGate Assay and hybridized product to both a Sentrix® Array Matrix and a Veracode Bead Pool. The genotypes generated from VeraCode were 99.988% concordant with GoldenGate and call rates were identical in 93.55% of the samples.

CIDR is beginning to evaluate a production version of the SNPs (OPA) for the SNP Barcode panel. This evaluation will include the ability to uniquely identify samples throughout lab processing and to determine sample quality without inflated false-pos/neg results.

2654/F

Copy number variant detection using Illumina BeadChip arrays. *D. Pinto[1, 2], J. Zhang[1, 2], B. Thiruv[1, 2], L. Feuk[1, 2], S.W. Scherer[1, 2].* 1) The Centre for Applied Genomics, Toronto, Canada; 2) Program in Genetics and Genomic Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada.

Understanding common genomic variation associated with disease susceptibility and population diversification is fundamental in human genetics. High-resolution SNP-based microarray technology now permits the simultaneous detection of SNPs and copy number variants (CNVs) on a genome-wide scale in a single experiment. This is achieved by using both the SNP allele calls and intensity of the allele-specific hybridization signals. The increase or decrease in hybridization signal for neighbouring SNPs can be used to identify regional patterns of structural genomic change. Currently, GeneChip (Affymetrix) and BeadChip (Illumina) are the platforms most widely used. Regardless of the platform used, identification of CNV regions is challenging, and the available tools have not been thoroughly tested. Illumina's Hap650Y BeadChip uses the Infinium assay to interrogate more than 655,000 tag SNPs, targeting common variation in four populations. To evaluate the performance of this array for the detection of both CNVs and SNPs, we examined 148 HapMap samples, various X-chromosome copy cell lines and the cancer cell line HL-60, and analyzed the data using various CNV calling algorithms, including BeadStudio, QuantiSNP and dCHIP. We also present a new CNV detection algorithm -iPattern- which uses a pattern recognition approach to analyze probe intensities of a group of samples and sliding windows to search for consecutive outliers. For any region, samples with probe intensities higher or lower than the average intensity of the majority samples are identified as gain or loss respectively. Preliminary results with Illumina Hap650Y were compared to Affymetrix GeneChip 500K data. This indicated that iPattern performs well for both platforms, showing significant concordance with another CNV calling algorithm, GEMCA. Here, we will review the CNV calling algorithms currently available and discuss results in light of previous studies.

2655/F

High-resolution whole-genome mapping of allele-specific human chromatin structure. *R. Sandstrom[1], M. Dorschner[1], M. Kuehn[1], S. Neph[1], J. Goldy[1], A. Haydock[1], M. Hirst[2], S. Jones[2], M. Marra[2], J. Stamatoyannopoulos[1].* 1) Dept. of Genome Sciences, University of Washington, Seattle, WA; 2) Genome Sciences Centre, BC Cancer Agency, Vancouver, BC.

WWe used Solexa sequencing to map >50 million individual in vivo DNaseI cleavage sites across the human genome at nucleotide resolution ('digital DNaseI'). In hundreds of human promoters and distal regulatory sequences marked by DNaseI hypersensitive sites in lymphoblast, neuroblast, and hepatocyte chromatin, the DNaseI cleavage patterns visualized by digital DNaseI are sufficiently dense to reveal the stand-specific 'footprints' of individual DNA binding proteins. In regions immediately flanking DNaseI hypersensitive sites, positioned nucleosomes can be readily identified by the characteristic ~10bp period of DNaseI cutting events in the minor groove of DNA. Dense sequence reads permit the recognition of known human polymorphisms, and thereby allele-specific assignment of DNaseI cleavage sites and analysis of chromatin structure. Outside of known imprinted regions, numerous genomic regions exhibit skewed allelic distributions of chromatin accessibility, including individual cis-regulatory sequences that are preferentially activated on maternal or paternal alleles. We also identified characteristic chromatin accessibility patterns at known structural anomalies of human chromosomes including translocation breakpoints, fragile sites, recombination hotspots, and radiation-sensitive domains. Digital DNaseI mapping has the potential to increase dramatically the scope and resolution of chromatin structural analyses of genome function and human disease.

2656/F

Highly parallel bead based DNA analysis. *D.N. Shinde, I. Tiemann-Boege, N. Arnheim.* Program in Molecular and Computational Biology, University of Southern California, Los Angeles, CA 90089, USA.

Rare genetic events, such as mutation and recombination, can be measured using PCR based techniques that selectively amplify the molecule of interest from a pool of genomes. This approach involves laborious optimization for each nucleotide examined. Recently, the introduction of emulsion based PCR technology has permitted the amplification of 10^6-10^7 individual molecules in parallel. Amplification occurs in microscopic aqueous compartments of an oil-buffer emulsion in a single reaction tube. PCR products from individual compartments containing a single template molecule and a single magnetic bead are captured by the bead and interrogated using different fluorophore labeled probes. We have modified the method to improve several aspects of the present protocols. First, we increased the proportion of oil/water compartments holding only single template molecules. Second, we increased the length of the product amplified on a bead to more than ~100bp by using alternative polymerases. Finally, we developed a detection method to examine simultaneously two SNPs located on the same molecule of DNA. The four alleles can be distinguished with minimal artifacts using four different fluorophores. These new implementations will allow us to measure rare mutations or recombination events at a high resolution with less effort.

2657/F

Estimating Allele Fraction or Allele Frequency using Unlabeled Probes and High Resolution Melting on the LightScanner. *M.D. Wall, L.L. Cutler, J.T. McKinney, D. deSilva, D.H.F. Teng.* Research and Development, Idaho Technology, Inc., Salt Lake City, UT.

The ability to estimate allele fraction of somatic mutations in primary tumor samples or allele frequency in a set of pooled DNA samples in a single reaction is desirable. We investigated the potential of using a new genotyping method involving an unlabeled probe and high resolution melting on the LightScanner instrument. Several common polymorphisms were chosen as targets and unlabeled probe assays developed to ascertain the genotype of several random DNA samples. For each locus, 3 samples were chosen representing each of the possible genotypes. The two samples representing the homozygous forms of the genotype were quantified and mixed at the following ratios: 95:5, 90:10, 75:25, 50:50, 25:75, 10:90, and 5:95. The 50:50 mixed sample was compared to the true heterozygote to validate the mixing ratios. Melting profiles of the unlabeled probes were converted to derivative peaks and the peak heights at each melting temperature of the probe were calculated. In all cases, discrimination of allele fraction down to 5% for both alleles was possible. Regression analysis of the observed peak height relative to the known allele fraction yielded R-squared values greater than 0.99, indicating that allele fraction can be reliably estimated to the level of 1:20 alleles. These results indicate that the use of high resolution melting with unlabeled probes can be an effective way to estimate the allele fraction in tumor samples or allele frequency in up to 10 pooled samples.

2658/F

Genome-wide approaches to T2D gene identification: how useful are assessments of biological candidacy? *N.W Rayner[1], E. Zeggini[1], N.J. Timpson[2], C.M. Lindgren[1], C.J. Groves[1], M.N. Weedon[2], T.M. Frayling[2], R.M. Freathy[2], J.R.B. Perry[2], H. Lango[2], B. Shields[2], A.T. Hattersley[2], M.I. McCarthy[1], K.S. Elliott[1].* 1) WTCHG, Oxford Univ, UK; 2) Peninsula Med Sch, Exeter, UK.

Linkage and association studies for type 2 diabetes (T2D) have implicated many genes, but there have, until recently, been few widely-replicated findings. However, large scale genome-wide association scans (GWAS) have led to the discovery of true disease loci. For many of these, prior evidence of their biological candidacy is limited. We can now take stock of the current knowledge of T2D candidacy and ask: how useful are assessments of biological candidacy in finding T2D loci? Using data from the Wellcome Trust Case Control Consortium GWAS (1924 cases, 2938 controls, 393,453 SNPs), we tested the relationship between T2D associations genome-wide and biological candidacy using the GeneSniffer program. GeneSniffer uses a range of online literature and databases to assign a T2D candidacy score based on the co-occurrence of gene-specific and disease phenotype-related terms. For each gene (coding seq +/- 50kb: n=18767) we derived the strongest SNP-specific association test statistic. Linear regression was used to test the association between statistic and GeneSniffer score. We excluded previously known T2D genes *TCF7L2*, *PPARG* and *KCNJ11*, as published studies would lead to secondary inflation of their candidacy. GeneSniffer scores for the 6 novel T2D loci were variable: *CDKAL1*[0], *SLC30A8*[304], *CDKN2A*[1073], *IGF2BP2*[609], *HHEX*[385], *FTO*[0] (average score=200 range 0-25354). We found a small but robust (r2= 0.04, p<10-4) positive correlation between the test statistic and T2D candidacy score. We accounted for the correlation correlation of gene length (and number of SNPs per gene) with the highest chi squared per locus (r2=0.25, p<10-4) by adjusting the statistic, and found a significant association with gene candidacy (p<10-4). The analysis showed that GeneSniffer score has some limited predictive value for gene association (goodness of fit for linear regression, r2=0.002). Our current understanding of diabetes aetiology remains a tentative predictor of the location of disease variants.

2659/F

A simplified single-step SNP genotyping assay - application to multiple ATP-binding cassette transporter SNPs. *C.G. Lee[1,2,3], Z. Wang[1], P.H. Sew[1], S.S. Chong[4].* 1) Biochemistry, Natl Univ Singapore; 2) DUKE-NUS Graduate Medical School; 3) National Cancer Centre; 4) Pediatrics, Natl Univ Singapore.

Several different methods have been developed for the simultaneous genotyping of multiple single nucleotide polymorphisms (SNPs). However, most of these techniques either require costly reagents or involve several steps. Here, we describe the development of a low cost, simple, rapid and sensitive SNP genotyping assay that involves a modified real-time allele-specific PCR. In this assay, only standard reagents for real-time PCR with SYBR Green dye are used except for 2 modifications. Instead of regular Taq DNA polymerase, a high fidelity DNA polymerase with 3'-5' exonuclease proofreading activity is used. Secondly, one of the amplification primers is allele-specific and modified with a 3'-phosphothioate end. With this modification, the 3'-5' proofreading activity of the polymerase is blocked when a 3' mismatch between primer and template occurs preventing the polymerase from extending from the mismatched primer. Thus, primer extension, and ultimately DNA amplification, will only occur when there is a perfect match between 3'-phosphothioate modified allele-specific primer and DNA template. Amplified product is readily detected in real-time via SYBR Green fluorescence emission. Through threshold cycle (C_t) analyses, the genotype of the DNA target can be readily determined. The entire genotyping process takes <2.5 hours in a standard real-time thermocycler and <1 hour in a Fast Real-Time PCR System. We evaluated the feasibility of this method in a pilot validation analysis of SNPs within several ATP-Binding Cassette (ABC) transporter genes displaying evidence of recent positive selection with potentially functional importance (Wang et al, 2007 HMG 16(11):1367-1380). For each SNP, >20 different genomic DNA samples were genotyped using this method in parallel with minisequencing and/or sequencing. Of 16 different SNPs examined in >400 DNA samples thus far, a genotype concordance rate of >97% was achieved. We conclude that this simple single-step genotyping assay is reliable and potentially amenable to high-throughput simultaneous analyses of multiple SNPs and mutations.

2660/F

Identification of *BMPR2* deletion/duplication breakpoints in familial pulmonary arterial hypertension (FPAH). *M. Pauciulo[1], K. Clark[1], L. Wheeler[2], J. Loyd[2], W. Nichols[1,3].* 1) Cincinnati Children's Hospital Medical Center; 2) Vanderbilt University Medical Center; 3) University of Cincinnati College of Medicine.

FPAH is an autosomal dominant disorder with reduced penetrance characterized by occlusion and remodeling of the pulmonary arteries leading to sustained elevation of pulmonary vascular resistance, progressive right heart failure, and death. Germline *BMPR2* mutations have been identified in approximately 70% of families. In a recent study of 30 FPAH families, *BMPR2* exonic deletions/duplications accounted for 48% (10/21) of the mutations identified. Nine of the 10 deletions/duplications involved one of the two large *BMPR2* introns, either IVS1 (>87 kb) or IVS3 (>46 kb). As the breakpoints of the dosage mutations were not determined, the origins of apparently similar deletion mutations identified for exons 1, 2, and 3 in each of two unrelated families could not be determined. The aim of this study was to identify the *BMPR2* deletion/duplication breakpoints and determine any common mutational hotspots leading to recurring mutations. Real-time PCR assays, designed in the introns of *BMPR2* surrounding the deletions/duplications, were used to map the extent of the dosage mutations. PCR primers were designed flanking the dosage mutations which enabled amplification, cloning, and sequencing of PCR fragments for determining the sequences at the deletion/duplication breakpoints. Breakpoints have been identified for seven of the 10 *BMPR2* deletions/duplications. Our results show that the two families carrying an exon 3 deletion have the identical 9,768 bp deletion which is flanked by a 42 bp identical sequence in introns 2 and 3. However, the breakpoints of the two exon 1 deletions and the two exon 2 deletions found in each of two families are different and thus represent independent mutational events. Identical sequences ranging from 30 to 47 bp were found flanking five of the seven identified deletion/duplication breakpoints, suggesting uneven crossing over as a common, but not an exclusive, mechanism of *BMPR2* exonic deletions/duplications. Our results also suggest that most *BMPR2* deletions/duplications may be independent mutational events and do not arise from intronic mutational hotspots.

2661/F

Interlaboratory validation study of High Resolution Melting Curve Analysis for mutation scanning of BRCA1 using the Idaho LightScanner. *N. van der Stoep[1], C.D.M. Paridon[1], P. Norambuena[2], A. Stambergova[2], M. Macek[2], T. Janssens[3], G. Matthijs[3], E. Bakker[1].* 1) Center of Human and Clinical Genetics (LUMC), Leiden, Netherlands; 2) Institute of Biology and Medical Genetics, Prague, Czech Republic; 3) Center for Human Genetics, Leuven, Belgium.

The current set up for mutation scanning of BRCA1 occurs through sequence analysis, DGGE, PTT and DHPLC. All these techniques are time consuming and expensive. Therefore we have evaluated the High-Resolution Melting Curve Analysis (HR-MCA) as a high-throughput mutation-scanning tool for the BRCA1 gene using the LightScanner from Idaho Technology (IT) and the LCGreen Plus+ mastermix. This study was implemented in the EuroGentest evaluation program for new techniques in genome diagnostics. Therefore the BRCA1 mutation scanning test was first set up at the LUMC in Leiden and subsequently partly re-evaluated by the laboratories in Prague and Leuven. Investigations were carried out using a panel of 189 variants and 327 wt controls. We optimized and evaluated HR-MCA of 48 primer sets that encompass all 24 exons of the BRCA1 gene. All heterozygous variants could be detected using the Call-IT 1.5 software (Idaho) and resulted in a 100% mutation detection sensitivity. These variants also include small DNA deletions and insertions. Out of 327 wts we observed 3,7% false positive curves (FP) resulting in a specificity of 96%. The detection of the homozygous polymorphisms depended on the used primer set, but due to overlapping fragments eventually all 9 homozygous variants could be detected as well. Moreover using unlabeled probes we were able to identify several frequent occurring polymorphisms, omitting unnecessary sequence analysis upon detection of these non-pathogenic variants. Finally we performed blind tests for 22 different BRCA1-amplicons and were able to detect all variants (except 1 homozygous variant) and observed a FP score of 1,8%. Re-evaluation of ten BRCA1-amplicons in the second diagnostic laboratory gave rise to identical results (third lab-study is ongoing). In conclusion, HR-MCA with the LightScanner appears to be a rapid and sensitive method for mutation scanning. More details will be presented at the meeting.

2662/F

Individualizing Cancer Therapies through the Optimized Selection of Compounds with Complementary Signatures. *E.O. Lillie[1,2], N.J. Schork[1,2].* 1) Scripps Genomic Medicine, TSRI, La Jolla, CA; 2) Center for Human Genetics and Genomics, UCSD, La Jolla, CA.

Each individual has unique genetic features that contribute to cancer risk and prognosis. Once cancer has been established, tumors display unique features due to stochastic somatic events during tumorigenesis. These unique features can be characterized by gene expression. We hypothesize that certain compounds produce a complementary gene expression signature that reflects biological activity opposite to the tumor's activity. Therefore, an individual's response to the compound can be predicted by how their unique tumor signature complements the signature of the compound. We tested this through the use of published gene expression datasets and the "Connectivity Map" (cmap), a collection of gene expression profiles from cultured human cells treated with bioactive small molecules (http://www.broad.mit.edu/cmap/). To test our hypothesis, we used 3 datasets from estrogen receptor positive breast cancer tissues obtained prior to tamoxifen (tam) therapy with follow-up data available (E-TABM-158, GSE4922, GSE2990) and compared them to normal breast tissue (GDS1096). Each subject's signature of the transcripts that were either up- or down-regulated was uploaded to the cmap for comparison with the tam signature in MCF-7 cell lines. A score between -1 and 1 for how similar the signature was to that of tam was output. For perfect complementarity we would expect a score of -1 suggesting that up-regulated transcripts in the tumor were down-regulated in response to tam. In the combined sample of 179 breast cancer cases, we observed a range of tam connectivity between -0.273 and 0.593. No associations between cmap score and risk of recurrence were observed with or without adjustment for other prognostic factors. Our null results may be explained by a number of factors: insufficient sample size, limitations in comparing expression in tumor tissue to immortalized cell lines, and the choice of transcripts to compare. We plan to further test these hypotheses using data from other cancers and compounds to further evaluate the utility of cmap for connecting drugs with disease.

2663/F

Identifying candidate markers for follow-up studies to genome-wide association: beyond nonsynonymous SNPs. *M.A. Levenstien, R.J. Klein.* Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

With the advent of cost-effective genotyping technologies, genome-wide association studies allow researchers to examine hundreds of thousands of single nucleotide polymorphisms (SNPs) for association with human disease. Recently, many researchers applying this strategy have detected strong associations to disease with SNP markers that are either not in linkage disequilibrium with any non-synonymous SNP or large distances from any annotated gene. In such cases, no well-established standard practice for effective SNP selection for follow-up studies exists. We aim to identify and prioritize groups of SNPs that are more likely to affect phenotypes in order to facilitate efficient SNP selection for follow-up studies. Based on the annotations available in the Ensembl database, we categorize SNPs in the human genome into functional classes including promoter regions, splice sites, and coding regions. Using SNP density and the distribution of derived allele frequencies within each class, we assess the relative strength of natural selection for each class. Within the HapMap ENCODE regions, we find that the SNP density for all three classes is significantly less than that for the genome as a whole. Interestingly, each functional class's distribution of its member SNPs' derived allele frequencies in these regions generally did not differ significantly from that of the genome. It will be important to explore additional classes such as regions which are highly conserved among related species for evidence of selective pressure.

2664/F

Validation of resequencing variants by Pyrosequencing. *M. Maheshwari, S. Scherer, B. Ng, G. Metcalf, K. Blankenburg, F. San Lucas, A. Garcia, D. Wheeler, G. Weinstock, R. Gibbs.* Human Genome Sequencing center, Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX 77030.

Baylor College of Medicine's Human Genome Sequencing Center has sequenced hundreds of candidate genes to identify alleles that may confer risk of various diseases like epilepsy, autism, cardiomyopathies, bipolar disorder and schizophrenia. Medical resequencing has facilitated the detection of thousands of single nucleotide polymorphisms (SNPs) and insertion-deletions (Indels) including coding and non coding variants. Because high throughput software is used to call variants, validation is needed to inform downstream functional analyses, broader population screening and accurate public database submissions. We adopted the PyroMark MD (BIOTAGE) pyrosequencing platform to validate SNPs and Indels discovered by Sanger sequencing based on verification studies demonstrating overall pass rates of 93% and correspondence rates of better than 99% using known HapMap SNPs and a CEPH DNA sample set. Pyrosequencing is sequencing by synthesis method, based on real time pyrophosphate detection. We chose pyrosequencing over alternative platforms as it generates both unambiguous genotyping results and some flanking sequence information beyond the SNP position, which serves as internal control. To date, we have validated hundreds of SNPs discovered primarily from our ion channelopathy and lung adenoma projects across tens of thousands of samples by re-amplifying each locus from the original DNA stocks. Overall concordance rates with Sanger platform/SNP Detector 3 derived SNPs and Indels is currently running between 75 and 80 percent. Further pipeline improvements in throughput, cost and data-tracking are currently under development.

2665/F

60-Plex Genotyping Reactions of Single Nucleotide Polymorphisms Using Single Base Primer Extension Coupled with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. *P. Oeth, G. del Mistro, G. Marnellos, T. Becker, S. Berkenkamp, C. Jurinke, S. Sur, D. van den Boom.* Research & Development, Sequenom, Inc., San Diego, CA.

We report the first example a of multiplexed genotyping reaction, greater than 40-plex, to be accurately resolved and typed using Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Our results are an extension of the number of genotypes achieved per reaction using the iPLEX genotyping reaction on the MassARRAY ® system with several important implementations to deal with assay design and the density of peaks per mass spectrum. Assay designs were created using proprietary software (MassARRAY ® Assay Designer) for homogeneous multiplexed PCR and primer extension reactions. Prior to assay design SNP sequences were screened for proximal SNPs against dbSNP (MassARRAY ® ProxSNP) and candidate assay primer triplets were screened against the human genome to establish uniqueness for assay hybridization (MassARRAY ® PreXTEND). Products were resolved on a linear mode MALDI-TOF MS within a mass window of 4500-9500 Da. In order to prevent any potentially confounding effects by salt adducts from parental allelic peaks, which might interfere with peak detection, an adduct reduction reagent was introduced prior to cation exchange desalting. Genotypes were called using a post acquisition clustering algorithm rather than in real-time (TYPER Analyzer 4.0). Preliminary studies using HapMap SNPs with a minor allele frequency of 40% or greater in the CEPH population of western European ancestry have established that 60-plex reactions provide a median assay coverage of 90-93%. Assay and genotype quality filters based on criteria for reproducibility, Hardy-Weinberg Equilibrium and Mendelian Error reduced assay coverage by approximately 5-10% resulting in a conversion rate of 80-88% per 60-plex with a HapMap genotype concordance of greater than 99.5%. Our data to date suggests that each designed 60-plex will yield a working 48-52 plexed reaction for large cohort typing of candidate gene regions during the fine mapping phase of association studies.

2666/F

A Nanofluidic System for Rapid and Reliable Genotyping. *R. Ramakrishnan[1], M. Pieprzyk[1], R. Welch[2], A. Crenshaw[2], B. Hicks[2], M. Yeager[2], S. Berndt[3], W-Y. Huang[3], R.B. Hayes[3], S.J. Chanock[3,4].* 1) Fluidigm Corp, S.San Francisco, CA; 2) Core Genotyping Facility, National Cancer Institute, Gaithersburg, MD USASAIC Frederick, Advanced Technology Program, NCI-FCRDC, Frederick, MD; 3) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 4) Core Genotyping Facility, NCI, Bethesda, MD.

Although remarkable advances have been made in low- and high-throughput genotyping platforms, there is a need for systems allowing medium multiplexing (30-300 SNPs) platforms with high throughput, excellent call rates, high concordance and low cost. In the current study we demonstrate the use of a unique nanofluidic genotyping system which is simple to use and exhibits these characteristics.

Fluidigm Corporation has developed Integrated Fluidic Circuits (IFCs) which reduce the amount of sample and reagents required for chemical reactions to nanoliter volumes.We demonstrate the use of specific IFCs called dynamic arrays, in a study to genotype 1000 unique human DNA samples on 48 different SNP assays, using nanoliter volumes of reagents. The DNA samples screened included 910 DNA case control samples extracted from blood from an incident adenoma project, and 90 HapMap samples extracted from cell lines. Each dynamic array IFC systematically combines samples and assays into 2,304 reactions. Each chip was thermocycled, imaged and analyzed using a BioMark™ system. Call rates of greater than 99.5% and high concordance values were achieved. Calls from the incident adenoma samples were validated by selected genotyping of the same samples on the ABI 7900, while calls from the HapMap samples were validated by concordance with results obtained by the HapMap project.

The excellent call rates and high concordance, combined with the massively parallel fabrication of valves in nanofluidic chips, provides a formidable genotyping tool. The development of this system profoundly impacts the ability to screen mid-range numbers of genotypes across multiple samples.

2667/F

HapMap SNPs in the UCSC Genome Browser. *H. Trumbower.* Genome Bioinformatics Group, Univ California, Santa Cruz, Santa Cruz, CA.

The UCSC Genome Browser (http://genome.ucsc.edu) is a web-based interface for displaying full genome data sets. HapMap SNPs are an important new genome-wide resource for the study of human polymorphism. The HapMap SNPs have been added to the Genome Browser with a set of customized visualization features.

For each of the 4 million SNP positions in HapMap Phase II, the display includes a summary of genotype results from each population, as well as orthologous alleles from chimp and macaque. For the four populations, the display uses a color gradient based on minor-allele frequency; the orthologous alleles are shaded based on quality score of the assembly at that position. When zoomed in, the major allele is shown for each population.

Software is provided that dynamically filters the data set. A location can be excluded based on whether the chimp allele is available, and whether it matches the human major allele, human minor allele, or neither human allele. This filter is only valid if an overall major allele exists, which is true for eighty percent of the HapMap SNPs. An independent filter with the same features is also available for macaque.

Another filter will exclude SNPs based on whether the major allele is consistent or mixed across the populations. Heterozygosity (2pq) is calculated over all populations at each position and has its own filter. Other filters include minor allele frequency minimum and maximum, monomorphism, population availability and quality score for orthologous allele.

2668/F

Development of a SNP Chip for genotyping of multiple single nucleotide polymorphisms simultaneously. *Y.H. Yang[1], T.L. Cheng[2,3], S.R. Lin[1,4], M.C. Hsieh[1,5].* 1) Graduate Institute of Medical , Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Faculty of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) National Sun Yat-sen University-Kaohsiung Medical university joint research center, Kaohsiung, Taiwan; 4) BioMedi Innovation Incubation Center, Kaohsiung Medical University, Kaohsiung, Taiwan; 5) Division of Endocrinology and Metabolism, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background and purpose: Recently, the relation between SNPs and diseases susceptibility are extensively studied. By multiple SNPs genotyping, people can know their susceptibility to certain disease. Development of a SNP Chip that is convenient, inexpensive, and fast for multiple SNPs genotyping is necessary. Methods: Four oligonucleotide probes are designed for each SNP. Two of them are SNP specific oligonucleotides, and the other two are the quality controls which contain only a point mutation. Those oligonucleotides were individually blotting on nylon membrane to form the SNP chip. Target SNP-containing regions of AGT (angiotensinogen), CETP (cholesteryl ester transfer protein), and APOE (apolipoprotein E) were amplified by multiple PCR. The multiple PCR productions were labeled with DIG-UTP. The DIG-labeled PCR products would hybridize with the SNP chip, following by Anti-DIG-AP (alkaline phosphatase) binding and color development by NBT/BCIP. The SNP genotypes are decided acording to the color expression on SNP Chip. Results: The genotypes of AGT, CETP and APOE analyzed by SNP Chip are highly consistent of direct sequencing. The accuracy of SNP Chip is more than 99 %. Conclusions: We have successfully established a SNP screening chip which is convenient, inexpensive, and high-throughput. This SNP chips can be used to screen patients for risk of disease and predict the onset and prognosis of disease.

2669/F

Massively Parallel Sequencing of Autism Candidate Genes. *S. Strom, J. ten Bosch, B. Merriman, Z. Chen, S.F. Nelson.* Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Newly available techniques allow for the re-sequencing of 1 billion base-pairs of DNA in three days at a reasonable cost. While extremely powerful, this technology has not yet been wielded in a way that illuminates complex human traits. This requires tractable methods to selectively analyze genomic regions of interest and methods to combine DNA samples from multiple individuals into one experiment. Here we report initial findings on a direct approach to identify DNA variants in candidate genes for Autism Spectrum Disorder using the Solexa 1G Genetic Analyzer. This study focuses on two positional candidate genes for autism spectrum disorder on chromosome 17q11 identified in a whole-genome scan (Stone et al. 2004): myosin 1D (Myo1D) and amiloride-sensitive cation channel 1 (ACCN1). The design of the Solexa instrument facilitates the analysis of eight DNA samples in tandem, allowing for the sequencing of a maximum of 6.5Mb per sample with twenty times coverage, a capacity which far exceeds the scope of this study as the coding regions of both genes combined is approximately 4.5kb. The test sample is comprised of 69 male probands who share two alleles identical by descent (Z2) with an affected brother in the linkage region. This study serves to demonstrate the feasibility of large-scale candidate gene sequencing using massively parallel sequencing technology.

2670/F

Common variation at 8q24 and prostate cancer risk. *M. Yeager-Jeffery[1,2], R. Welch[1,2], R.B. Hayes[2], P. Bouffard[3], N. Xiao[1,2], L. Burdett[1,2], N. Orr[4], A. Crenshaw[1,2], Z. Markovic[3], K.B. Jacobs[5], T.P. Jarvie[3], D. Hunter[2,6], R. Hoover[2], G. Thomas[2], T.T. Harkins[7], S.J. Chanock[2,4].* 1) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services; 3) 454 Life Sciences, Branford, CT; 4) Pediatric Oncology Branch, Center for Cancer Research, NCI, NIH, DHHS; 5) Bioinformed Consulting Services, Gaithersburg, MD; 6) Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; 7) Roche Applied Science, Indianapolis, IN.

Recently, several groups have reported strong associations between common DNA polymorphisms that span a segment of chromosome 8q24 and the risk of prostate cancer. There is evidence that at least three regions of this segment (chromosome 8: 126501167-128998553) are independently associated with risk and are also dependent on the ethnic origin of prostate cancer cases. As an extension of the Cancer Genetic Markers of Susceptibility project (http://cgems.cancer.gov), preliminary association studies of more than 4000 cases and 4000 controls of European origin have identified haplotypes on which disease-contributory mutations most likely exist. We have extensively characterized common genetic polymorphisms present for two of these regions, totaling > 148kb, using Roche/454 next-generation resequence analysis (chromosome 8: 128470954-128619305) of 40 prostate cancer cases and 40 controls of European origin and seven individuals from a CEPH family in which a common predicted susceptibility haplotype is segregating. The characterization of this region is important to rapidly identify common genetic polymorphisms so that they can be investigated for functional significance. There is growing evidence that these regions are also implicated in other cancer types; these observations underscore the importance of characterizing common genetic variation at 8q24. Funded by NCI Contract N01-CO-12400.

2671/W

The CIDR AutoCall Pipeline: An automated analysis pipeline for Illumina® Infinium Products. *M.W. Barnhart, K. Hetrick, C.W. Bark, D.R. Leary, G. Lowe, E. Hsu, J.L. Goldstein, K.F. Doheny, L. Watkins, Jr.* Center for Inherited Disease Research, Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) is a centralized facility established to provide genotyping and statistical genetics services for investigators seeking to identify genes that contribute to human disease. The large volume of data produced by genotyping products such as Illumina's Infinium group poses immense challenges for data storage, analysis, and retrieval. The CIDR AutoCall Pipeline, a Java application tightly integrated with the CIDR Infinium LIMS, is an automated analysis pipeline for data produced by Illumina's AutoCall utility for the HumanHap300, 550, and 650Y products. The tiny percentage of data that may be problematic is identified for human scrutiny; the rest is and archived for downstream data analysis and release. For a setof beadchip scans, the pipeline gathers and stores scanner information; generates a binary genotype call (GTC) file using Illumina's AutoCall utility; parses the GTC file to produce human-readable output; analyzes beadchip data quality; and archives the data. The pipeline first parses the final XML file produced by the scanner for each chip and uploads the data to the CIDR LIMS database. Next, AutoCall is used to generate a GTC file for each chip using the bead pool manifest (BPM) file, the cluster (EGT) file, and the chip intensity data (IDAT) files. Each GTC file is then read using CIDR's GTC file parser, a standalone software tool (available upon request) that retrieves detailed SNP data, computes normalized coordinates and theta, and writes a human-readable flat file. Overall data quality is calculated from the information in these files, and a BeadStudio "R SNP table" is used with it to generate Log R ratios across chromosome one. Finally, all the data is archived. Each chip's GTC file, its flat-file representation, and the metrics file containing QC data for each chip are written to project-specific folders for easy retrieval. This GTC archive provides all the data needed to perform a genetic analysis across a project.

2672/W

Representing genomic variation: a hierarchical database structure. *E. Bruford[1], T. Sneddon[1], M. Lush[1], M. Wright[1], S. Povey[2], E. Birney[1].* 1) HUGO Gene Nomenclature Committee, European Bioinformatics Institute, Hinxton CB10 1SA, UK; 2) Dept of Biology, University College London, Wolfson House, London NW1 2HE, UK.

The HUGO Gene Nomenclature Committee (HGNC) has to date approved over 24,000 unique symbols and names, the majority of which are for 'genes', i.e. genomic segments that are transcribed and translated into functional proteins. However, an increasing number of genes that were initially thought to be single copy in the human genome have been shown to be copy number variant (CNV) between individuals. This is especially true for genes encoding secreted, olfactory and immunity-related proteins, such as the amylase and defensin gene families. As individual copies of CNV genes are being discussed in the literature, and represented in the databases by alternative haplotypes (e.g. c5_H2 and c22_H2 in Build 36.1), there is an increasing need for a meaningful and systematic nomenclature system for them. Therefore the HGNC, in consultation with the scientific community and using data from the Database of Genomic Variants (http://projects.tcag.ca/variation/), has recently implemented a hierarchical structure in our gene nomenclature database (www.genenames.org). This facility allows for an approved gene record to contain sub-entries for each copy number variant e.g. the DEFB103 gene record links to DEFB103A and DEFB103B sub-entries. Examples of copy number variant genes that have been incorporated into our hierarchical database structure will be presented. We welcome requests from the research community to incorporate other experimentally verified CNV genes into our database. In addition to copy number variants, this new hierarchical database structure will also allow us to capture and represent information concerning other types of genomic variation, such as the complex gene loci encoding immunoglobulins, T cell receptors and protocadherins, and read-through/chimeric transcripts. We have recently relocated to the European Bioinformatics Institute EMBL Outstation at Hinxton, near Cambridge in the UK. For further information please email us at nome@ebi.ac.uk, or go to our new website, http://www.genenames.org. The work of the HGNC is supported by the NHGRI and the Wellcome Trust.

2673/W

Pilot Studies of the Human Variome Project. *R. Cotton[1], and Collaborators[2].* 1) Genomic Disorders Research Centre, St Vincent's Hospital, Fitzroy, Australia; 2) and Collaborators.

The concept of the Human Variome Project (1) (www.humanvariomeproject.org) was developed to draw attention to the importance of collection of variation and its phenotypic effect and to develop programs to put this into effect. The project was initiated in June 2006 (2). The project builds on work and concepts of the HGVS consortium over many years (www.hgvs.org) and will first focus on Mendelian disorder. The project intends to include all those discovering mutations and its effect and then collect the data so that it is instantly available for others who need it to inform clinical decisions and research. A major pilot study and plan has been developed by the inherited colon cancer community InSiGHT (www.insight-group.org) to develop procedures and systems to allow effortless flow of de-identified data from the patient/clinic/diagnostic laboratory via curated locus or gene specific databases to central databases/genome browsers such as dbGaP, UCSC, HGVbase and EBI. The system will be developed so that it is easily adaptable to other genes and to multiple laboratories, states, counties and countries around the world. Other pilot studies developed include specific ethical studies related to mutation collection, loading of LSDB content to dbGaP and funding of curation of LSDBs. 1. What is the Human Variome Project? Nat Genet 39, 423 (2007). 2. Ring, H.Z., Kwok, P.Y. & Cotton, R.G. Human Variome Project: an international collaboration to catalogue human genetic variation. Pharmacogenomics 7, 969-72 (2006).

2674/W

The Generic Genetic Studies Database: A Data Management System for Large Scale Genetic Studies. *A. Day*. Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Continuing advances in genotyping technology are fundamentally changing the way researchers investigate disease. These new technologies are allowing researchers to assay genetic variation at hundreds of thousands of markers across the entire genome simultaneously. Unfortunately, this progress in genotype data generation has not been matched with advances in the sophistication and capabilities of data management tools. The trend toward highly collaborative projects and the number of subjects, markers, and phenotypes being investigated in each project simply overwhelms the traditional ways of managing data with flat files.

The Generic Genetic Studies Database (GGSD) is an open-source data management software package for large scale genetic studies. GGSD is a web-integrated, relational database driven system that stores and appropriately links: (1)pedigree/individual information; (2)genotype data for both SNPs and microsatellites; (3)phenotype data for both quantitative and qualitative traits; and (4)disease definitions and individuals' status for those diseases. GGSD allows researchers to store and manage several projects all through a single interface, and manages the users of the system to track the project(s) each user has access to and their type of access (e.g. upload/delete/update or search/download). GGSD is an extremely flexible system. For example, it allows batch entry of data via file uploads, manual entry and editing of data through web forms, entity-centric web forms for complex querying of any table in the database, pedigree drawing, Hardy-Weinberg equilibrium determination for markers, data download in tab-delimited or linkage format, and sends emails to registered users when data is added/edited/deleted.

The GGSD system was designed around the LAMP and LAPP stack paradigm of constructing dynamic, interactive web-based applications. The entire system was designed and programmed using JavaScript, Perl, PHP, and ANSI SQL. Utilizing this open-source software design framework makes GGSD extremely versatile, portable, and economical.

2675/W

An integrated genome visualization tool for diagnostics and research. *B. Eussen, M. Moorhouse, T.A. Knoch, F. Grosveld, A. de Klein.* Clinical Genetics and Cell Biology, ErasmusMC, Rotterdam, South Holland, Netherlands.

In the past cytogenetics has illustrated that specific chromosomal regions are linked to genome structure related components of the DNA. The first genome wide approach was achieved with the identification of the first human duplicon set by E. Eichler et al 2002. These duplicons were not randomly spread on the genome but clustered on centromere and telomere regions. Also common microdeletion regions like Prader Willi and DiGeorge syndrome are flanked by these duplicon clusters. In principle all these duplicons regions can be pitfalls in the interpretation of genomic assays, a recent report of the HapMap project showed a nice correlation between duplicon regions and polymorphic regions in two different platforms: a tiled human BAC array and the Affymetrix 500K SNP (Redon et al. 2006). To visualize the actual complexity at array hotspots or candidate regions related to specific diseases, an integrated view on genomic and experimental data must be available in a customized way. For this we developed a generic visualization tool called 3D genome viewer. In a few examples we will show the flexibility, customization and the intuitive integration of experimental data from different diagnostic platforms (SNP/BAC arrays, QPCR, MLPA) with a selection of currently available public data sources like; segmental duplicons(Eichler et al. 2002), Genomic Variants data (Iafrate et al. 2006) and syndrome locations (DECIPHER). Also for future visualization requirements, high through put expression/SNP arrays, education purposes, nuclear organization, epigenetic and molecular imaging this genomic 3D viewer can be the basis of many visual concepts.

2676/W

Nucleosome Exclusion Regions across the Human Genome. *S. Khuri[1], A. Radwan[2], P. Luykx[3], A. Younis[3].* 1) Miami Institute for Human Genomics, University of Miami Miller School of Medicine; 2) Dept. Electical and Computer Engineering, University of Miami; 3) Dept. Biology, University of Miami.

Nucleosomes are DNA-protein complexes that are the building blocks of eukaryotic chromatin. They are involved in genome condensation, and play a significant role in transcriptional regulation. Each nucleosome comprises eight histone proteins that together form a compact unit that accommodates 147 nucleotides wound around it. There are certain DNA sequence patterns that are unlikely to be involved in nucleosome binding, due to a lack of flexibility inherent in their double helical structure. These sequence patterns include GC-rich motifs, as well as poly-A and poly-T tracts. Previously we developed a webtool (NXSensor) that was able to predict these nucleosome exclusion motifs. Using a pilot grid computing architecture, we developed an updated version of NXSensor and implemented it on the whole human genome (build hg18), where we observed three main trends. First, we were able to predict the location of Nucleosome Exclusion Regions on all chromosomes, and found that these were correlated with gene density. Second, we calculated Nucleosome Exclusion Scores (NXScores) for the promoter regions (-1500 to +500 bp) of all known genes, and found that there was a strong signal for nucleosome exclusion around the transcriptional start site. In addition, the high NXScores persisted on average 250 nucleotides into the gene, presumably to allow the transcription machinery to gain momentum, or to give leeway for alternative transcriptional start sites. Third, we correlated promoter-region NXScores with gene expression and tissue specificity. We found the more tissue specific a gene is, the more likely it is to have nucleosomes positioned along its promoter. Furthermore, gene expression was positively correlated with a high NXScore such that genes with median expression levels also had high NXScores, but expression level drops with very high NXScores. This may be a reflection of the slower movement of the transcriptional machinery through regions of a high GC content. These results provide further insights into the relationships between the human nucleosome landscape and gene regulation.

2677/W

Affymetrix Annotation Search, a tool for data mining whith Affymetrix data. *C.S. Rocha, I. Lopes-Cendes.* FCM, Unicamp, Campinas, Sao Paulo, Brazil.

Purpose

The Affymetrix Annotation Search is a web tool developed to facilitate for biologists to get annotation data from Affymetrix GeneChip. Starting with a list of identifiers (Probe Set IDs) this tool can search on a database the annotation corresponding to these identifiers. To make easier the data mining, was included links to others databases like: NetAffx, UniGene, Ensembl, SwissProt and OMIM. Was implemented too a filter by chromosome, this filter allows the user to diminish the amount of data to analyze directing his search to a especific chromosome.

Methods

This tool was developed to be web based. A SQL database was created with the Affymetrix annotation data, with the Probe Set ID field as primary key. The web tool was written in perl language using the CGI module for web aplication and the DBI module to database manipulation.

Summary of Results

The database has the annotation of all 54.675 Probe Sets presents in the HG-U133 Plus 2 Affymetrix GeneChip and can be fed with the others Affymetrix chips as requested.

Conclusion

This tool can help biologists that work with Affimetrix GeneChip, because it facilitate the analysis of the big amount of data that a microarray experiment generates, with the filter option, the user could have less but more specific data, with the external links the user have easily a lot of interesting information about his Probe Set.

The tool is freely aviable at: http://lgm.fcm.unicamp.br:9001/cgi-bin/affy/affy_annotation.cgi.

2678/W

Two Stage State-Space models for genetic networks. *X. Sun[1], L. Jin[1], M. Xiong[1,2].* 1) Dept Genetics, Fudan Univ, Shanghai, China; 2) Human Genetics Center, University of Texas, School of Public Health.

An essential issue for modeling genetic networks is how to model regulation of a gene. The genes are key components of the genetic networks. To address importance of the model of regulation of a single gene, in this report we propose two stage state-space models for genetic networks. Specifically, state-space models for genetic networks are decomposed into two parts. One part is to model its intrinsic regulation within the gene in which state space equations with two unobserved state variables are used to model a transcriptional process of the gene. Second part is to model the regulations between genes in which observed expressions of the other connected genes will be inputted to the state equations to regulate the expression of the gene. The extended Kalman filter is used to estimate the parameters in the models. However, the classical extended Kalman filter does not consider constraints due to the structure of the networks. To incorporate the structure of the networks into identification of the genetic networks we develop a new version of extended Kalman filter in which the constraints of the network structure are imposed in the parameter estimation. The proposed two-stage state-space models with constrained extended Kalman filter are applied to three published gene expression time course datasets. Our preliminary results show that the propose models and algorithms have much accurate precision in prediction of the gene expressions than the traditional methods.

2679/W

Global Analysis of Four Neural Tube Defect LongSAGE Libraries to Identify Anencephaly and Spina Bifida Candidate Genes. *A. Dellinger[1], S. Thomas[2], P.-T. Xu[1], H. Etchevers[2], M. Vekemans[2], J.R. Gilbert[3], M.C. Speer[1].* 1) Center of Human Genetics, Duke University Medical Center, Durham, NC; 2) INSERM U781, Hôpital Necker- Enfants Malades, Paris, France; 3) Miami Institute for Human Genomics, University of Miami School of Medicine, Miami, FL.

Neural tube defects (NTDs) are complex birth defects including anencephaly and spina bifida. Genetic contribution to NTD risk is well-established, yet major genes have not been identified. Evaluation of differential expression by LongSAGE (Serial Analysis of Gene Expression) can identify candidate genes. We made 4 LongSAGE libraries from caudal (CAU) and rostral (ROS) ends of normal microdissected human neural tube tissue at closure (Carnegie Stage 12) and post-closure (Carnegie Stage 13). (See Xu et al., this meeting). We hypothesize gene expression differences in library comparisons between ROS12 and ROS13 may identify candidate anencephaly risk genes; between CAU12 and CAU13 may identify candidate spina bifida risk genes; and between CAU and ROS may identify genes influencing the development of anencephaly vs. spina bifida.

P-values for tags in comparisons between stages and between CAU and ROS were computed using χ^2 or Fisher exact tests. 3294 genes had $p < .05$. Eliminating tags mapped to multiple genes left 2061 genes, with more genes in comparisons between stages (885 and 1086) than between CAU and ROS (70 and 293). To identify subsets of candidate risk genes we used genomic convergence, incorporating the 2061 genes with: linkage analysis in NTD families (236 genes); NTD pathway analysis (folic acid, retinol, Wnt signaling, cytokines) using KEGG, GO, and interaction databases (94 genes); and genes from known NTD mouse models (41 genes). CAU12 vs CAU13 has more pathway and linkage genes than ROS12 vs ROS13, including folate pathway genes DHFR (up in 13), FOLR1 (down), TYMS (up), and RBP1 (up). Folate and retinol pathway genes indicate a decrease in folic and retinoic acid levels, respectively, from stage 12 to stage 13. These data provide a rich source for the identification of lesion-specific candidate genes for neural tube defects.

2680/W

The effect of DNA extraction method, source (blood vs. archival tissue) and concentration on allele call rate in a candidate-gene, association study using the Illumina Infinium platform. *M. de Andrade[1], J.M. Cunningham[2], T. Petterson[1], J. Larson[1], J.A. Heit[3].* 1) Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 2) Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN; 3) Cardiovascular Diseases, Mayo Clinic College of Medicine, Rochester, MN.

iSelect, the new custom Illumina Infinium application, allows genotyping of 7.6K-60K SNPs in a single BeadArray. This will be employed for a candidate gene association study for venous thromboembolism (VTE) at the Mayo Clinic, consisting of 1500 clinic-based, objectively diagnosed VTE cases and 1500 age, sex and residence area matched controls. We selected 16,700 SNPs for 780 genes in pathways relevant to the pathogenesis of VTE using a haplotype tagging algorithm that incorporated Illumina's design score for iSelect. Prior to running the entire set of DNA samples, we wished to evaluate the effect of DNA from different sources on the performance of the BeadArray, in part to guide us in selection of suitable samples to include in the study. We first genotyped recently isolated high-quality DNA to generate a cluster algorithm. These DNAs were 88 Olmsted County controls with no VTE at the time of blood collection, and with DNA concentration greater than 51 ng/μL. The second used another 88 Olmsted County controls to investigate whether DNA extraction method, here PureGene (50%) and AGTC (50%), may affect the allele call. DNA extraction method was randomly assigned, using 6 of each per Illumina chip. The third was to investigate whether DNA concentration from lymphoblastoid cells and archive tissues may affect the allele call using 88 VTE cases that have either DNA extracted from archival tissue or lymphoblastoid cells. Controls include 2% samples replicates and a CEPH trio. These experiments are currently underway and we will report on the overall performance of this iSelect BeadArray and the effects of case status, age at blood draw, DNA extraction methods, type of DNA sample and DNA concentration on data quality.

2681/W

Optimization of whole genome amplification from FTA cards for genetic epidemiology studies. *I. Dimulescu, A.K. Smith, E.R. Unger, S.D. Vernon, M.S. Rajeevan.* Centers for Disease Control, Atlanta, GA.

Sample collection, processing, extraction and amplification of nucleic acids are key elements in bio-banking and conducting large-scale, multi-center genetic studies of public health importance. While blood dried on FTA cards offers a number of advantages, current methods recover low amounts of DNA which limits the number of genetic markers that can be tested. In this study, we evaluate the ability of different whole-genome amplification (WGA) protocols to generate a representative and renewable source of DNA from FTA cards. Peripheral blood was obtained from 33 anonymous volunteers in the CDC blood bank. Reference genomic DNA was extracted from whole blood or from PBMCs of each subject. Each sample was also used to prepare replicate FTA cards. Dried blood spots were extracted using the FTA, GenVault, and Qiagen lysate protocols, or used directly in the WGA. Two different WGA protocols were evaluated: Qiagen's REPLI-g kit based on phi 29 DNA polymerase mediated isothermal amplification and Sigma's GenomePlex kit based upon random fragmentation of the genome and amplification by PCR. A total of 80 single nucleotide polymorphisms were genotyped using either Applied Biosystems TaqMan allelic discrimination assay or the Sequenom iPLEX Gold assay. Performance of the WGA assays on each template source was determined based on the call rate and the concordance of genotype calls compared to gDNA. Based on the initial 130 genotypes determined by TaqMan, DNA extracted using FTA and GenVault protocols and amplified with REPLI-g had a call rate of 100% with concordance rates of 94.6% and 100%, respectively. In another set of 1180 genotypes derived from the iPLEX platform, DNA extracted from FTA lysate and amplified with REPLI-g resulted in 97.8% call rate and 99.9% concordance. DNA amplified with GenomePlex directly from FTA paper discs generated the highest call rate, 99.0% and were 100% concordant. Preparation of DNA template from stored FTA paper and selection of WGA protocol can influence call rate and allele drop-out. Studies are in progress to validate these pilot study results with FTA samples collected as part of a population-based genetic epidemiology study.

2682/W

Cloning of chromosome 3 inversion breakpoints in a 3-generation family with short stature reveal multiple repetitive elements. *U. Dutta, F. Matthes, I. Hansmann, D. Schlote.* Inst Human Gen & Med Biol, Halle/Saale, Germany.

Chromosomal rearrangements are often associated with a specific phenotype and they are a significant cause of human disorders. Mapping of breakpoints is a powerful tool for identification of such disease genes. Here we report a case of short stature in a girl with a karyotype of 46, XX,inv(3)(p24.1q26.1). Cytogenetic analysis had revealed a familial pericentric inversion 3, being heterozygous in the proband, her mother and grand mother, both of them also with short stature. In order to characterize the breakpoint physically, FISH analysis with large YAC and BAC clones were performed. Four p specific YACs and six BAC clones were used as probes for FISH. YAC clone CEPHy904H0787 (1090 kb) gave a split signal on the metaphase chromosomes of the proband. The split signal indicates that the target sequence carries the inversion breakpoint. Two BAC clones RP11666G20 and CTD2007B5 were identified spanning the breakpoint region, assigning the breakpoint to 3p24.1 and thus narrowed down the breakpoint region to 97.5 kb. Out of the 15 YACs and 10 BACs selected on the q arm, YAC CEPHy904G07889 (1610 kb) and BAC clone RP11-12N13 showed a split signal assigning the breakpoint to chromosomal band 3q26.1. Using sub cloned fragments of these BACs as well as Long range PCR products as probes the breakpoints were now located within a region of 3 kb and 5.3 kb on p and q respectively. Analysis of the genomic sequence surrounding the inversion breakpoints revealed 30% repetitive nature of the DNA containing LTR33A, MER67C, L2 and MER67D elements on p region and LTR16C, MER20, MLT2B1, LTR1B ,simple repeats and low copy repeats on q region. We determine that the breakpoints occurred between these repetitive regions. The presence of these repetitive elements, especially MER and LTR elements at the junction of the breakpoints suggest that the inversion may be the result of these repetitive elements. Our findings will help to provide a better understanding of the molecular mechanism underlining the spontaneous chromosome rearrangements in the human genome.

2683/W

Integrated genotyping of SNPs and copy number variation using Affymetrix array technology. *J.M. Korn[1,2,3,4], F. Kuruvilla[3,4], A. Wysoker[3], E. Hubbell[5], S. Cawley[5], S.A. McCarroll[3,4], M.J. Daly[3,4], D. Altshuler[3,4].* 1) Biophysics, Harvard University, Boston, MA; 2) The Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 3) Broad Institute of MIT and Harvard, Cambridge, MA; 4) Massachusetts General Hospital, Boston, MA; 5) Affymetrix, Inc., Santa Clara, CA.

Typically, whole genome association studies have treated SNPs as simple biallelic systems. However, a significant percentage of the genome is present at variable copy number, creating myriad possibilities beyond the canonical genotypes of AA, AB, and BB at SNPs in these regions, and impressing the importance of analyzing copy number variation alongside SNPs in studies of genetic variability. Joint analysis allows for high-resolution mapping of CNVs, unbiased estimates of SNP allele frequency, and clarification of chromosomal abnormalities ambiguous from SNP genotypes alone. The resulting SNP-CNV map is valuable for both case-control association and pedigree-based studies. We present two linked algorithms for integrated SNP genotyping and copy number analysis: Birdseed, a 2D Gaussian Mixture Model for SNP genotyping, and a novel HMM to detect copy number that seamlessly integrates both the SNP and non-polymoprhic probes on Affymetrix's SNP 6.0 array. Several customizations exploiting common features of SNPs ensure highly accurate genotyping. Birdseed achieves a 99.6% call rate and 99.7% concordance for the 270 HapMap samples. Imputation of copy-normal and copy-variant clusters not fit by the model allows for recovery of rare genotypes that can otherwise be lost. Imputed 1-copy cluster means were validated on chrX, and are accurate with 5% error. An in-silico gender mixing experiment simulated heterozygous deletions of varying sizes. For deletions spanning 3, 5, and 10 probes (corresponding to 5kb, 8kb, and 17kb on average) we discover 39%, 76%, and 84% of the events with 1 probe tolerance. Allowing a breakpoint error of 5 probes, we recover 96% of deletions spanning at least 10 probes. Together, these algorithms allow for accurate genotyping of SNPs both within and outside CNVs, and high resolution detection of deletions and duplications.

2684/W

Comparative analysis of chromosome 21 subtelomeric regions between human and chimpanzee. *Y. Kuroki[1], A. Toyoda[2], S. Tatsumoto[2], T.D Taylor[3], A. Fujiyama[2, 4], Y. Sakaki[1, 2, 3].* 1) Comparative Systems Biology Team, RIKEN Genomic Sci Ctr, Yokohama, Japan; 2) Sequence Technology Team, RIKEN Genomic Sci Ctr, Yokohama, Japan; 3) Genome Annotation and Comparative Analysis Team, RIKEN Genomic Sci Ctr, Yokohama, Japan; 4) National Institute of Informatics, Chiyoda-ku, Tokyo, Japan.

Subtelomeric regions are known as one of the most complicated regions in the human genome because of the interchromosomal segmental duplications and the existence of various repetitive sequences. Recent reports have shown complex patchwork of sequence blocks, human-genome specific segmental duplications, and higher frequency of gene transfer among the subtelomeres. Interestingly, some of these sequence blocks in the human subtelomeres are also found in the subtelomeres of other apes, chimpanzee or orangutan, for example. It means that the most part of the subtelomeric regions were duplicated in species-specific manner, although some sequence blocks were originated from common ancestral genome regions. To identify the origin and the evolutionary processes of the genome duplication in the subtelomeres, we isolated clones covering completely the chimpanzee chromosome 21 subtelomeric region. Comparative analysis of the high quality sequences derived from the subtelomeric regions of human and chimpanzee chromosome 21 revealed that the chimpanzee genome has longer subtelomeric region than the corresponding human subtelomere. The expanded fragment in the chimpanzee subtelomere was also located in other regions mainly in subtelomeres.

2685/W

AsiDesigner: siRNA design server considering alternative splicing. *Y.J. Kim[1], Y.K. Park[1], S.M. Park[1], Y.C. Choi[2].* 1) Medical Genomics Research Ctr, KRIBB, Daejeon, Korea; 2) 49-3 Moonpyung-dong, Daedok-gu, Bioneer, Co., Daejeon, Korea.

AsiDesigner is a web based siRNA design software system providing siRNA design capability to take into account alter-native splicing for mRNA level gene silencing. We developed a novel algorithm to retrieve a target region so that siRNAs can be designed dependent upon whether a user needed a specific mRNA isoform or combined mRNA isoforms from a target gene. The algorithm incorporates 1) selecting a target region; 2) using genome mapped results of all the isoforms; 3) extracting target sequences dependent upon selected isoforms. The developed algorithm and the AsiDesigner were tested and confirmed as very effective through-out many gene silencing experiments. It is expected that this exon-based siRNA design algorithm will play an important role in functional genomics, drug discovery, and other molecular biology studies.

2686/W

Discovery and genotyping of insertion/deletion variants from whole-genome SNP assay data. *T. Zerr[1], G.M. Cooper[1], J.D. Smith[1], E. Tuzun[1], J. Kidd[1], M.J. Rieder[1], E.E. Eichler[1,2], D.A. Nickerson[1].* 1) Department of Genome Sciences, University of Washington School of Medicine, Seattle WA; 2) Howard Hughes Medical Institute, Seattle, WA.

The potential phenotypic effects of common indel polymorphisms remain largely unexplored due to an inability to systematically and accurately genotype such variants. Whole-genome association studies have produced a wealth of data which should in principle allow such analyses; however, segmentation algorithms previously applied to quantitative whole-genome SNP data lack power to detect deletion variants spanning small numbers of probes, despite the fact that common deletions tend to be small in size. We have developed a computational approach to genotype biallelic insertion/deletion polymorphisms. Our algorithm uses mixture likelihood based classification to infer insertion/deletion genotypes from any number of SNP probes, and is capable of both ab initio deletion detection and directed deletion genotyping. We tested our approach by analyzing publicly available Illumina Infinium II SNP assay data from a panel of 120 HapMap samples. Inference of gender from X-linked marker data indicate that our algorithm can produce accurate insertion/deletion genotypes from as few as 60 samples, with as few as two probes within the variant region; in 6910 adjacent X-linked probe pairs, in samples of 54 females and 6 males (simulating a deletion allele frequency of 5%), males were correctly classified with a frequency of 98.3%, and females were correctly classified with a frequency of 99.1%. We were also able to validate our ab initio deletion detection using fosmid-end sequence data for three of the 120 samples. At least 33% of strongly scoring deletion allele predictions were supported by fosmid end sequence mapping, with 20% being supported for more weakly scoring sites. True validation rates are likely to be higher given the limited resolution of deletion detection via fosmid-end sequence mapping; we are sequencing a panel of our deletion predictions to confirm this hypothesis. We anticipate that our approach will facilitate analysis of common deletion polymorphisms in human genotype-phenotype studies.

2687/W

Proteomic Analysis of Retinoic Acid-induced Clubfoot-like Deformity in Rat Fetuses. *Z.G. Li[1,2], W.N. Fu[1], H. Ji[1], K.L. Sun[1].* 1) Medical Genetics, China Medical Genetics, Shenyang, Liaoning, China; 2) National Research Center of the New Drug Evaluation, Shenyang, 110021, P. R. China.

he etiology of idiopathic talipes equinovarus (ITEV) (clubfoot) is considered to be complex. Here we explore the expressions of clubfoot-related proteins and the change of the apoptosis rate in the clubfoot-like deformity model in rat fetuses induced by all-trans retinoic acid (ATRA). Clubfoot-like deformity model in rat fetuses were induced with ATRA (135mg/kg) in E10 pregnant Wister rats. Two-dimensional gel electrophoresis (2-DE) was applied to separate the total proteins of spinal cord, tibia-fibulae musculature, ankle joint tissue and ankle joint bone of the animal models. The Coomassie Brilliant Blue staining gels were analyzed by 2-DE software PDQuest 7.1.0. Selected differential protein spots were identified with peptide mass fingerprinting based on matrix-assisted laser adsorption/ionization time-of-flight mass spectrometry and database searching. XIAP, TNNT1 and Col2α1, three of the differential proteins, were identified furthermore. Apoptosis study was performed in terminal deoxynucleotidyl transferase nick end labeling. 23 protein spots were identified to be differential expressed in the clubfoot-like deformity model. In addition, three genes of XIAP, TNNT1 and Col2α1 were confirmed to be significantly down-regulated by the RT-PCR, and XIAP was further confirmed to be significantly down-regulated with immunohistochemistry. In ATRA-induced clubfoot-like deformity in rat fetuses, the rates of the apoptosis in the spinal, vertebra and muscle of the clubfoot-like deformity fetuses was 5.4, 10 and 3.7 times of those in the normal fetuses. The result suggests that certain differentially expressed proteins, such as XIAP and TNNT1, and Col2α1 showed a significant correlation with ITEV. Apoptosis plays a key role in the development of ITEV. The identification of protein alterations specify to ITEV would clarify the pathogenetic mechanisms involved in the disease and might be of prognostic and therapeutic benefit.

2688/W

Enhanced D-HaploDB: definitive haplotypes and extended haplotype information determined by genotyping complete hydatidiform mole samples. *K. Higasa, Y. Kukita, K. Miyatake, T. Tahira, K. Hayashi.* Res Ctr Gen Info, Kyushu Univ/Med Inst Bioreg, Fukuoka, Japan.

The Definitive Haplotype Database (D-HaploDB) is a web-accessible resource of genome-wide definitive haplotypes determined from a collection of Japanese complete hydatidiform moles (CHM), each of which carries a genome derived from a single sperm. Here, we strengthened the database by genotyping an additional SNP set (Affymetrix 500K) and some results of population genetical analyses were implemented. We particularly focused on extended haplotypes, because those common in a population may be the evidence of recent positive selection. In HapMap project, African and European samples were from trios, and the reconstructed haploytpes are highly accurate. However, the East Asian samples were from arbitrarily collected individuals, and haplotypes inferred from relatively small samples may contain significant error, when the data is used in error-sensitive analyses such as extended haplotype homozygosity (EHH) mapping. To assess the impact of this for detecting selected region, we compared some statistics. First, the integrated EHH (iHH) score was greater than those of HapMap. Second, unstandardized integrated haplotype score (iHS) showed more extreme values than HapMap, which means HapMap data is more likely to be neutralized. Third, the correlation (r) of iHS value between CHM and JPT was 0.79, which suggest that selected alleles are likely to show more outlier values in our data. Lastly, the comparison of length distribution of extended haplotypes of our haplotypes with those of HapMap showed serious fragmentation of long haplotypes in HapMap, and some consanguinity in three of the four HapMap populations. Taken together, selected haplotypes were more extended than previously reported. These haplotypes identified by D-HaploDB can be thought of as promising selection candidates and strongly complement the results from HapMap data. The D-HaploDB is freely accessible via the internet at http://finch.gen.kyushu-u.ac.jp.

2689/W

Initial investigations in genetical genomics. *R.M. Cantor-Chiu[1], B. Kerner[2].* 1) Human Genetics, UCLA School of Medicine, Los Angeles, CA; 2) Psychiatry, UCLA School of Medicine, Los Angeles, CA.

Genetical genomics is an emerging field of investigation focused on analyzing genetic variations, such as SNPs, to better understand genomic phenomena, such as gene expression levels. Sources of variation in gene expression are not well understood. We conducted analyses, as part of Genetic Analysis Workshop 15, to begin to identify and quantify these sources and assess their influence on regulation by cis acting elements. A variance components analysis of the expression levels of 65 genes in 14 CEPH pedigrees revealed that familiality (heritability plus common family environment) of gene specific expression varies considerably. The positively skewed familiality distribution ranged between 0 and .61 with a median of .23 and a mode of .15, indicating that environmental factors may play an important role in the expression levels of a large number of genes. To explore the process of mapping cis regulatory regions using SNP genotypes, we conducted quantitative trait linkage (QTL) analyses of these expression levels. For DDX17 at 43.4 Mb on 22p13, which had the highest estimate of famiality in this sample (.60), its mean expression levels varied widely among the families (7.2-8.6) and these family differences explained .25 of the variance of DDX17. Single point QTL variance components linkage analyses of DDX17 expression, found 6 SNPs (between 32.8 and 46.6 Mb) out of 57 along the entire chromosome linked with LODs > 3.0. Alleles of these SNPs explain .09 of the variance in expression. Stepwise regression was conducted to narrow this potential cis regulatory region, and 2 SNPs (rs80533 at 39.4 Mb, p<.003 and rs760482 at 37.7 Mb, p<.046) were retained. In multiple regression analyses of DDX17 expression that included factors for family and SNP genotype, family and its interaction with SNP genotype were significant for each SNP, although allelic differences were not. We conclude from these initial studies that gene expression is genetically complex, with multiple genetic and environmental contributions. As with other complex phenotypic traits, these factors are likely to complicate the detection of regulatory elements in genetical genomics investigations.

2690/W

Purine/Pyrimidine Motif Differences in Recombination Hotspots and Coldspots. *J. Cai[1], P.R. Calkins[2], J.C. Cohen[3], A.F. Wilson[1].* 1) Genometrics Section, NHGRI, NIH, Baltimore, MD; 2) Dept of Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 3) Dept of Pediatrics, Stony Brook University Health Science Center, School of Medicine, Stony Brook, NY.

During the past few years, it has become apparent that the locations of recombination events are clustered in a small proportion of the human genome. Recombination hotspots are regions of one or two thousand base pairs of DNA where the recombination rate is significantly higher than elsewhere in the genome. Hotspots are often flanked by coldspots, regions of lower than average frequency of recombination. With the identification of a large set of hotspots in the human genome, the frequency and distribution of specific sequence motifs can be compared in hotspot and coldspot regions. To date, relatively few sequence motifs have been associated with hotspots. The frequency of every 7bp motif was compared in hotspot relative to coldspot regions, and paired t-tests were calculated; however, based on previous unpublished work, sequence composition was considered at the purine/pyrimidine level (RY) rather than at the ACGT nucleotide level. Of all possible 7-mers at the purine/pyrimidine level, 0.86 fewer copies of the RYYRRYR/YRYYRRY motifs were found in the hotspots relative to the coldspots ($p < 1.37x10^{-23}$), while the RRRRRRR/YYYYYYY motifs were more frequent in the hotspots than in the coldspots (1.82 copies, $p < 1.83x10^{-30}$). When the regions flanking hotspots were compared to the regions flanking the coldspots, the differences between the frequencies of both motifs in hotspots relative to coldspots approached zero as the distance from the hotspot/coldspot boundary increased. Permutation tests, where motif frequencies in hotspot or coldspot regions were compared to those of size-matched random sequences from the genome, confirmed that the RYYRRYR/YRYYRRY motifs were less frequent in hotspots while the RRRRRRR/YYYYYYY motifs were more frequent in coldspots. Studying the composition of recombination hotspots may help elucidate the factors that affect recombination and understand the molecular mechanism and regulation of crossover events as well as the evolutionary forces affecting recombination.

2691/W

A Novel Sequence-Based Typing Method for Identification of Killer Immunoglobulin-Like Receptor (KIR) Genes and Alleles to Identify Their Associations with HIV-1 Infection. *R. Hardie[1], T.B. Ball[1], M. Luo[1], D. La[2], J. Kimani[3], C. Wachihi[4], E. Ngugi[4], F.A. Plummer[1,2,3].* 1) Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada; 2) National Microbiology Laboratory, Winnipeg, MB, Canada; 3) Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya; 4) Department of Community Health, University of Nairobi, Nairobi, Kenya.

Host genetics are important in HIV-1 infection and disease progression, but the role of natural killer (NK) cell receptors has not been studied comprehensively. NK cells act in the innate immune response against viruses by producing cytokines/inducing cytotoxicity. KIRs are NK receptors with HLA class I as their ligands, which activate or inhibit NK function. KIR-encoding genes have evolved and are polymorphic, suggesting a role for KIRs in infectious disease pathogenesis. We hypothesize that KIRs play a role in susceptibility to HIV-1 in highly-exposed but uninfected women in a sex worker cohort from Nairobi, Kenya. To genotype these KIRs, PCR primers were designed in introns flanking the most polymorphic typing exons of KIR 2DL2/2DL3 and 3DL1/3DS1. DNA was isolated from 1154 women defined as HIV resistant/susceptible, with varying disease progression within the susceptible individuals, genes were amplified, then sequenced with nested sequencing primers. Taxonomy-based sequence genotyping software CodonExpressTM was used to genotype KIR genes. Of thoses typed, 10% had only 2DL2 genes and alleles, 35.3% had both 2DL2/2DL3 and 3DL1/3DS1, 46.3% had only 2DL3 and 7.3% had no sequence. About 10% of sequences did not match currently identified alleles, which are potentially new alleles. Next, SPSS 13.0 will be used for correlation and Kaplan-Meier analysis of seroconversion and progression. In conclusion, this method is superior to sequence-specific PCR methods used by others because it is rapid, requires less template, requires fewer reactions, is able to co-amplify two genes and all alleles of each gene simultaneously, is highly specific and has the potential to identify new alleles. This novel technique will facilitate large-scale associations analysis of KIR genotypes and susceptibility to HIV and other infectious agents.

2692/W

Multiplexed genotyping using a novel digitally inscribed bead-based system. *J. Yeakley[1], E. Chao[2], J. Velasquez[2], M. Lopez[2], T. McDaniel[1], I. Lewis[1], H. Chen[1], S. Oeser[1], R. Smith[1], M. Graige[1], S. Barnard[1], J. Sirkis[1], J. Moon[1], S. Lipkin[2].* 1) Research & Development, Illumina, Inc., San Diego, CA; 2) Dept. of Medicine, University of California Irvine, Irvine, CA.

Genotyping of clinical samples has been limited to low levels of multiplexing, ranging from one to a few dozen single nucleotide polymorphisms (SNPs) per sample. By increasing multiplexing levels, a clinical lab can increase information content per sample, decreasing costs and sample material requirements. We have adapted the GoldenGate® Assay for simultaneously genotyping 96 to 1536 SNPs to the BeadXpress System, a new high-throughput platform that utilizes microbeads with unique, digitally inscribed holographic codes. Genotyping on this platform ranges from 96 to 384 multiplexing, using the same GoldenGate Assay that has proven highly robust for millions of genotypes. In preliminary tests, we have observed greater than 99% call rates, and greater than 99.5% rates for reproducibility and heritability. In a test of 96 SNP genotypes chosen for a study of colorectal cancer, a point mutation in the MSH2 gene, previously implicated in predisposition to several cancers, was correctly genotyped when compared to qPCR analysis of the same samples. Together with genotyping data from reference samples, the GoldenGate Assay on the BeadXpress System has yielded highly reproducible and accurate genotypes, suggesting that this approach will prove useful for rapid refinement of SNPs for development of clinical genotyping tests.

2693/W

Genomewide Association Studies: Performance of genomic and WGA DNA sources on Illumina® GWA arrays. *K. Hetrick, C. Bark, J. Gearhart, E. Kwasnik, M. Zilka, C. Ongaco, Y. Tsai, J. Romm, E. Pugh, K. Doheny.* Center for Inherited Disease Research (CIDR) and Genetic Resources Core Facility (GRCF) SNP Center, IGM, JHUSOM, Baltimore, MD.

CIDR is a centralized facility established to provide genotyping and statistical genetics services for investigators seeking to identify genes that contribute to human disease. Currently we offer GWA SNP genotyping services using Illumina Infinium® products. Optimal data quality is obtained from lymphoblast cell line or peripheral blood DNA sources; however, many studies have a limited amount of blood-derived DNA, or only have DNA from other sources. We have conducted a few small-scale controlled experiments exploring the impact of DNA source on data quality and compiled retrospective empiric performance data for all samples run on Illumina Infinium II GWA arrays that we have analyzed to date. Genomic DNA derived from blood samples results; 98.7% (5,463 out of 5,534) sample completion rate (sample call rate > 96.5%) after one assay attempt per sample (cut-off set by ≤ 0.1% error rate after two assay attempts); average call rate of successful samples, 99.7%. 35.2% (25 out of 71) of the 'failed' blood samples appear to contain multiple genomes when reviewing theta value distributions across the genome. In a completed study of 2,563 blood samples, 61.1% (11 out of 18) of the samples below a 96.5% call rate were recovered after re-attempting them. WGA samples (Amersham Biotech, conc. 100 ng/ul) derived from blood DNA results; 91.7% (143 out of 156) sample completion rate (sample call rate > 90%) after clustering the WGA samples with blood samples (cut-off set by call rate distribution); average call rate of successful WGA samples, 97.9%. In a controlled experiment using the HumanHap550 BeadChip, paired WGA/genomic samples were analyzed using a SNP cluster definition derived from a study where both blood and WGA samples were analyzed together. 7 WGA (QIAGEN, conc. 100-200ng/ul)/blood pairs (a trio and a tetrad) results; average call rate, 98.94%; reproducibility between pairs, 99.99%; Mendelian heritability, 99.96%. Analysis of WGA samples derived from other sources is ongoing.

2694/W

Multiplex SNP typing method: DigiTag2. *N. Nishida[1], T. Tanabe[2], M. Takasu[1], A. Suyama[3], K. Tokunaga[1].* 1) Dept Human Genetics, Univ Tokyo, Tokyo, Japan; 2) Bio Business Division, Olympus Corporation, Tokyo, Japan; 3) Dept Life Sciences, Univ Tokyo, Tokyo, Japan.

DigiTag2 assay performs multiplex SNP typing by encoding all of the SNP genotypes to the well-designed oligonucleotides, named DNA coded numbers (DCNs). The assignment of the DCNs to the target SNPs is unconstrained, therefore, the DNA chips prepared to read out the types of DCNs are universally available for any types of SNPs. And, the DigiTag2 assay uses non-labeled primers and probes, which lead to save the cost of the assay. We investigated the feasibility of the DigiTag2 assay by genotyping 96 SNPs, located in the 610 kb genomic region including IL-4 and IL-13 genes, using 936 individual genomic DNA samples. The conversion rate, which is defined by the proportion of successfully genotyped SNPs in the total number of SNPs examined, was revealed to be over 90%, and the typing result was 100% identical to the result from direct sequencing. To verify the applicability of DigiTag2 assay, we genotyped 19 SNPs that were miss-genotyped by the other SNP typing method. Sixteen of 19 SNPs were revealed to be successfully genotyped by the DigiTag2 assay. We estimated the running cost for the DigiTag2 assay (for oligonucleotides, reagents, DNA microarrays, etc.) is less than $0.06/genotype. The DigiTag2 assay can use the same set of DCNs for any set of target SNPs, thereby enabling 96-pelx genotyping with the same assay protocols and the same DNA chip having the same set of probes. We expect that the DigiTag2 assay will be used for high-resolution mapping of primary genes after genome-wide search for disease susceptibility regions.

2695/W

Applications of Next-Generation Sequencing in Genetic Epidemiology. *F.M. De La Vega[1], J. Sorenson[1], F. Hyland[1], K. McKernan[1], W. Kim[2], S.J. Finch[2], D. Gordon[3].* 1) Applied Biosystems, Foster City, CA; 2) Stony Brook University, Stony Brook NY; 3) Rutgers University, Piscataway, NJ.

An important application for next-generation sequencing (NGS) is the deep resequencing of targeted regions and whole genomes for the discovery of a range of sequence variants including SNPs, rare mutations, indels, copy number, and large scale genomic rearrangements. Ultimately one goal is to provide "genometypes" of individual patient samples for identification of disease susceptibility alleles. In such experiments, a shotgun sequencing of template DNA is performed, where reads are derived from clonal fragments and genotypes are derived by counting. We sought to understand the relationships between number and length of reads and per-base sequencing error to the overall genotyping accuracy through simulations and through experimental results from the Applied Biosystems SOLiD system. Since NGS platforms typically produce short reads (25-35bp), coverage needs to increase to 15-20X to reduce heterozygous misclassification errors to acceptable rates, whereas homozygote calling requires less coverage (10-15X). Error rate significantly influences coverage requirements highlighting its importance in genometyping. Due to shotgun coverage some missing data will persist, suggesting a role for statistical imputation methods. A critical question in association studies is the power to detect genetic association with a fixed sample size. Recent work has focused on two-stage designs, where subsets of individuals and markers are typed each in screening and replication stages. A typical assumption is that the disease variant is contained in the screening SNP panel. Here, we examined situations where the disease variant is initially not typed (as is more typical), but can be discovered and simultaneously typed by NGS of the leading candidate regions in cases and controls. We discover that while significant power loss may occur when the disease variant is absent from the screening phase, power can be recovered by full SNP ascertainment by resequencing. These results suggest that NGS could become a valuable tool in the identification of susceptibility genes for complex disease.

2696/W

Methods for comparative sequence analysis using mass spectrometry. *C. Honisch, Y. Chen, T. Shi, D. van den Boom.* Molecular Applications, Sequenom, Inc, San Diego, CA.

Mass spectrometry has become a standard tool for automatic identification of proteins and mapping of proteomes using peptide mass fingerprinting and pattern matching against established databases. We recently developed new algorithms and software tools for nucleic acid mass fingerprinting that enable automated, high-throughput comparative sequence analysis by MALDI-TOF mass spectrometry including the detection of single nucleotide changes. Large-scale genome DNA-sequencing projects provide a rapidly increasing number of reference sequences for the approach. As part of our comparative sequencing method, reference patterns are simulated based on the imported sets of reference sequences and mass spectra are acquired after PCR, in vitro transcription and base specific-cleavage of 500-800 bp genomic regions. In theory, samples can be identified by finding the best match of the detected peak pattern with the simulated pattern of the references, but missing and additional peaks due to deviations between the sample and the best match, contaminating adduct peaks, intensity variations and the overall spectra quality required the implementation of an iterative identification process and the development of a quality scoring scheme. Using these new algorithmic approaches, we developed analysis routines reporting on sequence identification results, confidence levels and single nucleotide changes. Normalization and scaling of acquired data allow for cluster analysis and grouping of samples based on their specific patterns. Sample mixtures are reflected in the data and can be analyzed. We will present data on the successful application of the algorithms to comparative sequence analysis for mutation detection, haplotyping, mixed population analysis and microbial typing.

.

2697/W

Genomic Initiative at the Javeriana University: Human genetics and biological diversity, the necessity of Metagenomics. *J. Bernal, F. Suarez, A. Ordoñez, I. Zarante.* Inst Genetica Humana, Univ Javeriana, Bogota, Colombia.

The Colombian Genomics Platform project integrates the strengths of six Colombian well-known research groups with experience in Environmental Metagenomics and Human Genomics of Mendelian and multifactorial diseases. Colombia is considered to be the second country in diversity in the planet. Its area, which is less than 1% of the planet, includes 10% of the world biological diversity of the world and 50% of the existing plants. Colombia is actually in a full process of epidemiological transition and the birth defects are becoming the principal cause of mortality in early childhood, at the same time multifactorial diseases like cancer or cardiovascular diseases are increasing its prevalence. Objectives: To establish a Genomic platform in Colombia in order to promote and make easier the development of projects in different research groups that aim to describe and use biodiversity and genetic resources of our country. To determine the interaction, between genes and environment, in the clinical and non-clinical aspects. To develop clear policies related to the use of genetic resources in legal and economic aspects and develop a group of bioethical aspects related to Metagenomics in a biodiverse country. Methods: integration of research groups under a unique genomics work platform under the direction of the Instituto de Genética Humana at the Pontificia Universidad Javeriana. Results: the different research projects of Human Genomics, e.g. SNPs Mapping: The codifying regions of the following genes will be sequenced and regulated: L1CAM for hydrocephaly, CRELD1, GATA4, ACVR2B, GJA1 and JAG1 for heart congenial malformations, MTR, y MTRR for neural tube disorders and IRF6 for cleft lip and palate; Environmental Genomics e.g. analysis of Metagenomics in a highly intensive shrimp farming system and characterize the bacterial populations by means of 16S rRNA analysis, bioinformatics and bioethics are further described and discussed.

2698/W

Identification of an exon 15 duplication in BRCA1 in a familial ovarian cancer patient using an improved method: upQMPSF. *S. Azrak[1], R. DiCioccio[1], K. Rodabaugh[2], P. Liang[1].* 1) Dept Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) Dept Gynecologic Oncology, Roswell Park Cancer Inst, Buffalo, NY.

Genetic mutations such as SNPs and small deletions/insertions do not explain all genetic causes for cancer. Recent studies have demonstrated that other types of genetic changes are also responsible for cancer etiology. Among these are the large genomic rearrangements existing as deletions or duplications involving one or more exons of a gene. This type of aberration is believed to be more frequent than currently known due to the limited availability of superior methodologies for their detection. We have, therefore, developed an improved version of Quantitative Multiplex PCR Short fluorescent Fragment (QMPSF), called Universal Primer QMPSF (upQMPSF). The improvements include replacing the individually labeled fluorescent primers by one universal fluorescent primer and maximizing the multiplex PCR capacity to allow up to 15 products per set. For example, we covered all exons of BRCA1 using only two sets. Furthermore, the primers were designed to cover the entire exon regions, such that the method can also be used to detect small deletions and insertions in the exons. These improvements dramatically improve the cost efficiency, making it possible to use the method for the first round screening of genomic rearrangements, as well as small insertions/deletions. Using upQMPSF, we screened 88 familial ovarian cancer patient samples, which had previously tested negative for point mutation and small deletions/insertion in BRCA1, for large genomic rearrangements. We detected a novel 3 kb duplication spanning exon 15 of BRCA1, which is predicted to generate a truncated protein. The result was verified using MLPA and Mutation-specific Multiplex PCR (MM-PCR) and the breakpoints of the duplication were fully characterized at the sequencing level. Validation of several additional genomic rearrangement cases are in progress. Our results validate the utilities of upQMPSF and important contribution of genomic rearrangements to cancer susceptibility. This research is supported by grants from NCI (CA16056 and CA101515) and from Roswell Alliance Foundation and Gynecological Cancer Foundation.

2699/W

New process using next generation sequencing for the fine mapping of variations in genomic DNA regions identified by whole genome association studies. *P. Bouffard[1], M. Yeager[2,3], R. Welch[2,3], Z. Markovic[1], B. Desany[1], T.P. Jarvie[1], T.T. Harkins[5], S.J. Chanock[3,4].* 1) 454 Life Sciences, Branford, CT; 2) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick, Inc, NCI-Frederick, Frederick, MD, 21702; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services; 4) Pediatric Oncology Branch, Center for Cancer Research, NCI, NIH, DHHS; 5) Roche Applied Science, Indianapolis, IN.

We developed a process that uses the power of next generation sequencing to screen for known and novel genetic variants within chromosomal regions identified by whole genome association studies.

Upon identification of a genomic region, PCR primers are designed to amplify overlapping amplicons ranging in size from 2 to 15 kb. PCR conditions are optimized on a control human genomic DNA sample to obtain a single agarose gel band of appropriate size for each primer set.

The resulting panel of amplicons is amplified from samples of interest, visualized on an agarose gel, quantified and pooled at an equimolar ratio. The pools are then fragmented into 300 to 800 bp fragments and sequenced on a next generation sequencing platform. We developed and tested this new process on a 136 kb region of human chromosome 8. Thirty two amplicons ranging in size from 2,059 to 5,439bp were amplified from four samples from the HapMap - CEPH panel. The 32 amplicons cover all but three small regions within the 136 kb segment. The sequencing run generated an average mapped depth of 160 and average read length of 250 bp, which was sufficient for detecting all known SNPs within the region covered.

Funded by NCI-Contract N01-CO-12400.

2700/W

Algorithm for conversion of TaqMan® genotyping assays to unlabeled probe assays. *D. de Silva[1], M. Wall[1], J. Blackett[1], F. le Calvez[2], S. Tavtigian[2], J. McKinney[1], D. Teng[1].* 1) Idaho Technology, Inc, Salt Lake City, UT; 2) International Agency for Reasearch on Cancer, Lyon, France.

In the past, using PCR methods for genotyping has required fluorescently labeled oligonucleotides. These methods can be costly and time consuming. TaqMan® probes are one such example where a different probe is required for each allele. LunaProbes, an extension of the high-resolution DNA melting technique, now provides a simple, inexpensive alternative for genotyping. With LunaProbes, all three possible genotypes that result from a given polymorphism can be detected with a single unlabeled oligonucleotide probe. A LunaProbe is a simple oligonucleotide blocked at the 3′ end to prevent extension. LunaProbes are designed to sit over a SNP of interest and are included in the PCR reaction prior to amplification. Genotyping is accomplished by monitoring the melting of the probe-target duplex post PCR. Key to the success of this method is the use of asymmetric PCR, where one primer is used in excess resulting in the over-production of the target strand recognized by the probe and the use of LCGreen® Plus dye that is capable of producing a strong fluorescent signal from the probe-target interaction. A simple decision tree is presented that allows rapid assessment of an existing fluorescent probe design for conversion to a LunaProbe assay. The steps required take into consideration: the sequence and Tm of oligonucleotides being used to amplify region of interest, and the sequence and Tm of the probe in use. This assessment will also define which DNA strand is optimal for the probe to anneal to, helping a user design the asymmetric PCR reaction. LunaProbes are a simple inexpensive alternative for genotyping, easily replacing current expensive methods of genotyping. The algorithm presented was validated on ten TaqMan assays where a single LunaProbe replaced two fluorescently labeled probes with a 100% success rate.

2701/W

Rapid genotyping methods for detection of the novel *CYP2A6*12* hybrid allele and *CYP2A6* copy number variation using Pyrosequencing technology. *D. Koontz, A. Spencer, J. Huckins, M. Gallagher.* CDC, NCEH, DLS, Molecular Biology Branch, Atlanta, GA.

CYP2A6 is the primary enzyme that metabolizes nicotine to cotinine. Genetic variability in this gene contributes to much of the inter-individual variability in *CYP2A6* enzyme activity. Several allelic variants arise from unequal crossover events that occur between the *CYP2A6* gene and the inactive *CYP2A7* gene. The *CYP2A6* deletion allele is created by such an event with *CYP2A6* gene duplication as the reciprocal outcome. A *CYP2A7/CYP2A6* hybrid (*CYP2A6*12*) is another variant created by an unequal crossover. Traditional methods for genotyping these variants are laborious, unreliable and not suitable for large sample sizes. We developed Pyrosequencing assays for each of these variants that use sets of PCR primers to co-amplify common regions of these genes. To detect copy number variation, the *CYP2A7*-specific peak heights serve as the reference by which to compare *CYP2A6*-specific peak heights. Genotype calls are derived from quantitative peak height ratios that give unique values for each genotype. To detect the *CYP2A6*12* hybrid allele, both *CYP2A6* wild-type and hybrid sequences are co-amplified and the presence of the hybrid sequence is determined by sequence analysis. We genotyped 4 ethnic groups: Hispanic (N=32), Asian (N=42), African American (N=35), and European Caucasian (N=43) from the Coriell Human Variation Collection. Samples were previously genotyped by 2-step long range-PCR for *CYP2A6*12* and by PCR-RFLP and TaqMan for *CYP2A6* copy number. Results were 100% concordant with the Pyrosequencing assays. Despite small sample sizes, a striking inter-ethnic difference in the distribution of the *CYP2A6* deletion allele was noted and is consistent with previous reports of a higher prevalence in Asian populations. These assays will be used to assess *CYP2A6*12* and *CYP2A6* copy number variation in a large-scale study of 7,300 multi-ethnic DNA samples from the U.S. population for prevalence estimates and associations with variability in nicotine metabolism. With appropriate robotics, over 1,900 samples can be genotyped in 8 hours.

2702/W

Integrated Detection and Analysis of SNPs and Copy Number Variation for Genomewide Association and Cancer Studies. *F.G. Kuruvilla[1,3,4], S.A. McCarroll[1,4], S. Cawley[2], J. Korn[1,4,5], A. Wysoker[1], J. Blume[2], M.J. Daly[1,4], S. Lincoln[2], S.B. Gabriel[1], R.P. Rava[2], D.M. Altshuler[1,4].* 1) Broad Institute of Harvard and MIT, Cambridge, MA; 2) Affymetrix Inc, Santa Clara, CA; 3) Brigham & Women's Hospital, Boston, MA; 4) Massachusetts General Hospital, Boston, MA; 5) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA.

While the roles of SNPs and Copy Number Variants (CNVs) both should be assessed in each disease study, their measurement platforms have until now been separate. We have developed a next-generation microarray platform with 906,600 SNPs, capturing 3.8 million SNPs with an average maximum r squared of 0.85, as well as 202,000 high-density probes of regions of known copy-number variation and 744,000 probes tiled throughout the genome for identification of novel or de novo CNVs. SNP genotyping is 99.6% concordant with Hapmap data with an average 99.7% call rate across all SNPs. The arrays make it possible to accurately genotype clinical samples for more than 500 common CNVs, allowing common CNVs to be systematically assessed, for the first time, for association with clinical phenotypes. Moreover, we find that integrated analysis of SNPs and CNVs improves the analysis of both forms of variation, allowing us to fill in physical coverage gaps in LD across the genome, allowing copy number to be inferred at an allelic level, and allowing inference of the actual copy numbers that underlie reported regions of copy number variation. Integrated analysis of SNPs and CNVs will enable powerful and novel inference in whole genome association studies.

2703/W

Epitope-tagging of endogenous proteins in somatic cells for ChIP-chip. *P.C. Scacheri, X. Zhang, Z. Wang.* Department of Genetics, Case Western Reserve University, Cleveland, OH.

Chromatin immunoprecipitation coupled with DNA microarray (ChIP-chip) technology offers enormous potential for genome-wide identification of transcription factor binding sites. The success of ChIP-chip relies heavily on antibodies with high affinity and specificity, yet such antibodies are not available for most proteins. In principle, this problem can be circumvented by constructing epitope-tagged proteins recognizable by well characterized antibodies. However, expression of tagged proteins at non-physiological levels can reduce the efficiency of ChIP and produce a genomic distribution of the tagged protein that differs from that of the endogenous proteins. To surmount this problem, we developed a strategy whereby recombinant adeno-associated virus (rAAV) is used to epitope tag endogenous loci by homologous recombination-mediated "knock-in". The tagging approach is fast, can be applied to numerous loci and multiple somatic cell lines, and facilitates western, immunofluorescence, and immunoprecipitation analyses of targeted proteins. As proof of principle for use in ChIP-chip, we introduced a triple FLAG tag into the C-terminus of STAT3, a transcription factor that is constitutively activated in a multitude of tumors. ChIP-chip analysis of FLAG-tagged STAT3 in colon cancer cells enabled discovery of hundreds of STAT3 binding sites, and ChIP-chip analysis of untagged STAT3 indicated that the locations of the STAT3 sites were not affected by the presence of the tag. The majority of STAT3 binding sites are located far from promoters, suggesting that the regulatory role of STAT3 in colon cancer is highly complex. This knock-in approach can be used to target virtually any locus, obviates the need for cloning tagged full-length cDNAs, insures normal expression profiles, and provides a general solution for the study of proteins for which antibodies are substandard or not available.

2704/W

Patterns of genetic variation in miRNA genomic regions: haplotype block structure and its application to association studies. *Y. Espinosa-Parrilla[1], M. Muiños-Gimeno[1], M. Montfort[1], M. Bayés[1], X. Estivill[1, 2].* 1) Genes and Disease Program, CeGen and CIBERESP (CRG-UPF), Barcelona; 2) Pompeu Fabra University, Barcelona, Catalonia, Spain.

MicroRNAs (miRNAs) have a crucial role as posttranscriptional regulators of genes, being involved in the regulation of at least a third of mammalian genes. Allelic variants involving miRNAs or their regulator machinery may be an important source of genetic variation and contribute to complex disease susceptibility. Association studies using single SNPs in miRNA genomic regions might help to evaluate miRNA allele variants with respect to disease. We constructed a panel of SNPs covering miRNA regions and studied their pattern of variation in the population. We first analyzed the genomic organization of miRNAs and have defined 164 regions spanning 2 Mb of genomic DNA and containing 326 known human miRNAs (MiRBase, 7.1), including the precursor sequence as well as at least 5 kb upstream and downstream of the miRNA. Forty-nine clusters containing 192 miRNAs were defined at a 2-kb inter-miRNA distance with two large clusters in chromosome 14 and 19, containing 23 and 44 miRNAs, respectively. Considering the SNP coverage of HapMap data (Rel 19/phase II), the SNP density of miRNA regions was considerably lower than in the average of the genome, with only 8 SNPs in miRNA precursor sequences (0.3 SNPs/kb). For an optimal selection of informative SNPs, we combined tagSNP and random SNP selection methods to design a panel of 768 SNPs. Only 18 out of the 768 SNPs were located in premiRNAs sequences (8 SNPs from HapMap and 10 from dbSNP). Genotyping in 340 Spanish unrelated individuals was performed using a custom Golden Gate assay from Illumina. Half of the SNPs located in the premiRNAs were monomorphic in the studied population, which is suggestive of strong selective constraint on miRNAs. Similar allele frequencies and LD patterns between the Spanish population and the HapMap CEU sample was shown confirming the applicability of our SNP panel to the study of the association of miRNAs in complex disorders and common traits. Supported by Spanish Government (FI05/00061, R&C program) and Generalitat de Catalunya.

2705/W

SNP genotyping in the presence of copy number polymorphisms. *T. LaFramboise[1], M. Kothari[1], L. Macconaill[2], M. Gould[1].* 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) The Broad Institute of Harvard and MIT, Cambridge, MA.

Genotyping SNPs using microarrays has become increasingly high-throughput and cost-effective. The methods associated with these arrays generally assume two copies of each SNP locus per cell, for a diallelic genotype. Given the recent discovery of widespread copy number variation in the human genome, however, this assumption is no longer always valid. For example, if an A/G SNP is contained within a genomic region that is duplicated in a significant proportion of the human population, this SNP's genotype may be AAA, AAG, AGG, or GGG for some individuals. Similarly, an individual may carry an A- or G- genotype in a region harboring a germline deletion, or a "--" genotype if chromosomes harboring the deletion are inherited from both parents. This "generalized genotype" is unrestricted by the usual diallelic assumption that results solely in AA, AG, and GG genotypes, and succinctly provides both copy number and SNP allelic information.

Most available software would call an AAG genotype as AA or AG, an A- genotype as AA, and a -- genotype as No Call. These erroneous calls can lead to incorrect phasing and apparent Hardy-Weinberg deviations. Moreover, the impending growth in genome-wide association studies will heavily rely on accurate SNP genotyping, whether the SNPs are used as markers or as putative causal variants.

We have developed methods to infer generalized genotypes from both the Affymetrix and Illumina arrays. Each of these platforms interrogates over 500,000 human SNPs across the genome. Our generalized genotypes were verified using a variety of experimental assays, and demonstrated a high level of accuracy. The interrelationship between SNP allele and copy number variation provides insight into the history of the point mutation and duplication events that resulted in these variants. Our analysis of thousands of duplicated SNPs implies that the duplication is more recent than the point mutation in most, if not all, cases. Furthermore, the duplication events seem to be recurrent in human history in many cases.

2706/W

Multi-plexed TaqMan®-based miRNA Profiling of Cancer and Stem Cells. *Y. Wang[1], C.Y. Park[2], I.L. Weissman[2], J. Weidhaas[3], O. Loudig[4], R. Tan[1], C. Chen[1].* 1) Molecular Biology, Applied Biosystems, Foster City, CA; 2) Stanford Institute for Regenerative Medicine and Stem Cell Biology, Stanford University, Palo Alto, CA; 3) Yale University School of Medicine, New Haven, CT; 4) Albert Einstein College of Medicine, Bronx, NY.

Developing microRNA (miRNA) profiling strategies that are efficient and quantitative has proven to be challenging because of their small sizes and the sequence similarity among family members. We have developed multi-plexed TaqMan®-based MicroRNA Assays and TaqMan Arrays that provide simple, rapid, quantitative and sensitive tools for miRNA profiling. We have applied such strategy to profile expression of 366 miRNAs in seven breast cancer cell lines as well as paired samples derived from FFPE and fresh frozen breast tumor tissues. Unsupervised hierarchical analysis separated the stromal-derived and luminal epithelial-derived breast cancer cell lines into two distinct groups. Cell-type specific miRNA signature includes miR-200, miR-203 and miR-222 etc, representing potential miRNA biomarkers for characterization of sub-types of breast cancer. In addition, the FFPE sample showed similar miRNA profiles as the paired freshly frozen samples, demonstrating our miRNA profiling strategy can be successfully applied to analyze archived FFPE samples. Coupled with multiplex preamplification, the sensitivity of the multi-plexed TaqMan-based miRNA assays can be further extended to quantifying single or very few cells. We demonstrated the application of this method in studying the development of human hematopoietic stem cells (HSC). We have evaluated the expression profile of 315 human miRNAs using FACS-purified normal human bone-marrow derived HSC and three committed progenitor populations. Distinct miRNA signatures were identified that distinguish each of these cell populations. Characterization of these candidate miRNAs may lead to better understanding of the regulatory roles of miRNAs in development of hematopoiesis.

2707/W

Validation of Applied Biosystems 3730 genetic analyzer for STR-based relationship testing. *J. Cummings[1], J. Kibler[2], M. Roche[2], M. Marfori[2], T.D. Kupferschmid[2], D.D. Einum[1].* 1) Sorenson Genomics, Salt Lake City, UT; 2) Sorenson Forensics, Salt Lake City, UT.

Background: The Applied Biosystems (ABI) 3130 and 3700 genetic analyzers are capillary electrophoresis-based instruments utilized in the relationship testing community for fragment analysis. However, the 3130 has limited high-throughput capabilities and the 3700 often involves unpredictable capillary failures. With the release of the next-generation 3730 platform, high throughput processing is achievable while maintaining low failure rates. This study's objective was to validate the Identifiler multiplex (ABI) on the 3730 for relationship testing applications in a high throughput laboratory. **Methods**: Genomic DNA used in this validation were isolated from buccal swabs using Qiagen's QIAamp Swab BioRobot Kit. The samples were then normalized using a modified PicoGreen quantitation assay and amplified using the Identifiler PCR kit on GeneAmp 9700 thermocyclers. Amplified products were processed on a 3730 48-capillary array and the resulting genotypic data was analyzed with GeneMapper. Data quality parameters examined in this study included crosstalk, concordance, precision, sensitivity and stutter. **Results**: Crosstalk was assessed with "zebra" and "checkerboard" plate configurations whereby signal was examined in blank wells and was detected only when input DNA was increased to 6.5ng. Data concordance was examined by analyzing controls and ladders with published genotypes as well as examining samples from NIST. No instances of non-concordance were observed. Run-to-run and capillary-to-capillary precision was evaluated by examining migration of a series of allelic ladders. For all alleles, the detected size ranges were less than 1.0 base pair. Sensitivity studies involved input DNA quantities of 6.5ng, 3.25ng, 1.62ng, 0.81ng, 0.4ng, 0.2ng and 0.1ng. With addition of less than 0.4ng, stochastic effects became prominent. Stutter percentages all fell within thresholds recommended by the manufacturer. **Conclusion**: This study demonstrates that the 3730 yields reliable and consistent results within the context of high-throughput fragment analysis for relationship testing laboratories.

2708/W

Single nucleotide polymorphisms and comparative sequence analysis of the *TNFA* gene in a pedigreed colony of vervet monkeys (*Chlorocebus aethiops*). *S.B. Gray, T.D. Howard, C.D. Langefeld, G.A. Hawkins, A.F. Diallo, J.D. Wagner.* Pathology/Comparative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

Single nucleotide polymorphisms (SNPs) in the human *TNFA* gene have been associated with differential *TNFA* expression or susceptibility to various complex diseases. Vervet monkeys are becoming an important animal model for such complex diseases as obesity, Type 2 diabetes, and atherosclerosis. At present, there is little genetic information available for the vervet. This study represents one step toward further genetically characterizing the vervet in order to refine it as a robust animal model. This data will also permit future association studies and facilitate phylogenetic analyses. *TNFA* is a candidate gene that is putatively involved in an inflammatory cascade involving obesity and its comorbidities. We have sequenced the promoter, exons and intronic regions of the *TNFA* gene in 265 individuals from a pedigreed colony of vervet monkeys, which is currently in the 5th-7th generation, with 24 original matrilines. This resulted in a contiguous sequence of ~4 kb. These 265 individuals were previously phenotypically characterized for obesity and associated risk factors, most of which were shown to be significantly heritable. Sequencing revealed a total of 11 SNPs, with 5 in the promoter region, 4 in the intronic regions, and 2 in the 3'/5' untranslated region, and minor allele frequencies between 0.08 and 0.40. An intronic tetranucleotide repeat polymorphism was also detected. Comparisons of the vervet *TNFA* gene sequence with that of humans and rhesus macaques indicated 93.6% and 98.4% sequence identity, respectively. A comparative sequence map of the *TNFA* gene, reported herein, for humans, vervets, and rhesus monkeys, illustrates that the number and relative position of SNPs is similar among all 3 species. This sequence data will contribute to refining the vervet model of complex polygenic disease, allow future association studies, facilitate phylogenetic analyses, and represents the first report, to our knowledge, of *TNFA* or any other cytokine gene sequence data for the vervet monkey.

2709/W

High Resolution Melt Curve Analysis of Genomic and Whole Genome Amplified DNA. *M.H. Cho[1,2,3], B.J. Klanderman[1], D. Ciulla[1], B.A. Raby[1,2,3], E.K. Silverman[1,2].* 1) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 2) Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 3) Division of Pulmonary and Critical Care Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA.

Background: High resolution melt curve analysis is a post-PCR technique that can be used for mutation detection. Recent advances in instrumentation and double strand specific DNA dye have improved the accuracy of this method. To our knowledge, this method has not been tested on whole genome amplified (WGA) DNA. Methods: Whole genome amplification (REPLI-g®, Qiagen) was successfully performed on genomic DNA samples from 39 subjects from the Boston Early-Onset COPD study. 24 amplicons from 9 genes were PCR amplified in paired genomic and WGA samples and subsequently analyzed by high resolution melt curve analysis using the LightScanner® (Idaho Technology). Selected samples from each melt curve were bidirectionally resequenced. Results: Melt patterns were concordant between the genomic and WGA samples in 92% of successfully analyzed sample pairs. Of the discordant patterns, there was an overrepresentation of alternate melt curve patterns in the WGA samples suggesting the presence of a mutation (false positives). Targeted resequencing was performed in 140 genomic and 136 WGA samples and revealed 43 SNP. Heterozygous variants were identified by non-wild type melt pattern in 100% of genomic and 92% of WGA samples. Wild types were correctly classified in 93% of genomic and 54% of WGA samples. As expected, the technology lacked sensitivity for homozygous variants. Conclusion: High resolution melt curve analysis is a sensitive tool for SNP discovery in genomic DNA. Performance appears to be substantially less in whole genome amplified DNA. Acknowledgments: We thank all the study participants. Work was supported by U.S. National Institutes of Health (NIH) grants R01 HL075478 (Silverman) and K08 HL74193 (Raby).

2710/W

High resolution melting analysis (HRM) for rapid and sensitive detection of mutations in *BRCA1&2*: comparison of Lightscanner (Idaho Technology) and LightCycler 480 (Roche). *K. Claes, K. De Leeneer, I. Coene, B. Poppe, A. De Paepe.* Centre for Medical Genetics, Ghent Univ Hosp, Gent, Belgium.

HRM is an emerging technique for detection of nucleic acid sequence variation. It involves the precise monitoring of the change in fluorescence caused by the release of an intercalating DNA dye from a DNA duplex as it is denatured by increasing temperature. Developments in instrumentation and fully saturating intercalating dyes have made accurate HRM analysis possible. We evaluated two HRM instruments for screening *BRCA1&2* mutations. To cover the complete coding region and splice sites we designed 112 PCR amplicons (lengths: 136-435bp), amplifiable with a single PCR program. LCGreen® Plus was used as intercalating dye. HRM was performed on the 96-well Lightscanner (Idaho Technologies) (LS) and the Lightcycler® 480 (Roche) (LC) instruments. The sensitivity was evaluated by analysing 212 positive controls scattered over almost all amplicons and the specificity by a blind screening of 22 patients previously screened by other techniques. All 212 known heterozygous sequence variants were detected on the LS by analysis on "normal sensitivity". On the LC the standard sensitivity setting of 0.3 had to be increased to 0.5 to detect all variants, hereby decreasing the specificity (96.2% vs. LS: 97.3%). A few amplicons were responsible for the majority of the false positives and we are currently optimizing these fragments. Previously, we screened *BRCA1&2* by direct sequencing of the large exons 11 and DGGE for all other coding exons. By introducing HRM our reporting time can be decreased 3 times: no post-PCR handling is required and the software allows fast analyses. Also the cost price of the consumables is limited (LCGreen® Plus: ±0.25EUR per reaction; 96-well PCR plate suitable for HRM: ±3.5 (LS) -4.8 (LC) EUR). The cost price of the LS instrument is about half of the cost price of the LC, however, the latter can also be used for real-time Q-PCR. HRM is a rapid, cost-efficient, sensitive methodology simple enough to be readily implemented in a diagnostic laboratory. The sensitivity of both instruments evaluated was 100%; the LS scored slightly higher for specificity than the LC.

2711/W

Improved homozygote detection through internal calibration of high-resolution melting data. *C.N. Gundry[1], S.F. Dobrowolski[1], Y.R. Martin[1], T.C. Robbins[1], L.M. Nay[1], C.T. Wittwer[1,2], D.H-F. Teng[1].* 1) Biochemistry R&D, Idaho Technology, Salt Lake City, UT; 2) University of Utah, School of Medicine, Department of Pathology, Salt Lake City, UT.

Background: Genotyping techniques using high-resolution DNA melting are both simple and powerful, but they can be limited by sample-to-sample variations in temperature, buffer and volume. Heterozygotes are easiest to detect in melting because of the presence of heteroduplexes, which alter the shape of the transitions significantly, within the re-annealed product. Homozygote differentiation, however, often depends more on temperature reproducibility for accurate genotyping because of similar melting shapes. In addition, some types of homozygotes (e.g., A/T or G/C) are inherently difficult to detect, even with the best instruments because of small Tm differences. Methods: Calibrator oligonucleotides were synthesized and included at equimolar concentrations with their complementary sequences. 96 well PCR was performed in conventional thermocyclers with LC Green® Plus dye in the presence of calibrators. PCR plates were moved to the LightScanner and melts were performed from 55 to 97 °C. Calibrator melting signitures were aligned, within each reaction, and used to transform the fluorescence data. Genotypes were determined by sequencing and/or probe-based genotyping for each sample. We assessed homozygote genotyping error rates before and after calibration by measuring the distance (°C) of each sample's apex to the mean Tm value of the group to which its genotype corresponded. Errors were observed when the Tm of a sample was closer to the mean Tm of the incorrect group. Results: Across multiple plates and PCR targets calibration improved the homozygote genotyping error from 10% to less than 1%. Tm standard deviations lowered from approximately 0.056 to 0.027 °C, after calibration. Conclusion: Internal calibration improved discrimination of homozygotes. Our calibration system enabled the correct genotyping of symmetric base-pair neutral homozygotes, in apparent exception to nearest neighbor thermodynamic assumptions.

2712/W

Identifying Disease-causing Non-coding Mutations by Medical Sequencing. *T. Hefferon[1], S.Q. Lee-Lin[1], J. Idol[1], V. Maduro[1], S. Terry[2], A. Sharp[3], E.D. Green[1], NIH Intramural Sequencing Center.* 1) NHGRI, NIH, Bethesda, MD; 2) PXE Int'l, Washington, DC; 3) Dept. Genome Sciences, U. Washington, Seattle, WA.

The comparison of genome sequences from diverse vertebrate species has enabled the identification of highly conserved regions that are under negative selection. Having resisted mutation over evolutionary time, such regions are likely to contain functional genomic elements that are important for the survival of organisms. We are using a comparative genomics approach to identify highly conserved non-coding regions in and around known human disease genes, and then screening those regions by medical sequencing for possible disease-causing mutations. In two related projects, we are studying patients with cystic fibrosis (CF) or pseudoxanthoma elasticum (PXE). The genomic regions encompassing both genes mutated in these disorders (*CFTR* and *ABCC6*, respectively) have been sequenced in multiple species, allowing the identification of multi-species conserved sequences (MCSs). We are using a medical-sequencing approach to screen DNA samples from patients where one or both mutations remain unidentified after rigorous screening of coding, splice, and promoter regions; since these patients do not appear to have two coding mutations, they may carry disease-causing changes in non-coding functional sequences. We have found multiple variants in both genes, and are following them up with further studies to define their possible functional roles. Our *CFTR* studies are being aided by the rich data sets for the corresponding genomic regions generated by the ENCODE project; these data are providing important insights about the possible function of the conserved non-coding regions being examined. Meanwhile, our *ABCC6* studies are complicated by the presence of two partial pseudogenes in the genomic region of interest, which are products of segmental duplications; this raises the possibility that copy-number changes may account for the disease in some patients. Together, these projects illustrate the complexities associated with the search for disease-causing mutations in some genetic diseases and the important interface between comparative genomics and medical sequencing in human genetics studies.

2713/W

Interlaboratory validation of High Resolution Melting (HRM) for BRCA1 and BRCA2 on the LightCycler® 480. *T. Janssens[1], N. van der Stoep[2], R. Buser[3], G. Michils[1], A. Corveleyn[1], P. Maillet[3], E. Bakker[2], G. Matthijs[1].* 1) Center for Human Genetics, Leuven, Belgium; 2) Center for Human and Clinical Genetics, Leiden, The Netherlands; 3) Laboratory of Oncogenetics, Geneva, Switzerland.

EuroGentest is a network funded by the European Commission that aims to improve and harmonize the overall quality of genetic services throughout Europe. The validation of new technologies is one of many contributions to accomplish this. High Resolution Melting (HRM) was selected as one of the technologies for which a thorough validation would be very timely. In a collaborative study, we extensively tested HRM on the LightCycler® 480 (Roche Applied Science). HRM is a fast, simple and cost effective high-throughput scanning method to detect sequence variations. PCR is performed in the presence of a saturating fluorescent dsDNA binding dye. Single-base variations in the amplicon result in altered melting behavior after heteroduplex formation, which affects the curve shapes in HRM plots. The LightCycler® 480 performs both PCR and HRM in a single multistep run. BRCA1 and BRCA2 were chosen as target genes, because their size and mutation spectrum represent a challenge in molecular diagnostics. The current methods, like e.g. dHPLC and DGGE, despite their good performance, are limited by their throughput or labour-intensity. HRM is potentially useful for solving these problems. However, it needs to be shown that the sensitivity and the ease of use are at least as good as the current state of the art. Therefore, an extensive validation was set up in parallel in 3 labs. The first objective was to design a complete primer set for BRCA1 and BRCA2, which was derived from the dHPLC and sequencing primers, but optimized for HRM. Indeed, the performance of HRM is largely depending on the quality of the PCR. Critical criteria were specific banding patterns after gel electrophoresis, nice sigmoid curves on the real-time PCR plots and no more than 2 melting domains per amplicon. For the final validation, at least 150 known variations will be tested in a blinded way. This will allow us to determine whether HRM reaches a sensitivity close to 98%, which would make it a suitable new method for diagnostic use.

2714/W

Identification of novel mutations and sequence variation in the Zellweger syndrome spectrum of peroxisome biogenesis disorders. *W.Y. Yik[1], S.J. Steinberg[2], P.K. Dranchak[1], A. Moser[2], H. Moser[2], J.G. Hacia[1].* 1) 1Department of Biochemistry and Molecular Biology University of Southern California 2250 Alcazar Street, IGM 240 Los Angeles, CA 90089, USA; 2) 2Peroxisomal Diseases Laboratory Kennedy Krieger Institute Baltimore, MD.

Peroxisome biogenesis disorders (PBD) are a complex group of autosomal recessive diseases that result in neurological, skeletal, hepatic, and renal abnormalities. Approximately 80% of PBD patients are in the Zellweger syndrome spectrum (PBD-ZSS). In turn, 90% of PBD-ZSS patients are caused by mutations in the *PEX1*, *PEX6*, *PEX10*, *PEX12* and *PEX26* genes which are essential for the assembly of functional peroxisomes. Here, we have developed cost-effective sequencing assays to survey the mutational spectrum of these five *PEX* genes in a cohort of 60 PBD-ZSS patients. A total of 54 unique sequence variants were identified, including 18 novel mutations predicted to disrupt protein function.Overall, direct sequencing provides a reasonable approach for the molecular diagnosis for PBD-ZSS patients and for refining our knowledge of PEX gene functional domains.

2715/W

TaqMan® Drug Metabolism Genotyping Assay Panels on TaqMan® Low Density Arrays. *T. Hartshorne, J. Au-Young, T. Ceccardi, R. Padilla, J. Ziegle.* Molecular Biology, Genomics; Applied Biosystems, Foster City, CA.

TaqMan® Low Density Arrays from Applied Biosystems are 384 well micro fluidic cards that offer a convenient and easy to use platform for running panels of TaqMan® Gene Expression Assays. The arrays are pre-loaded with assays, which greatly simplifies the experimental workflow, eliminates the need for liquid-handling robots, and facilitates high reproducibility of results. Investigators engaged in pharmacogenetic research often need to repeatedly screen a given set of Drug Metabolism Enzyme (DME) polymorphisms. To determine if TaqMan Arrays could be used with panels of TaqMan® DME Genotyping Assays, we conducted benchmark tests to compare the performance of genotyping assays on TaqMan Arrays and on conventional 384 well plate platforms. Two arrays were tested: these had the configuration of 48 assays spotted 8 times and contained a total of 91 distinct TaqMan Validated SNP and DME Genotyping Assays. One array contained 48 DME assays to Cytochrome P450 SNPs in CYP2D6, CYP2C9 and CYP2C19 genes, many of which were challenging targets for assay design due to the presence of pseudogenes and copy number variations. 45 African American and 45 Caucasian Coriell genomic DNA samples were run on the arrays and the resulting genotyping data was compared to data from assay validation studies run on 384 well plates. Assay performance was found to be equivalent between platforms: all assays tested on arrays performed well (100% pass rate), and call rates and call accuracy were > 99 % in 3 separate experiments. Data from both arrays and 384 well plates was analyzed using Autocaller Software (in development). This interactive software tool enables overlaying and viewing cluster plots from multiple plates or arrays for easy analysis and editing of genotyping data. TaqMan® Low Density Arrays could offer a simple workflow and accurate genotyping platform for routine, reliable and cost-effective pharmacogenetic screening of DME polymorphisms.

2716/W

Genotyping Triallelic SNPs in Drug Metabolizing Enzymes. *T. Ceccardi, T. Hartshorne, C. Chen.* Molecular Cell Biology, Applied Biosystems, Foster City, CA.

Applied Biosystems currently offers over 2,500 TaqMan® SNP Genotyping Assays for Drug Metabolizing Enzyme (DME) genes. These genes are involved in the processes that breakdown and eliminate chemicals, such as drugs or carcinogens, in the human body. Single nucleotide polymorphisms (SNPs) or mutations in these genes can affect their functionality. Knowing their genotypes can be critical during pharmaceutical development and clinical trials. Although commercially available TaqMan® DME Genotyping Assays detect many of the most important genetic mutations, some highly-valued SNP assays were not yet designed since they did not follow the "rules" necessary for successful automated design. One of the rules broken by these high-value SNPs is that, rather than being typical biallelic SNPs where one nucleotide base is substituted for another, three different nucleotide bases or tri-alleles are seen in the human population. When testing these triallelic SNPs, a researcher would like to know the frequency of all three alleles. However, standard FAM/VIC®-labeled TaqMan assays only measure two alleles. Here we examined two different approaches for triallelic SNP genotyping. The first was by running two FAM/VIC-labeled assays, each with a probe for the major allele and one of the minor alleles, and analyzing the resulting two cluster plots in concert, with a "map" of expected cluster positions. Alternatively, a probe for the third allele was labeled with a third dye (NED), and mixed together with FAM and VIC® probes which target the first two alleles. We designed assays for two different triallelic SNPs to test the two alternate workflows and analysis methods. We found that although the workflow is simpler for a 3-probe triallelic SNP assay, it is complicated in data analysis to resolve all possible allele/dye combinations.

2717/W

Development of a SNP Chip for prediction of steroid-induced osteonecrosis. *W.T. Huang[1], S.R. Lin[2,3], G.J. Wang[4], F.Y. Chung[1].* 1) Graduate Institute of Medicine Kaohsiung, Taiwan; 2) Graduate Institute of Medical Genetics, College of Medicine; 3) BioMedi Innovation Incubation Center, Kaohsiung Medical University; 4) Department of Orthopedics Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background: Osteonecrosis (ON) is also called the Avascular Necrosis (AVN) which is an impairment happening in the blood stream in bones and may finally cause bone cell death. There are many risk factors for ON such as the trauma, steroids therapy, alcoholism, blood disease and smoke. The exact pathogenesis mechanism of ON is still unclear so far. The specific aim of this study was to establish a SNP chip to distinguish the risk genotypes of ON for detecting the possible risk factors causing this disease. Methods: The subjects of this study included 106 patients with SLE who had been treated with steroid for at least two years or more. Twenty out of 106 patients were ON patients, and eighty six were non-ON patients. Genomic DNA was isolated from the blood samples of SLE patients. The genotypes were determined by PCR-RFLP and confirmed these results by DNA sequencing. We use membrane array which developed in our lab before to construct the SNP chip. Result: The genotyping of BMP2, VEGF and MTHFR were done and the results were shown as follows BMP2 genotypes of ON vs. non-ON: CC(2.8% vs. 15%), TC(11% vs. 44%), TT(5.5% vs. 22%); VEGF genotypes of ON vs. non-ON: AA(9.6% vs. 22%), AG(9.6% vs. 39%), GG(1% vs. 18%); MTHFR genotypes of ON vs. non-ON: CC(11% vs. 44%), TC(6.7% vs. 33%), TT(1.9% vs. 2.9%). We have also designed the oligonucleotide probe of these candidate genes for constructing the SNP chip. The results of SNP chip are highly consistent with RFLP and DNA sequencing. Conclusion: We have now accomplished the genotyping of three candidate genes. Due to small number of cases, we can not calculate correlation between these risk genotypes and ON. Therefore we may increase case number and investigate more related genes in the future. We believe that the development of the SNP chip in this study can be used in the prediction of ON and with the combination of medical therapy; it may reach to the goal of preventing ON.

2718/W

A genome-wide map of 8.27 million SNPs in the laboratory mouse genome. *B. Karlak[1], K. Frazer[1], C. Wade[2,3], M. Bogue[4], D. Hinds[1], E. Beilharz[1], R. Gupta[1], J. Montgomery[1], M. Morenzoni[1], G. Neilsen[1], S. Osborn[1], C. Pethiyagoda[1], L. Stuve[1], F. Johnson[5], M. Daly[2,3], D. Cox[1].* 1) Perlegen Sciences, Mountain View, CA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 4) The Jackson Laboratory, Bar Harbor, ME; 5) Toxicology Operations Branch, NIEHS, Research Triangle Park, NC.

A dense map of genetic variation in the laboratory mouse genome will provide insights into the evolutionary history of the species and lead to an improved understanding of the relationship between inter-strain genotypic and phenotypic differences. Using our high-density oligonucleotide array-based technology, we re-sequenced the genomes of four wild-derived and eleven classical inbred strains and identified 8.27 million high quality SNPs. The wild-derived inbred strains are separated from one another by over 3 million discordant SNPs uniformly distributed across the genome, while pairs of classical inbred strains are separated on average by 936,000 discordant SNPs. Analysis of the genetic contributions of the four Mus subspecies to the classical strains surprisingly revealed that collectively the contributions of the three Asian subspecies molossinus (27%), musculus (14%), and castaneus (12%) is larger than that of domesticus (46%). The re-sequencing data, mapped to build 36 of the NCBI Mouse Genome, is available at our web site (http://mouse.perlegen.com/), and includes information on SNP location, genotypes, gene assignment, PCR primer composition, trace files and quality information. The website allows the data to be viewed interactively or downloaded in batch format.

2719/W

Proteomic analysis to identify candidate genes influencing high-density lipoprotein particle size in obese individuals. *L.A. Collins[1], S.P. Mirza[1], L. Martin[2], A.H. Kissebah[1], M. Olivier[1].* 1) Medical College of Wisconsin, Milwaukee, WI; 2) Children's Hospital, Cincinnati, OH.

Obesity is associated with a significant risk for cardiovascular co-morbidities. This effect is primarily mediated by an atherogenic dyslipidemic profile, specifically a preponderance of small, dense high-density lipoprotein (HDL) particles. However, the molecular basis for the altered HDL particle size distribution in obesity is poorly understood. A genetic analysis in a family-based cohort of 2207 individuals identified strong quantitative trait locus (QTL) (LOD = 3.15) for HDL median particle diameter on human chromosome 12. The interval contains 213 annotated genes, none of which have a known role in cholesterol or lipid metabolism.

Here, we describe a proteomic analysis using tandem mass spectrometry in conjunction with an enzymatic peptide labeling technique to identify differentially expressed proteins in HDL particles. Recent research suggests that the HDL proteome is altered in dense HDL particles.

We isolated HDL fractions from plasma samples using non-denaturing fast protein liquid chromatography, with the use of a single Superose 6 30/100 GL column. A chloroform extraction procedure allows for the efficient isolation of lipid-embedded or associated proteins from HDL particles compatible with subsequent mass spectrometric analysis. Preliminary data will be presented on quantitative profiling of the HDL proteome. Using proteomic data, in conjunction with pathway and protein interaction analyses, we will highlight connections between alterations in protein expression and genes in the QTL region on human chromosome 12.

2720/W

How is mRNA expression predictive for protein expression? - a correlation study on human circulating monocytes. *Y.Z. Liu[1], P. Xiao[2,4], Y.F. Guo[2,3], S.F. Lei[2], F.Y. Deng[2], X.D. Chen[2], L.M. Li[2], S. Wu[2], Y. Chen[2], H. Jiang[2], L.J. Tian[2], J.Y. Xie[2], X.Z. Zhu[3], S.P. Liang[2], H.W. Deng[1,2,3].* 1) Univ. of MO -Kansas City; 2) Hunan Normal Univ., China; 3) Xian Tiaotong Univ., China; 4) Creighton Univ., Omaha, NE.

A key assumption in studying mRNA expression is that mRNA expression is informative to predict protein expression. However, only limited studies on yeast or human tissues explored how much information the mRNA expression could provide for the corresponding protein expression. To investigate the relationship between mRNA and protein expression levels in vivo in normal human cells, we performed correlation analyses on mRNA-protein expression in freshly isolated human circulating monocytes from thirty unrelated females. The expressed proteins for 71 genes were quantified and identified by two-dimensional electrophoresis coupled with mass spectrometry. The corresponding mRNA expressions were quantified by Affymetrix gene expression array technology. Significant mRNA-protein expression correlation ($r = 0.235$, $P < 0.0001$) was observed for the whole data set including all studied genes and all the samples. The correlations vary in different biological categories of gene ontology. For example, the highest correlation was achieved for genes for extracellular region in terms of Cellular Component ($n = 15$, GO: 0005576, $r = 0.643$, $P < 0.0001$) and the lowest correlation was obtained for genes for regulation ($n = 16$, GO: 0050789, $r = 0.099$, $P = 0.213$) in terms of Biological Process. At the genome level, fifty percent of the samples showed significant positive correlation for the 71 genes and the average mRNA expression value in all the samples was significantly correlated with the average protein expression level ($r = 0.296$, $P < 0.01$). However, at the population level, only five studied genes demonstrated significant positive correlation across all the samples. Our results showed an overall positive correlation between mRNA and protein expression levels. However, the moderate and varied corrleations suggest that mRNA expression may be sometimes useful but certainly far from perfect in predicting protein expression levels.

2721/W

Study of evolutionary conserved regions on the vicinity of COL18A1 reveals putative functional sequences. *E. Kague[1], S. Fisher[2], S.L. Bessling[2], M.R. Passos-Bueno[1].* 1) Department of Genetic and Evolutive Biology, Universidade de Sao Paulo, Sao Paulo, Brazil; 2) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, USA.

COL18A1 has 43 exons that transcribe three isoforms from two different promoters. The three isoforms display complex patterns of tissue-specific expression, including in kidney, lung, brain, and retina. Mutations in COL18A1 lead to Knobloch Syndrome, an autosomal recessive disease characterized by vitreoretinal and macular degeneration and occipital encephalocele. We analyzed the promoter of COL18A1 short isoform with luciferase assays and transfections in HEK293T cells of 5` COL18A1 deletion constructs. It revealed a core promoter between -103 and -270bp from the transcription start site, and two other important regions between -540 and -750, and -919 and -1030. We also evaluated non-coding sequences associated with COL18A1 for transcriptional regulatory activity using in vivo assay in transgenic zebrafish. Sequences were selected based on evolutionary conservation using VISTA (>100pb and >75% identity with mouse) and PhastCons programs, resulting in 21 regions. These regions and 4 promoter constructs were assayed for their ability to drive EGFP expression in mosaic injected embryos. For comparison, whole embryo in situ hybridizations were carried out to evaluate endogenous col18a1 expression in zebrafish embryos. None of the human promoter constructs leads to expression in zebrafish embryos. However, 9/21 conserved non-coding regions drove expression consistent with endogenous zebrafish col18a1. Four constructs drove EGFP expression in pronephic duct at 1 dpf, a prominent site of col18a1 expression. Additional tissues where expression was observed included notochord, cartilage, retina, forebrain, otic vesicle, pectoral fin, cranial blood vessels and connective tissue of the fin. The regions selected with VISTA program were also analyzed by transfections in cells with high (HEK293T) and low (HeLa) endogenous expression of COL18A1. 9/13 increased luciferase expression at least 4 fold, and there were no difference between transfections in HEK293T and Hela cells.

2722/W

In vivo screen for enhancer activity using lentivirus-mediated transgenesis. *M. Friedli[1,5], I. Barde[2,5], C. Attanasio[1,5], M. Arcangeli[1,5], A. Quazzola[2,5], S. Verp[2,5], F. Spitz[3,4,5], D. Duboule[3,5], D. Trono[2,5], S.E. Antonarakis[1,5].* 1) Department of Genetic Medicine and Development,University of Geneva,Switzerland; 2) School of Life Sciences, EPFL,Lausanne,Switzerland; 3) Department of Zoology and Animal Biology,University of Geneva, Switzerland; 4) EMBL Heidelberg,Heidelberg,Germany; 5) National Research Centre Frontiers in Genetics.

Finding sequences that control spatial and temporal expression of genes is important to understand genome function. In this study, we aim to perform an in vivo unbiased screen for enhancer activity in a 200-kilobase DNA fragment using lentiviral-mediated transgenesis. While previous studies have used evolutionary conservation as an indicator of regulatory potential, our study is without any bias towards a particular sequence feature. We chose a mouse BAC corresponding to a region of human chromosome 21; this region was selected because it contains the olig1 and olig2 genes that are expressed specifically in the CNS and thus potentially interesting for Down Syndrome. In order to screen this fragment systematically for enhancer activity, we generated a library of overlapping clones by digesting the BAC with CviJ and cloning upstream of a LacZ reporter in a lentiviral vector. The library contains 87 clones to date, with sizes ranging from 2-4 kb covering 70 percent of the BAC sequence. We are generating lentivectors for each segment of the library and injecting them in pools of 10 in mouse oocytes. E11 Embryos are recovered from foster mothers and stained for LacZ in order to identify expression patterns. The first three pools injected yielded 10 of 62 LacZ positive embryos with an average of 3.5 transgene integrations per embryo. The inserted segments were sequenced from all embryos in order to correlate a pattern of expression with a specific sequence. Different fragments, (some containing evolutionary conserved sequences) were identified that potentially contain gene expression regulators. Injection of individual sequences will be used to confirm enhancer activity of candidate clones. This approach will enable us to probe a substantial genomic segment for enhancer activity in mouse embryos.

2723/T

Genomic analysis of mouse inner ear organogenesis. *S.A. Sajan[1], M. Lovett[1], M. Warchol[2].* 1) Division of Human Genetics and Department of Genetics, Washington University School of Medicine, St. Louis, MO; 2) Department of Otolaryngology, Washington University School of Medicine, St. Louis, MO.

Mammalian organogenesis is a complex process demanding the activity of thousands of genes. To identify novel genes, networks of genes, and pathways active during inner ear (IE) development, we expression profiled mouse IE and non-IE (NIE) tissues beginning at E9, progressing at half-day intervals, up to E15. Various sub-structures of the IE were profiled separately as they became distinguishable during development, resulting in 29 IE samples being collected. Particular attention was paid to micro-dissection and data quality control. More than 5,000 genes varied in expression temporally, spatially, or both, and these defined 28 distinct patterns. Of these genes, 8% currently lack biological annotations, thus representing novel candidates required during IE development. Pathway analysis identified 53 significant signaling cascades enriched in at least one of the 28 expression patterns. Known pathways, including *Notch*, *Wnt*, and *TGF-beta*, and pathways as yet not implicated in IE development such as *Amyloid processing*, *Circadian rhythm*, and numerous immune-related cascades, were identified. Also observed was a down-regulation at E14 of ribosomal protein genes and of those involved in mitochondrial energy production. Whole mount RNA in-situs confirmed the differential expression of selected genes, and also delineated distinct spatial patterns of expression. Moreover, 54 human genomic intervals linked to uncloned non-syndromic deafness were identified that contained genes whose mouse orthologs were expressed and/or differentially expressed in our dataset. These serve as candidates for mutational analysis. Our study contributes significantly towards a comprehensive mouse IE gene expression database, and creates additional opportunities for computational and genomic approaches in understanding IE development.

2724/T

Finding Mouse Models of Human Disease In MGI. *S.M. Bello, D.L. Burkart, M.A. Updegraff, L.L. Washburn, B. Richards-Smith, A. Anagnostopoulos, M.N. Knowlton, R. Babiuk, H. Onda, M. Tomzuk, I. Lu, H. Dene, C. Smith, J.T. Eppig.* The Jackson Laboratory, Bar Harbor, ME.

Mammalian models of human disease are critical to increasing our understanding of disease mechanisms and discovering potential new therapies. The use of the mouse to create such models is facilitated by the wealth of genetic tools available, including high-resolution genetic maps, a multitude of inbred strains, a sequenced genome, and well-developed transgenic techniques, as well as the accessibility of all life stages of the mouse to investigation. The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org/) provides integrated access to genetic and phenotypic data for mouse models of human disease.

There are multiple ways to find disease models in MGI. Users can search or browse Online Mendelian Inheritance in Man (OMIM) disease terms to find published models for a specific disease. In addition, the integration of phenotypic and genetic data allows users to search for potential disease models using key characteristics of the disease alone or in combination with other criteria, such as chromosomal location or gene function. For example one can ask "What mutation or combination of mutations result in coloboma along with heart and ear abnormalities?". Finally, if a user is interested in a model involving a specific gene, they may search by gene name or synonym to find all known mutant alleles for that gene, as well gene trapped ES cell lines. These searches return information about the allele(s) including, details of the molecular mutation, descriptions of genotype specific phenotypic characteristics, any known human disease model associations, and a bibliography of relevant papers.

MGI currently includes almost 2000 genotypes associated with OMIM disease terms and over 10% of OMIM terms have one or more associated mouse models.

Supported by NIH grant HG000330.

2725/T

Global gene expression analysis using 7081 publicly available microarrays identifies 26 novel genes selectively expressed in fetal cartilage. *V. Funari[1], A. Day[2], D. Krakow[1, 2], S. Nelson[2], D. Cohn[1, 2].* 1) Medical Genetics Institute, Cedars-Sinai Medical Ctr, 8700 Beverly Blvd, Los Angeles, CA; 2) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Cartilage plays a fundamental role in the development, function and maintenance of the human skeleton and mutations in many genes selectively expressed in cartilage are associated with skeletal dysplasia phenotypes. To identify new genes which may play important roles in human skeletogenesis, microarray analysis was used to identify 90 uncharacterized or unannotated genes that were expressed at least 5-fold higher in fetal cartilage than in normal non-cartilage tissues. These genes were then further evaluated for their global gene expression patterns in 7081 publicly available microarrays. An approach was developed to determine which of these genes exhibited null or minimal expression with little variation within non-cartilage tissues, hypothesized to represent cartilage selective genes. 26 of the 90 novel genes exhibited the least variation in gene expression among non-cartilage tissues, and displayed expression patterns similar to a set of cartilage-selective control genes. All 26 genes were significantly down-regulated in cultured de-differentiated chondrocytes, indicating that these genes in part characterized the differentiated state of cartilage. Analysis of regional expression within the cartilage growth plate demonstrated that two of the genes, LOC200118 and C10ORF49, were down-regulated in the hypertrophic zone. In addition, orthologous proteins have been identified in other vertebrates, consistent with an evolutionarily conserved biological role. The protein structure of LOC200118 suggests it is a transcription factor, while C10orf49 encodes a protein predicted to contain a signal peptide and a protein-protein interaction domain suggesting it may interact with other proteins within the extracellular matrix. This study indicates that the cartilage transcriptome contains a rich resource of novel genes likely to have roles in skeletal development. These genes also represent novel candidate disease genes for inherited skeletal disorders.

2726/T

PhenCode: Linking Human Mutations and Phenotype. *B. Giardine[1], C. Riemer[1], W.J. Kent[2], W. Miller[1], R.C. Hardison[1].* 1) Center for Comparative Genomics and Bioinformatics, Pennsylvania State Univ, University Park, PA; 2) Center for Biomolecular Science and Engineering, University of California, Santa Cruz, CA.

PhenCode is a collaborative project to connect phenotype and clinical data with information on genome sequences, evolutionary history, and function. The phenotype data are located in various locus-specific databases (LSDBs) and centralized repositories, whereas genome data are located in browsers such as the one at UCSC. The project currently incorporates data from 119 LSDBs and SwissProt, including mutations associated with cystic fibrosis, phenylketonuria, immune disorders, muscular dystrophy, anemia, and cancer. The Locus Variants track at the UCSC Genome Browser displays genomic positions of the variants. By viewing Locus Variants in register with other tracks, users can integrate information from multiple data types. The detail page, accessed by clicking on a mutation in the display, presents summary information about the genotype and phenotype of the variant, along with links back to the data source for greater detail. One example shows different phenotypic severities of deletions that do or do not remove distal enhancers. The UCSC Table Browser provides the ability to query across the summary data from the LSDB's as well as queries comparing data across tracks. An example of this would be getting the substitutions from the Locus Variants track that are not in dbSNP but are in conserved regions. Websites:

Documentation for the PhenCode project: http://www.bx.psu.edu/

Locus Variants track (hg17, hg18): http://genome.ucsc.edu/.

2727/T

Transcriptome plasticity through mammalian RNA editing. *S. Maas, W. Gommans, N. Tatalias, D. Dupuis.* Biological Sciences, Lehigh University, Bethlehem, PA.

An important mechanism for the generation of molecular diversity in mammals is pre-mRNA editing by A-to-I modification. It increases RNA and protein diversity and regulates key functional properties of neurotransmitter receptors in the central nervous system by changing single codons in pre-mRNA. The deficiency or misregulation of editing has been implicated in the etiology of neurological diseases, such as epilepsy, amyotrophic lateral sclerosis (ALS), depression, and tumor progression.

We have recently identified Alu repeat elements in the human genome as a major target for A-to-I RNA editing. These findings suggest additional roles for RNA editing and links it to other RNA processing phenomena, such as alternative pre-mRNA splicing as well as siRNA mediated gene silencing and miRNA function. We and others have further mapped RNA editing events to micro RNA precursor sequences that suggest changes for the processing of miRNAs and miRNA target specificity.

In all known and characterized cases of recoding by A-to-I editing the ensuing amino acid substitutions have been linked to alterations in protein function. A few additional cases recently identified involved edited nucleotides that had previously been annotated as a single nucleotide polymorphism (SNP) according to the NCBI dbSNP database. In each case the validation for the SNP was solely based on expressed sequence data. This observation raises the possibility that the pool of A/G SNPs that have not been genomically validated might contain more cases of A-to-I RNA editing.

Here we present the results of a bioinformatics approach to delineate A-to-I RNA editing events in the human genome that lead to non-synonymous amino acid changes based on the subset of non-genomically validated SNPs. Out of several hundred potential targets we experimentally confirmed the occurrence of RNA editing in vivo for high scoring candidate genes and predict additional targets.

2728/T

THE RNA SPLICER GENE SRRM2 IS STRONGLY ASSOCIATED WITH PARKINSON DISEASE. *L.A. Shehadeh[1], N.F. Tsinoremas[4], J.M. Vance[3], N. Adi[2], S. Papapetropoulos[2].* 1) Mol and Cell Pharmacology, University of Miami Miller School of Medicine, Miami, FL; 2) Neurology,UM; 3) Human Genetics, UM; 4) Informatics, Scripps Florida Research Institute.

OBJECTIVES: Parkinson's disease (PD) is a complex neurodegenerative disorder with both genetic and non-genetic factors involved in its etiopathogenesis. Our hypothesis is that by interrogating transcriptome-wide expression data from different PD tissue sources we can discover susceptibility genes that may have sizeable public health benefits. METHODS: We performed extensive analysis on 3 microarray experiments. A total of 148 raw Affymetrix data (cel) files available through GEO were utilized: (A) 22 genechips from substantia nigra (SN) in postmortem brain of PD and controls, (B) 21 from rotenone-treated neuroblasoma cells (an in vitro model of PD), and (C) 105 from blood of PD versus healthy and neurological disease controls. Raw expression files were normalized using GC-RMA processor and analyzed using GeneSpring software. RESULTS: There were 174 transcripts corresponding to 160 genes that were overlapping in at least 2 out of the 3 experiments with only one gene overlapping among all the three PD experiments: the RNA splicing gene SRRM2 (or SRm300), (sereine/arginine repetitive matrix 2. It has been previously reported in two other PD expression studies as significantly upregulated in postmortem substantia nigra of PD patients versus controls. SRRM2 transcript was upregulated by 80% ($p<0.01$) in the substantia nigra of PDs versus controls and by 40% in the 4 wk rotenone treated cells versus the controls. Interestingly, while SRRM2 transcript was upregulated by 20% in the blood of female PDs versus female healthy controls, and 30% in the blood of male PDs versus male healthy controls, it was not changed in the blood of neurological diseased female or males versus the healthy controls. CONCLUSION: The consistent upregulation of the RNA splicer gene SRRM2 in two different PD neuronal sources and in PD blood but not in blood of neurologically diseased patients makes SRRM2 a strong candidate gene for Parkinson disease and draws attention to the role of RNA splicing in the disease.

2729/T

***VaryGene*, a new satellite database of annotated human polymorphism in the integrated human transcriptome database H-InvDB.** *M.K. Shimada[1,2], Y. Yamaguchi-Kabata[2], C. Yamasaki[1,2], T. Imanishi[2], T. Gojobori[2,3].* 1) Japan Biological Information Research Center, Koto-ku, Tokyo, Japan; 2) Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan; 3) Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Shizuoka, Japan.

The H-Invitational Database (H-InvDB; http://hinv.jp/) is an integrated database of human transcriptome based on extensive annotation of human full-length cDNA (FLcDNA) clones. The latest release contains annotation of 175,542 mRNAs and FLcDNAs extracted from the public DNA databank. We determined 34,701 gene clusters, which could define 34,093 (98.3%) protein-coding and 608 (1.8%) non-protein-coding loci, while 860 (2.5%) protein-coding loci overlapped with predicted pseudogenes. We provide in-depth annotation of alternative splicing isoforms, functional non-coding RNAs, functional domains of proteins, subcellular localizations, metabolic pathways, predictions of protein 3D structure, mapping of SNPs and microsatellite repeat motifs, co-localization with orphan diseases, gene expression profiles, evolutionary features and protein-protein interactions. Here we present a new satellite database of H-InvDB for annotated human polymorphism, "*VaryGene*". We reviewed publicly available polymorphism by mapping onto H-Inv transcripts and evaluating the quality of SNP information. *VaryGene* shows annotated polymorphism (SNPs and indels) information including location in transcripts as well as in the reference genome sequence, original classification of SNPs for each transcript by effects on gene products, relations with functional domains and links to public databases. *VaryGene* includes 40,484 synonymous SNPs, 53,754 nonsynonymous SNPs, 1,258 nonsense SNPs that cause 593 NMD events, and 159 SNPs that read through original termination codons, as well as 1,535 indels that cause 1,331 frameshift events. We believe that the release of *VaryGene* as a satellite database of H-InvDB may facilitate the progress of human genetic researches.

2730/T

Functional organization of the transcriptome in human cerebral cortex, caudate nucleus, and cerebellum. *M.C. Oldham[1], S. Horvath[2], K. Iwamoto[4], T. Kato[4], D.H. Geschwind[3].* 1) Neurosci. PhD Program; 2) Biostat., Human Genet; 3) Human Genet., Neurology, & Semel Inst., UCLA, Los Angeles, CA; 4) Lab. for Mol. Dynamics of Mental Disorders, Brain Sci. Institute, RIKEN, Saitama, Japan.

Microarrays have emerged as a powerful tool for exploring the functional identities of tissues by enabling comparisons at the level of the transcriptome. In the brain, transcriptional profiling is complicated by significant cellular heterogeneity, which can cloud functional context. New analytic methods that treat microarray data as a holistic system instead of a collection of discrete measurements have shown great promise in illuminating the higher-order structure of biological networks. Here we apply one such method, weighted gene coexpression network analysis, to microarray data derived from human cerebral cortex, caudate nucleus, and cerebellum. Through detailed exploration of gene coexpression relationships we provide an integrated view of the transcriptome in each brain region. We demonstrate that the network structure of gene coexpression in human cerebral cortex is highly reproducible across microarray platforms and individuals, suggesting a fundamental organization to the cortical transcriptome that has not been previously recognized. Through comparisons with cerebellum and caudate nucleus we identify many aspects of network structure that are conserved across brain regions, and some that are not. We characterize modules of co-expressed genes that correspond to each of the major cell classes of the brain: neurons, oligodendrocytes, astrocytes, and microglia. Other modules distinguish additional cell types, organelles, synaptic function, and gender differences. We introduce a quantitative metric that describes how strongly each gene "belongs" to each module in a given gene coexpression network. Our analysis provides a new foundation for neurogenetic inquiries and reveals the existence of a previously unrecognized functional organization to the human brain transcriptome. Support: Postmortem brain tissue was donated by The Stanley Medical Research Institute's brain collection courtesy of Drs. Michael B. Knable, E. Fuller Torrey, Maree J. Webster, Serge Weis, and Robert H. Yolken.

2731/T

A genetic and genomic approach to neurofibromin function. *A. Pemov[1], C. Park[1], L.M. Messiaen[2], K. Reilly[3], D.R. Stewart[1].* 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) Dept. of Genetics, UAB, Birmingham, AL; 3) NCI-Frederick, Frederick, MD.

Introduction. Neurofibromatosis type 1 (NF1) is a monogenic disorder of dysregulated tissue growth. The causative gene, *NF1*, encodes the tumor suppressor neurofibromin. Its other putative functions are not well understood. We hypothesized that the function of neurofibromin can be determined by comparing differences in gene expression between affected (A) and unaffected (U) individuals in human lymphoblastoid cell lines (LCLs). We also examined expression differences in B-cells from *Nf1+/-* mice. *Methods.* Three age- and sex-balanced LCL datasets (18 adults, 9 children, 8 adults) were balanced for number of A and U individuals. The mouse dataset included 6 *Nf1+/-* C57BL6/J animals and 6 WT animals. All animals were ~ 8 months old and sex-balanced. Total RNA was isolated from LCLs and mouse spleen B-lymphoblasts and analyzed on Illumina platform. A permuted t-test comparing A vs. U gene expression within a dataset, overlap analysis of the top 500-700 differentially-expressed genes among the datasets, and Gene Set Enrichment Analysis (GSEA - Broad/MIT) was performed. *Results.* 1) Permuted t-test and overlap analysis found few statistically-significant genes; 7 of 14 genes validated by qPCR were significant in at least one dataset. The genes are involved in a variety of biological processes, including protein kinase cascade and G-protein coupled receptor signaling. 2) GSEA analysis of the 4 datasets revealed enrichment of our datasets with numerous gene sets (FDR < 0.05) mostly related to cell cycle regulation, DNA replication and interferon signaling. *Discussion.* Traditional microarray analysis techniques (e.g. permuted t-tests) found few relatively high-scoring differentially expressed genes within and among our 4 sample sets; this is likely due to the small sample size and modest expression differences. The GSEA approach, which identifies biological processes rather than highly significant individual genes, allowed us to detect additional transcriptional profiles (e.g. cell cycle regulation and interferon signaling) that are perturbed in NF1-affected patients and *Nf1+/-* mice.

2732/T

Potential Association between DUF1220 Domains and Autism, Autism Spectrum Disorders, and Mental Retardation. *M.C. Popesco[3], L. Dumas[1], M. Cox[1,2,3], J. Hopkins[1,2,3], A. Karimpour-Fard[4], J.M. Sikela[1,2,3].* 1) Human Medical Genetics Program, University of Colorado at Denver & Health Sci Ctr, Aurora, CO; 2) Neuroscience Program; 3) Dept of Pharmacology; 4) Dept of Preventative Medicine and Biometrics.

The 1q21.1 region appears to be one of the most complex and dynamic regions in the human, containing a disproportionately high number of sequence gaps. We have identified 10-12 genes (e.g. NBPF8, NBPF11, NBPF20, and PDE4DIP) in the 1q21.1 region encoding DUF1220 domains, a protein domain of unknown function, which appears to be highly amplified in humans. In previous work, we used Western and immunocytochemical analysis to show strong expression of DUF1220 domains in neurons of the human neocortex, a region potentially involved in higher cognitive abilities. A previously reported genome-wide study of copy number variation in individuals with unexplained cognitive dysfunction identified de novo deletions in a 3-Mb region of 1q21.1, suggesting that this may be a recurrent pathological syndrome. More recently results from a comprehensive search for Autism Spectrum Disorder (ASD)-related genes support the possible involvement of DUF1220 domains in ASDs. Specifically, a 1.1 Mb copy number gain was identified at 1q21 in three ASD individuals. Four independent studies show that a 1.1-2.1 Mb region of 1q21 that contains a number of DUF1220-encoding genes (NBPF family) is deleted or duplicated in individuals with idiopathic mental retardation (IMR) autism or ASD, supporting the potential association of DUF1220 domains with these cognitive disabilities. The most recent human genome assembly predicts that, of 44 genes in this region, 6 are NBPF genes and the region encodes 57 DUF1220 domains. In addition, we have used DUF1220-specific Q-PCR to demonstrate that DUF1220 sequences are deleted (43 copies vs. 62 in a normal control) in an IMR individual with a 1.47 Mb de novo deletion of 1q21.1 sequences. In summary we demonstrate a potential link between DUF1220 domains and diseases of human cognition, a finding that, if substantiated, further supports the involvement of DUF1220 domains in higher cognitive function in humans.

2733/T

Overexpression of Helicobactor pylori-associated urease mRNAs in human gastric cancer. *M.Y. Huang[1], C.H. Wu[2], C.S. Yeh[2], T.L. Cheng[3], J.Y. Wang[4], S.R. Lin[5].* 1) Department of Radiation Oncology, Kaohsiung Medical University, Faculty of Medicine,College of Medicine,Graduate Institute of Medicine,and Kaohsiung Medical University; 2) Graduate Institute of Medicine; 3) Faculty of Biomedical Science and Environmental Biology; 4) Departments of Surgery; 5) Graduate Institute of Medical Genetics,and Kaohsiung Medical University,Taiwan.

Background and Purpose: Urease is involved in H. pylori infection and survival in acid circumference. This study explored the overexpression of H. pylori-associated urease mRNAs in human gastric cancers by using a well-established membrane array analysis method in our lab. Method: Analysis of 30 gastric cancer tissue specimens and 30 paired adjacent normal tissues demonstrated that urease genes involved in H. pylori infection were up-regulated in gastric cancer tissues. UreA, G and I are predominant genotypes found in gastric cancer tissues. However, the mRNA levels of UreC and UreE were hardly to be found in both gastric cancer and normal tissues in our study. In addition, we treated NIH-3T3 cells with two kinds of H. pylori exudates (weak urease activity (HP-W) and strong urease activity (HP-S)) which contained 1.6, 3.1, 6.5, 13 and 25.9 pg/ml urease of HP-W exudates and 18, 36, 75, 150 and 300pg/ml urease of HP-S exudates. NIH-3T3 cells were treated with these different concentration components for 24 hours. Result: Cell proliferation rate was elevated 2.7%, 9.9%, 18.9%, 36.6%, and 42.9% respectively after HP-W exudates were treated, and elevated 8.1%, 31.9%, 45.9%, 74.9%, and 81.3% respectively after treatment with HP-S exudates. In further investigation of the time course of NIH-3T3 cells treated with 50ug/mL H. pylori, the exudates revealed that the proliferation rate was elevated 14%, 23.7%, 38.7%, 31.6% and 29% respectively after HP-W treatment and elevated 29.8%, 50.4%, 78.5%, 62.3% and 55.9% after HP-S treatment for 6, 12, 24, 48 and 72 hours respectively. Conclusions: Membrane array promises a new diagnostic tool to detect H. pylori more sensitively than the CLO test. These results suggest that urease may play an important role in the development of gastric mucosal hyperproliferation in H. pylori-induced gastritis.

2734/T

Association between high myopia and PAX6 promoter polymorphic region. *C.Y. Lam, P.O.S. Tam, D.S.P. Fan, S.W.Y. Chiang, D.Y. Wang, B.J. Fan, G.H.F. Yam, D.S.C. Lam, C.P. Pang.* Ophthalmology & Visual Sci, Chinese University of Hong Kong, HKSAR.

Myopia is the most common eye disorder worldwide and the prevalence in Asia may exceed 65%. High myopia (HM) is defined as refractive error ≤-6.00 D. There is a high and increasing prevalence of HM in Hong Kong. Apart from impaired vision, it is a common cause of severe complications of macular diseases and retina detachment. A myopia locus was reported on chromosome 11p13 and the PAX6 gene located at that region was postulated to be associated with myopia development. This study aims to investigate the association of the PAX6 gene with HM in a Hong Kong Chinese cohort. The promoter, coding sequences and adjacent splice-site regions of the PAX6 gene were screened in 191 unrelated HM patients with refractive error -6.00D or below and 161 unrelated control subjects by direct sequencing after PCR. Three sequence changes were identified. R81R (678A>G) was found in one HM patient and S412S (1617C>T) was found in one control subject. Another polymorphism in the non-coding region, IVS10-12C>T, occurred in both patients and control subjects with P > 0.05. Twenty polymorphisms were identified in the promoter region but none of them showed association with HM (P>0.05). Two dinucleotide repeats (AC)m and (AG)n in the promoter region were found to be highly polymorphic. Association was observed between these two repeats with HM in which HM patients had high repeat number in both (AC)m and (AG)n (p-value=0.0001317 and p-value=0.001). Our results indicated that the Chinese population does not show association between HM and polymorphisms in PAX6 coding region but the AC and AG dinucleotide repeats in the promoter region was significantly associated with HM. These two repeats in the promoter region may affect the transcription activity of PAX6 and therefore contribute to myopia. We are conducting functional analysis of the PAX6 promoter polymorphic variants to confirm investigate the effect of the length variations of these repeats in transcriptions.

2735/T

Disruption of MATR3 and AHDC1 in Noonan-like syndrome. *F. Quintero-Rivera[1,2,8], A.W. Higgins[3,8], A. Roberts[4,6], R. Kucherlapati[4,6], G. Bruns[4], I. Seong[2], B. Gelb[7], H. Ferguson[3], R. Maas[5,8], C.C. Morton[3,8], J.F. Gusella[2,8].* 1) Pathology & Lab Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Center for Human Genetic Research, Massachusettes General Hospital, Boston, MA; 3) Departments of Pathology, Brigham & Women's Hospital, Boston, MA; 4) Genetics Division, Children's Hospital Boston, MA; 5) Genetics Division, Brigham and Women's Hospital, Boston, MA; 6) Harvard Partners Center for Genetics and Genomics, Boston, MA; 7) Pediatrics and Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 8) Harvard Medical School, Boston, MA.

To uncover genes critical in human development, patients with congenital anomalies and apparently de novo chromosomal rearrangements are being studied through the Developmental Genome Anatomy Project (DGAP). Here, we report a 5-yo male who presented with a Noonan syndrome (NS)-like phenotype and 46,XY,t(1;5)(p36.11;q31.3)dn. We cloned and sequenced the junction fragments and found disruption of 3' UTR of Matrin3 (MATR3) on 5q31.3 and the AT hook DNA binding motif containing 1 locus (AHDC1) on 1p36.11. The Matrin3 protein (MAT3) localizes to the nuclear matrix and interacts with other components of the internal fibrogranular network. In Xenopus oocytes, Matrin 3 is part of a complex that regulates RNA editing. However, little is known about its expression and function in other organisms. In situ hybridization of mouse embryos demonstrated that Matr3 is predominantly expressed in the maxillary prominence, eye, second branchial arch and limb buds. In humans, the transcripts are highly expressed in heart and brain, which are affected in our patient. MAT3 levels are variable across normal controls in lymphoblastoid cell lines. In addition, a knock-out mouse model in which Matr3 is disrupted showed embryonic lethality of Matr3-/- at E9. We screened for mutations in AHDC1 and MATR3 in 120 individuals affected with NS that were negative for mutations in PTPN11, KRAS and SOS1. No pathogenic mutations were identified. Our data confirms that mutations of MATR3 and AHDC1 do not account for a significant proportion of Noonan-syndrome cases. This is the first report of a constitutional disruption of MATR3 and AHDC1.

2736/T

Global gene-expression analysis of eczema skin; a focus on skin barrier function. *A. Sääf, A. Liedén, E. Ekelund, M. Nordenskjöld, M. Bradley.* Center for Molecular Medicine, Karolinska University Hospital, S-171 76 Stockholm, Sweden.

Atopic eczema (AE) is a common skin disorder currently affecting up to 20% of children in some countries (1). AE usually begins in infancy or early childhood with a significant proportion of children having continued problems into adult life. Patients with AE suffer from itchy, dry and inflamed skin, often in combination with other atopic manifestations such as allergic asthma and allergic rhinoconjunctivitis (hay fever). Twin studies indicate a strong genetic contribution in the development of AE (2,3) and genetic linkage analyses have identified several chromosomal regions linked to AE (4-7). However, very little is known about specific genes involved in this complex skin disease and the underlying molecular mechanism is not yet identified. We used human cDNA microarrays to identify a molecular picture of the programmed responses of the human genome to the pathological condition of AE. Among the genes consistently over-expressed in AE skin as compared to skin from healthy control individuals were members of the transglutaminase family (TGM1 and TGM3) and corneodesmosin (CDSN) that play a central role in forming the outermost layer of the skin, the cornified envelope. These genes are localized to known susceptibility chromosomal regions for eczema (TGM1; 14q11, TGM3; 20p13, CDSN; 6p21.3). It is not known, however, if genetic polymorphisms in these genes contribute to skin barrier dysfunction in eczema patients. To answer this question, we investigated the role of genetic variation at these loci in the development of eczema. In summary, we here present a global gene signature of eczema skin, and furthermore genetic polymorphisms are described in candidate AE susceptibility genes identified by the microarrays. In conclusion, our data supports the hypothesis that barrier dysfunction is an important factor in eczema pathogenesis. 1. Morar 2006, J Allergy and Clin Immun 118(1):24-34 2. Larsen 1986, J Am Acad Dermatol 15:487-494 3. Schultz-Larsen 1993, J Am Acad Dermatol 28 :719-723 4. Lee 2000, Nat Genet 26:470-473 5. Cookson 2001, Nat Genet 27:372-373 6. Bradley 2002, Hum Mol Genet 11:1539-1548 7. Haagerup 2004, Allergy 59(1): 88-94.

2737/T

A Practical Approach: Multiplex Ligation-Dependent Probe Amplification (MLPA) Analysis of Deletions/Duplications in CFTR Gene Using Whole Genome Amplification (WGA). *L. Chou[1], K. Sumner[1], E. Lyon[1,2].* 1) Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City UT 84108-1221, USA; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132, USA.

In clinical diagnostic laboratories, a common challenge in validating a genetic test is the lack of positive controls. Positive controls are hard to obtain, because they are rare and/or have limited amounts (e.g. prenatal samples). Such samples are inadequate for reproducibility studies or as controls in routine testing. Copy number variations (CNV) techniques such as array CGH usually require large quantities of initial DNA input. There are reports describing the success of using whole genome amplified (WGA) samples with array CGH. However, there is no study regarding the use of WGA samples with MLPA, a small scale, high-resolution technique to detect CNV. Here, we first report the results of using WGA with MLPA, with the CFTR gene as a disease model. After initial optimization, we found exonic deletions could be reliably identified by MLPA using WGA samples. However, we observed consistent failures of one chromosomal control probe and one target specific probe. The reason of this failure is unknown; possible explanations include unexpected biased amplification affecting the quantitative analysis. In order to obtain reproducible results, three parameters need to be considered and optimized: DNA extraction method, post WGA purification, and initial DNA input for MLPA (>200 ng/uL). Based on these initial data, we conclude that it is possible to use WGA samples with MLPA technique.

2738/T

Quantitation of Fusion Transcripts Using TaqMan® Gene Expression Assays. *K.Y. Lee[1], F. Hu[1], E. Langit[1], C. Preud'Homme[2], J.M. Cayuela[3], B. Cassinat[4], M. de Graaf[1], S. Guenther[1], G. Marcus[1], P. Brzoska[1].* 1) Applied Biosystems, Foster City, CA; 2) Laboratoire d'Hematologie, Lille, France; 3) Laboratoire Central d'Hématologie, Hôpital Saint-Louis, Paris, France; 4) Unité de Biologie Cellulaire, Hôpital Saint-Louis, Paris, FRance.

Chromosomal aberrations such as translocations are frequently found in human cancer cells. Chromosomal translocations may result in a chimeric gene expressing a fusion transcript which is then translated into a fusion protein that affects normal regulatory pathways and stimulates cancer cell growth. A well known example is the BCR/ABL chimeric mRNA which is the result of a translocation of ABL on chromosome 9 to the BCR breakpoint cluster on chromosome 22. The resulting fusion transcript is the cause for 90% of chronic myelogenous leukemia. Current methods for identifying translocations include FISH and karyotyping, neither of which can be used to quantify the expression level of the fused gene. We have designed TaqMan Gene Expression Assays for a set of known fusion transcripts for quantitative analysis. We collected 214 fusion transcript GenBank mRNA Accessions representing 165 unique translocation events from two data sources (Chimer D:http://genomce.ewha.ac.kr/ChimerDB/ and Hahn et al, PNAS 2004;101;13257-13261. The transcripts were annotated and fusion breakpoint locations were identified or verified. Assays were designed such that the primers and probe spanned the breakpoint region and were not placed directly on the breakpoint. The transcript breakpoint region (~10bp), SNPs and repetitive sequences were masked before the assay was designed using the Applied Biosystems assay design pipeline. As proof of principle, several assay designs were tested against plasmids and patient samples known to contain the translocation variant. Only those samples containing the fusion transcript were amplified indicating the specificity of the assay. From a large number of assay designs, we selected 165 TaqMan Gene Expression Assays targeting each of the 165 translocation variants. These 165 assays for quantitating fusion transcripts are currently published on the Applied Biosystems Website (http://www.appliedbiosystems.com).

2739/T

Physical mapping of human chromosome 21p. *J. Doering[1], R. Ennesser[1], Z. Flener[1], E. Miller[1], S. Bracken[1], M. Cummings[2].* 1) Loyola University Chicago; 2) University of Illinois, Chicago.

While 10-15% of the human genome is composed of heterochromatic DNAs, these regions are not included in the completed sequence. We are creating a detailed physical map of the centromere and p arm of HC21 as a model for the organization of such regions. Our previous work demonstrated that the proximal short arm of HC21 contains a copy of the chAB4 duplicon that is actually part of a larger duplicon ~200kb in size. Copies of this duplicon are highly conserved on HC21, Y, 17 and 22. Further sequence analysis now reveals that there is a 47kb inverted repeat within this duplicon as well as poorly conserved copies of rDNA spacer sequences and acrocentric chromosome subtelomeric sequences. This duplicon on proximal 21p is located in the midst of the α21-II region, which contains multiple clusters of monomeric alphoid DNA more than 2Mb away from the centromere. Such an organization of alphoid sequences has not been found on other chromosomes to date. We have identified an HC21p BAC containing the 9kb subtelomeric repeat known to be on distal 21p13 and have constructed a nearly 400kb contig including that BAC by identifying sequences in the database that had not been previously placed in the overall genome map. This region contains complex rearrangements of portions of the same duplicon found in the proximal area of 21p. We have also constructed an overall physical map for most of 21p13. It contains a single 0.8Mb cluster of satellite I, adjacent to one major satellite III cluster of 0.5Mb, which in turn is adjacent to a small cluster of β satellite. Regions between these clusters appear to be composed of low copy number sequences. The overall organization of HC21p appears to be much more complex than originally proposed, and it indicates that extensive sequence rearrangements have been involved in the evolution of this chromosomal region.

2740/T

Zygosity determination using DNA prepared from saliva. *K. Duvefelt[1,2], A. Lindstedt[1], U. Hannelius[2], C. Lagerberg[2], G. Tybring[2], J. Kere[1,2].* 1) Karolinska University Hospital; 2) Karolinska Insitutet.

We investigated how DNA derived from saliva, collected with Oragene DNA kit, performed in SNP genotyping using the Sequenom MALDI-TOF technique. Subsequently zygosity was determined using our SNP panel.

The motivation for the analysis was to find out:

a) How did this DNA work with the genotyping methodology?

b) Was it possible to genotype using a large range of DNA concentrations?

c) How did the zygosity analysis perform with the generated data?

Saliva was collected using Oragene DNA self-collection kit from 44 twin pairs and DNA extraction was performed on the Gentra Autopure LS instrument using Puregene. The DNA concentration was measured with OD as well as PicoGreen. The correlation between these measurements was: R^2=0,59, probably due to impurities leading to absorbance giving an overestimation of the DNA content. One concern with saliva DNA is contamination of bacterial DNA that could lead to incorrect measurements of human DNA content. The PicoGreen quantification gave a range from 0-1002 ng DNA /μl with a mean of 97 ng/μl. Corresponding OD measurement ranged from 7-1364 ng/μl, mean 221 ng/μl. We selected 10 samples with PicoGreen measured concentrations from 6 to 1002 ng/μl for genotyping, with 24 different SNPs in one multiplex reaction, using eight different concentrations ranging from 2,5 ng to 320 ng. The success rate of genotyping for the different concentrations ranged from 87 to 98% (mean 97%). There was a 100% correlation of the genotypes over the concentration range. Subsequently all 88 samples were genotyped with our zygosity panel of 47 SNPs distributed in three multiplex reactions. The measured amount of DNA per reaction ranged from 0,25 ng to 250 ng. The success rate per sample was between 77 and 100% (mean 99%); per marker it had a mean of 98% (range: 93 to 100%). Zygosity could unambiguously be determined from 43 of the 44 twin pairs studied. We conclude that SNP genotyping of DNA originating from saliva collected by Oragene kit yields reproducible results using the Sequenom MALDI-TOF technique. The analysis could be performed over a wide range of DNA concentrations and subsequent analysis of the zygosity can be performed with reliable results.

2741/T

Delivery and retention of an episomal Herpes Simplex Virus type 1 amplicon vector containing the entire *HPRT* genomic locus in embryonic stem cells. *P. Edser[1], M. Quail[2], D. Adams[2], R. Wade-Martins[1].* 1) Univ of Oxford,UK; 2) Sanger Institute,Cambridge,UK.

Regulation of gene expression is controlled by a variety of elements found within genes including promoters, introns, and cis acting sequences. Many viral vectors used for gene delivery have a limited transgene capacity and cannot deliver an entire genomic DNA locus resulting in unregulated transgene expression. Herpes simplex virus type 1 (HSV-1) amplicons are able to carry inserts of up to ~150 kb. This capacity means that 95% of human and mouse genes can be delivered using the infectious bacterial artificial chromosome (iBAC) delivery method. HSV-1 amplicons can be packaged by a helper virus-free system and infect many different cell types. Inclusion of *oriP/EBNA1* Epstein Barr virus retention elements allows the HSV-1 iBACs to remain episomal, avoiding transgene silencing or cell transformation by insertional mutagenesis. Multipotent stem cells offer the possibility of cell replacement therapy. Embryonic Stem Cells (ESCs) are pluripotent and unrestricted as to which cells they can differentiate into given the correct signals. For example, ESCs are known to differentiate efficiently into neurons following established protocols. The ability to transduce ESCs would allow gene correction prior to cell transplantation and differentiation. We have previously infected mouse ESCs with a HSV-1 iBAC amplicon with an efficiency of up to 50%. Here we have infected the hypoxanthine phosphoribosyltransferase (*HPRT*) deficient ESC line, E14tg2a, with an iBAC containing the entire *HPRT* genomic locus. We used MOIs from 2 to 20 and saw high levels of delivery. Vector transduction was confirmed using the *EGFP* reporter gene. Infected cells were selected using hygromycin for prolonged periods and individual stable clones containing the iBAC were isolated. The long term retention of the episome is being assessed. We plan to differentiate ESC containing an HSV-1 iBAC vectors into neurons to follow transgene expression and phenotype correction. Our work demonstrates for the first time the transduction of mouse ES cells with a complete BAC genomic locus via the HSV iBAC delivery system.

2742/T

Human neural crest cells share a complex molecular signature with embryonic stem cells. *S. Thomas[1], M. Thomas[2], P.T. Xu[4], J. Poulain[3], C. Golzio[1], P. Wincker[3], M.C. Speer[4], A. Munnich[1], S. Lyonnet[1], M. Vekemans[1], H.C. Etchevers[1].* 1) INSERM U781 Hôpital Necker, Paris, France; 2) L'Oreal Recherche, Aulnay, France; 3) Genoscope, Evry, France; 4) Center for Human Genetics, Duke Univ, Durham, NC.

A fundamental question of developmental biology is how a wide variety of functionally different mature cell types arises from a unique cell population. Human neural crest cells (hNCC) form in the embryo during the third to fifth weeks of pregnancy. While partially competent progenitors continue to reside in some adult tissues in animal models, most NCC differentiate into all components of the peripheral nervous system, melanocytes, some types of endocrine cells as well as connective and structural tissues in the head. Abnormal development of hNCC leads to malformations and tissue dysplasias known collectively as neurocristopathies. To identify new candidate genes for neurocristopathies, we made a Long-SAGE library from hNCC derived from a normal embryo at Carnegie stage 13. Data analysis showed expression of multiple Gene Ontology functional classes that were anticipated based on animal studies and/or human disease. Hierarchical clustering was performed. A high degree of similarity was found between hNCC and human embryonic stem cells (hESC) compared to 14 other tissues or cell types, including more tissue-restricted stem cells, such as mesenchymal stem cells. Functional annotation of hNCC and hESC SAGE libraries shows that the same molecular cascades are statistically enriched in both cell types, such as transcription regulation, cellular proliferation and growth factor pathways. Furthermore, TPE analysis (Moreno et al., 2001) identified about 400 genes specific to either hNCC or hESC relative to 14 other tissues. Interestingly, half of the genes found preferentially expressed in hNCC were common to hESC and vice versa, with a number of molecules implicated in pluripotency. In addition to highlighting myriad candidate disease genes, this study demonstrates that premigratory hNCC present hallmarks of stem cells, paving the way for further studies of hNCC cell biology and therapeutic potential.

2743/T

Massively Parallel Sequencing of cDNA as a Strategy for Genomic Resequencing. *S.F. Nelson, B.L. Merriman, Z. Chen.* Dept Human Genetics, UCLA Medical Ctr, Los Angeles, CA.

The genome-wide resequencing of coding regions holds great potential value for disease research. For a specific example, Velculescu, et al (2006) sequenced coding regions of 13,026 genes in 11 tumor genomes in order to identify mutations contributing to tumor genesis, and more generally, in complex disease genetics the trend is generally towards resequencing ever larger numbers of candidate genes in affected individuals. The natural, universal limit of these efforts would be to simply resequence all exons as a general purpose screen for disease-relevant variants. Recent advances in sequencing technology make this plausible, but the current strategies employed for genome-wide exon surveys rely on PCR-related methods to capture the coding regions for sequencing, which is a costly, laborious, and often problematic component of the procedure. In contrast, here we suggest using cDNA as a means to effectively extract the coding portion of the genome for sequencing, and this is also well suited to the new massively parallel sequencing technologies which can sequence complex DNA pools. To explore the practicality of the approach, we use empiric data from deep sequencing of full length cDNA from gliomas and glioma derived cell lines, and using the Solexa sequencing system to generate depths of up to ~120,000,000 short reads per library. Specific issues we address are the number of genes that are effectively resequenced in this approach, and whether dis-regulated expression---as present in tumor samples or induced cell cultures---enables better sequencing coverage of genes that are not commonly transcribed. The strengths of this approach are the simplicity with which it targets all exons for sequencing, and its ability to properly utilize the enormous sequencing capacity of the new sequencing technologies.

2744/T

How good is whole-genome amplified DNA for genome-wide association studies? *N. Akula[1], S. Cichon[2], M. Noethen[2], F. McMahon[1].* 1) Genetic Basis of Mood and Anxiety Disorders, National Institutes of Mental Health/National Institutes of Health, Bethesda, MD; 2) Department of Genomics, Life & Brain Center, University of Bonn, Germany.

The quality and quantity of available DNA samples remains a limiting factor for genome-wide association studies. This issue becomes even more critical if cell lines are not available or samples were collected under sub-optimal conditions. Whole-genome amplification is a cost-effective solution, but the performance of whole-genome amplified DNA on genome-wide single-nucleotide polymorphism chips is not well established. We genotyped four unrelated DNA samples derived from whole blood on the Illumina HumanHap550 genotyping chip both before and after whole-genome amplification, and compared the results. The genotype discrepancy rate between genomic DNA and whole-genome amplified DNA was less than 0.02% in all samples we tested. One SNP in an inter-genic region on chromosome 2p was discrepant in all 4 samples between the genomic and whole-genome amplified DNA. Call rates for genomic DNA samples were all greater than 99%, whereas call rates for the corresponding whole-genome amplified samples were 95%-96%. This study demonstrates that whole-genome amplified DNA performs well on a widely-used genome-wide genotyping platform.

2745/T

Performance of whole genome amplified DNA in genome-wide association studies. *M. Inouye[1], Y.Y. Teo[2], K.S. Small[2], A.E. Fry[2], S.C. Potter[1], S.J. Dunstan[3], M. Seielstad[4], I. Barroso[5], N.J. Wareham[6], K.A. Rockett[2], D.P. Kwiatkowski[1,2], P. Deloukas[1].* 1) Human Genetics, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; 3) Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam; 4) Agency for Science, Technology and Research, Singapore; 5) Metabolic Disease Group, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom; 6) MRC Epidemiology Unit, Strangeways Research Laboratories, United Kingdom.

Research into the genetic basis of common diseases is expanding rapidly with the advent of genome-wide association studies. Experimental success in these studies relies on accurate SNP typing with high-density probe arrays thus requiring high quality DNA, which is often irreplaceable and available in finite amounts. Whole genome amplification allows nanogram quantities of DNA to be rescued for SNP genotyping. However, previous studies have not accurately reflected how robustly panels of 300,000 to 1 million SNP sets will perform with amplified DNA, thus highlighting the importance of a genome-wide map of amplified DNA performance on high-density genotyping arrays. We performed an extensive comparison of amplified DNA with genomic DNA from 4,995 individuals from 4 separate cohorts genotyped at three facilities in America, Britain and Singapore. These samples were run on multiple genome-wide arrays from both Affymetrix's GeneChip and Illumina's BeadArray. Amplified DNA exhibited a substantial decline in call rates when compared against genomic DNA, and in selected regions of the genome we observed correlated differential rates of probe hybridization and signal loss on both platforms. These regions are highly dependent on GC content and segmental duplications, thus resulting in a biased set of lower quality SNPs which lowers genome coverage. We also highlight the promise of genotype imputation to recover lost performance.

2746/T

Massively Parallel cDNA Resequencing For Transcriptome Analysis. *Z. Chen, B.L. Merriman, Y. Lee, B. O'connor, S.F. Nelson.* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

The new massively parallel resequencing technologies are well suited to gene expression profiling and transcriptome analysis. By adopting suitable methodologies, it is possible to simultaneously do basic transcript counting for quantitative gene expression analysis, as well as resequence transcripts to detect alternative splice variants, allelic expression differences, and coding sequence mutations. Here we investigate the viability of these various goals within the context of resequencing a large number of tumor cDNA libraries using the Solexa massively parallel sequencing technology, which allows us to sequence cDNA libraries with a depth of up to ~100,000,000 36-mer reads. We also consider important related issues such as the impact of protocols for cDNA library generation and specifics of the sequencing protocol on the quality of the resulting data.

2747/T

Large highly conserved non-coding transcripts are mutational targets in cancer. *D.I Smith[1], D.S. Perez[1], A.L. Ducharme-Smith[1], J. Pritchett[1], S. Ganapathiraju[1], D.A. Ahlquist[2].* 1) Lab Med & Path/Exper Path, Mayo Clinic, Rochester, MN; 2) Department of Medicine, Mayo Clinic, Rochester, MN.

Whole-genome tiling arrays are a powerful tool to identify novel transcripts across the entire human genome. We utilized these arrays with normal human bronchial epithelial cells (NHBE) comparing the transcriptional expression of untreated cultures to those exposed to either growth under hypoxic conditions or to the cigarette carcinogen NNK. Our initial results demonstrate the existence of numerous non-coding transcripts (NCTs) that are either transcriptionally active and/or stress-responsive across the genome. Of the non-coding sequences identified, the ratio of transcriptionally active regions (TARs) to stress-responsive regions (SRRs) is approximately 5 to 1. We focused on characterizing novel, large (>400 bp), abundantly-expressed, and highly-conserved non-coding (nc) TARs. Using real-time RT-PCR we observed that some of the ncTARs displayed tissue-specific expression while others were more ubiquitously expressed. Many of these large conserved ncTARs were also found to have aberrant expression in different cancers. A subset of the ncTARs examined were also mutated in different cancers. The role of these ncTARs in the normal cell, as well as the role that mutations in these sequences play in cancer development is presently unknown. We have also begun to identify a novel group of large, highly-conserved sequences whose expression is apparently altered by cellular stress. A number of these ncSRRs also have aberrant expression in different cancers and are being similarly examined to determine if they are also targets of mutation in different cancers. Collectively, this analysis has only examined the most abundantly-expressed or most differentially altered transcripts across the human genome, thus, there are potentially thousands of additional large, highly-conserved NCTs across the human genome. Future studies will attempt to determine the mechanistic action of such non-coding sequences in normal cellular function and disease, and how they might interact with protein-coding genes within crucial signaling pathways.

2748/T

Identification of nuclear genes associated with mitochondrial functions by transcriptional profiling of human cell lines lacking mtDNA. *R. Mineri[1], N. Pavelka[2], P. Ricciardi-Castagnoli[2], M. Zeviani[1], V. Tiranti[1].* 1) Molecular Neurogenetics Unit, IRCCS Foundation Neurological Institute C. Besta, Milan, Italy; 2) Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy.

Mitochondrial biogenesis is under the control of two different genetic systems: the nuclear and the mitochondrial genome (mtDNA). To identify which nuclear transcripts were subjected to retrograde regulation in a mammalian system, we performed a comparative microarray analysis of global RNA expression profiles in two immortal human cell lines and in their rho° cell derivatives, i.e. cells that had been completely depleted of mtDNA by exposure to ethidium bromide (EthBr) a DNA intercalating agent. Affymetrix HG-U133A GeneChips, designed to interrogate the expression of more than 22000 genes, were used to probe cRNAs extracted in triplicate from rho° and parental cells. Using a highly stringent statistical approach, we identified 191 genes the expression of which was significantly and consistently different in both rho° cell lines, versus their parental cell lines. In order to determine whether our gene cohort were significantly enriched in gene products associated with specific molecular functions, subcellular compartments or biological processes, we analyzed the functional annotations of the 191 differentially expressed transcripts by using their associated Gene Ontology terms. Four functional categories were identified: protein synthesis (14 genes), mitochondrial proteins (16 genes), mRNA processing (4 genes), and cell cycle and chromatin structure (12 genes). Three major achievements were obtained from this analysis indicating that the absence of mtDNA determines: i) a reduction of the cell replication rate, ii) a down-regulation of nuclear-encoded subunits of complex III of the respiratory chain and iii) a down-regulation of a gene described as the human homolog of Elac2 of E. coli, which encodes a protein that we show to also target to the mitochondrial compartment by standard immunofluorescence and mitochondrial import assays in transfected cell lines.

2749/T

The Jackson Laboratory Repository: Models of Human Disease. *S. Rockwood, C. Lutz, M. Sasner, L. Donahue.* Genetic Research Science, The Jackson Laboratory, Bar Harbor, ME.

The Repository at The Jackson Laboratory (TJL) serves as a centralized source for mouse models of human disease. The longevity of the constituent programs organized under the Repository (est 1960's) has fostered the evolution of an infrastructure supporting the efficient importation, cryopreservation and distribution of mouse models to the biomedical community. Approximately 300 new strains are added annually to the over 3500 strains in the Repository. Although a broad spectrum of strain types are imported, several specific disease areas have been targeted for expansion, most notably Parkinson's Disease, Alzheimer's Disease and Spinal Muscular Atrophy (SMA). The Parkinson's Disease Mouse Model Repository (PDMMR) and Alzheimer's Disease Mouse Model Repository (ADMMR) projects serve similar functions for their respective disease areas within the main Mouse Repository. Under these programs, mice will be rederived to a high health status; key alleles moved to standard genetic backgrounds and distributed. When appropriate, aged mice will be made available. A database is available for researchers to retrieve information related to strains. Search results contain information describing phenotype, development, maintenance, licensing and references. To facilitate searches for strains related to human diseases, queries can be executed using OMIM search terms. Alternatively, researchers can conduct searches using the Mammalian Phenotype Ontology developed by the MGI group at TJL. In anticipation of a continued increase in the use of cre-lox mutants in human disease research, characterization of cre-expressing strains is being added. Using a lacZ reporter strain, photomicrographs of expression patterns in embryonic and postnatal tissues are being made available on-line. Donating a strain to the Repository fulfills the NIH's requirements for sharing of mice. Researchers wishing to have strains considered for inclusion in the Repository are encouraged to submit their strains at: http://www.jax.org/grc/index.html. Support is provided by the NCRR (RR09781, RR11083, RR16049), NIA, HHMI, The Ellison Medical Foundation and from private charitable foundations.

2750/T

Defining the Ciliary Proteome. *Y. Liu[1], J.L. Badano[1], N. Katsanis[1,2,3].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; 3) Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD.

The cilia are hairlike organelles projecting from the surface of the cell that distribute nearly ubiquitously in all the vertebrate cells. They are not only in the context of fluid of cell motility as thought tranditionally, but also major sensory function centers to mediate the transmission of several key morphogenetic pathways. These broad roles for cilia are highlighted by the fact that ciliary dysfunction leads to a broad range of human phenotypes.

Considering the importance of the cilium, and its potentially central role in numerous cellular processes, we and others have sought to define and experimentally validate the mammalian ciliary proteome. 10 independent protein sets, each enriched for ciliary molecules have been generated, which have recently been integrated by the Katsanis lab into a single proteome, consisting of some 1,200 human proteins and deposited into a custom-generated unrestricted database (http://www.ciliaproteome.org/, Gherman et al. Nature Genetics 2006).

However, the contents of the ciliary proteome largely remain a computational prediction, requiring validation. Towards that goal, we have initiated a large-scale cell localization study to determine a) which proteins localize to the cilium and/or the basal body and b) how are they perturbed upon introduction of lesions that disrupt ciliary function. We studied their cellular localization by imaging live and fixed ciliated mammalian cells, costained with known centrosomal markers, such as γ-tubulin. Among the first test set of 96 proteins, 23 show specific patterns of cellular localization. Some of them are centrosomal and basal body proteins, some are aggregate around centrosome and basal body, some are localized in cilium, and some others are expressed in nucleus. We may go on to study the relationship between these proteins with specific cellular localization and the previously known ciliary proteins or microtubule network components.

2751/T

Genome wide identification of transcriptional start sites in human cancer cell lines with SOLiD sequencing technology. *S. Hashimoto[1], K. Matsushima[1], S. Morishita[2], R.C. Nutter[3].* 1) Graduate school of Medicine, The University of Tokyo, Tokyo, Japan; 2) 2Department of Computational Biology, Graduate School of Frontier Sciences, The University of Tokyo, Japan; 3) Applied Biosystems, Foster City, CA.

Genomic and full-length cDNA sequences provide opportunities for understanding human gene expression. Although the established functional genomic technologies, for example, DNA array and SAGE, can identify coding and noncoding RNA transcripts, identification of genes across the whole genome is still problematic. We and others have developed high-throughput methods, such as 5'SAGE that enable genome-wide identification of transcription start sites (TSSs) and that allow quantification of mRNA transcripts. Determination of the TSS would be the first step in identifying the promoter region, which pivotally regulates transcription of the gene. Although the TSS of most genes show heterogeneity, this may reflect physiological, developmental, and pathological states of the particular cells or tissues. The use of these methods markedly facilitates identification of full-length protein-coding and noncoding RNAs, and also of their promoter regions. We have recently improved these methods for the newest sequencing method, Oligonucleotide ligation and detection(SOLiD) technology, so called "5'end-SOLiD". An important factor in tumor development seems to be the epigenetic effects on tumor suppressor genes. Because of its ability to suppress tumor cell proliferation, angiogenesis, and inflammation, the epigenetic drug such as histone deacetylase (HDAC) inhibitor is currently in clinical trials. However, how epigenetic drugs mediate its effects is poorly understood. To assess the effects of epigenetic drugs, the gene expression by 5'end-SOLiD in colon cancer cell lines treated with epigenetically affecting agents, 5-aza-2'-deoxycytidine, a potent inhibitor of DNA methylation and trichostatin A, a HDAC inhibitor was investigated. Epigenetic modification induced not only the change of expression of the cell cycle progression-associated genes in human colon cancer cells but also the gene expression with aberrant start sites.

2752/T

Global assessment of microRNA related variation on expression QTLs. *C.M. Lindgren[1,2], F. Pettersson[1], M. Jain[1,3], J.M. Taylor[1], J.L. Min[1], A.L. Gloyn[2], J.C. Barrett[1], J. Broxholme[1], M.I. McCarthy[1,2], K.T. Zondervan[1], L.R. Cardon[1].* 1) WTCHG, Oxford, UK; 2) OCDEM, Oxford, UK; 3) NHGRI, NIH, USA.

MicroRNAs (miRNAs) are endogenous, small noncoding RNAs that regulate target mRNA by binding to regulatory sites in 3' untranslated regions (UTR) of their targets. Genetic variation in miRNA sequences or their targets has been shown to disturb their interaction and result in diverse phenotypes spanning from massive meatiness in sheep to Tourette's syndrome in humans. We hypothesize that genetic variations in and immediately surrounding the 475 miRNA sequences are associated with expression quantitative trait loci (eQTL) in humans, which could subsequently contribute to various phenotypic differences. Thus, we tested the genotypes of 147 miRNA related single nucleotide polymorphisms (SNPs) in HapMap against expression levels from ~47,000 different gene transcripts collected from lymphoblastoid cell lines of 60 unrelated CEU HapMap individuals (public data, Stranger et al Science 2007) using linear regression. Nominally significant results were further tested in 90 Chinese and Japanese (CHB+JPT) and 60 Yoruba (YRI) unrelated HapMap samples. Our analyses in the CEU population show 42 SNPs that are nominally associated with 90 transcript levels ($p<10^{-5}$). Expression levels of two transcripts in the mitochondrial ribosomal protein gene L43 (*MRPL43*) are associated with a SNP (rs4919510) in the mature *miRNA-608* sequence ($p<10^{-6}$ & $p<10^{-8}$, respectively) of which one replicates in both the CHB+JPT ($p<10^{-13}$) and YRI ($p<10^{-13}$) populations, after Bonferroni corrections. The data indicates expression of *miRNA-608*, which is needed for regulation of target genes and *MRPL43* is non-conservatively predicted to harbor a *miRNA-608* binding site (RNA22, IBM). Thus, we report cis-regulation of *MRPL43* by variation located in the mature *miRNA-608* sequence replicated in replicated in three independent and ethnically diverse populations. Effects on eQTL's through genetic variation in miRNA could provide further insights to how they contribute to phenotypic variation, including disease susceptibility in man.

2753/T

RNAi of human transcription factors for analysing regulatory networks. *M. Sultan[1], I. Piccini[1], D. Schmidt[1], D. Balzereit[1], A. Fiebitz[1], W. Wruck[1], R. Herwig[1], I. Ulitsky[2], F. Buchholz[3], H. Lehrach[1], M.L. Yaspo[1].* 1) Max Planck Institute for Molecular Genetics, Berlin, Germany; 2) Tel Aviv University, Tel Aviv, Israel; 3) Max Planck Insitute for Molecular Cell Biology and Genetics, Dresden, Germany.

Systematic analysis of transcription factors (TFs) and associated gene regulation networks are of central relevance to developmental biology and medicine. In order to get insights into the nature and complexity of gene regulatory networks we analysed global gene expression profiles after knocking down specific transcription factors by means of RNAi technology in human cell lines. We initially focused on the TFs encoded by human chr.21 (TFs21) that are endogenously expressed in Hek293T17 cells. In order to evaluate off-target effects affecting the expression of unintended gene targets, we performed independent experiments using three different types of silencing molecules (siRNAs, esiRNA). Silencing efficiencies for the different molecules were evaluated by qRT-PCR, and whenever possible by Western blot. We retained for further analysis samples showing a knock-down at the mRNA level of at least 75%. Global transcriptome analysis are carried out on the Affymetrix platform. The subset of genes that are found dysregulated with all silencing molecules represent a high-confidence set of potential direct as well as indirect TF target genes. For BACH1 we identified a subset of 157 genes that are found dysregulated with all silencing molecules, representing a high-confidence set of potential target genes. Among those we observed the up-regulation of HMOX1 (Heme oxygenase 1), a known target of BACH1. We will present data on the set of 10 knocked down TFs21, and show how the data sets are used to chart potential regulatory networks, integrating other levels of information of ChIP on chip experiments and promoter informatics. In a systematic effort, we are currentely knocking down the expression of ca. 200 additional human transcription factors potentially involved in key developmental processes and human pathologies for understanding associated gene regulation networks.

2754/T

A Pipeline for Designing Custom TaqMan® Assays for Small RNA Genes. *L. Wong, Y. Wang, D. Ridzon, L. Bahreinifar, C. Chen.* Assays and Arrays R&D, Applied Biosystems, Foster City, CA.

MicroRNAs (miRNAs) are a new class of non-coding RNAs that mediate post-transcriptional gene silencing. A growing number of novel miRNAs and other small RNA genes are being discovered and there is a significant need for custom assays to determine the level of their expression. An automated bioinformatics pipeline has been developed to design TaqMan® MicroRNA Assays to enable quantitation of miRNA expression by real-time PCR. A set of 446 miRNA assays were designed and their performance evaluated based on assay linearity and no template control (NTC) signal. From this initial test set, we observed an assay performance success rate of approximately 90%, with NTC failures contributing to the majority of the remaining 10%. Through examination of oligo interactions, new design rules were implemented to the pipeline intended to reduce the NTC failure rate. In a first phase validation, 30 "failed" assays and 30 "passed" assays from the initial test set, were redesigned with the optimized pipeline. These 60 redesigned assays were then tested in parallel with the original 60 assays for a direct comparison of assay performance. From the "failed" set, we observed nearly 50% improvement for assays designed with the optimized pipeline. For a second phase validation, over 1,000 mammalian miRNA genes were selected from Sanger miRBase (release 9.1) and submitted for design using the optimized pipeline. A subset of these newly designed assays will be evaluated to confirm the success rate for assay performance. As research interest in small non-coding RNAs is rapidly expanding beyond miRNAs, the capability of the pipeline to design assays for other small RNA genes including siRNAs, shRNAs, and piRNAs will be tested. Results of TaqMan® assays for siRNAs will be presented.

2755/T

Large-scale transcriptional profiling for the identification of genes influencing biological aging. *J.C. Charlesworth, J.W. Kent Jr., J.E. Curran, M.P. Johnson, H.H.H. Göring, T.D. Dyer, S.A. Cole, J.W. MacCluer, E.K. Moses, J. Blangero, S. Williams-Blangero.* Southwest Foundation for Biomedical Research, San Antonio, TX.

The genetic architecture of biological aging is complex, involving multiple genetic and environmental factors and their interactions; however the specific genes involved in the biological pathways of aging are largely unknown. In this study, we employ an integrative genomic approach that utilizes large-scale transcriptional profiling to rapidly identify novel genes influencing differential aging. Using RNA extracted from lymphocytes, we obtained genome-wide transcriptional profiles from 1,240 individuals in the San Antonio Family Heart Study. High-dimensional endophenotypic search procedures identified 4,136 transcripts that were significantly correlated with chronological age (corrected for multiple testing using a FDR of 0.05). Functional annotations of genes within this set indicate significant over-representation of genes in several critical pathways including reactive oxygen species production ($p=1.36\times10^{-4}$), immune response ($p=3.08\times10^{-4}$) and DNA replication/repair ($p=4.20\times10^{-4}$). Of these age-associated genes, 781 exhibited expression levels that showed significant evidence for genotype-by-age (G×A) interaction as determined by a quantitative genetic test. G×A interaction is interpretable as evidence for a heritable basis of biological response to aging. Of the transcripts that showed both a significant relationship with age and evidence for G×A interaction, 144 also showed nominal evidence for *cis*-regulation, as inferred from quantitative trait linkage analysis. Examples of these *cis*-regulated genes include *VSTM1* (evidence for *cis*-effects: $p=2\times10^{-23}$; evidence for G×A effects: $p=2.5\times10^{-6}$) and the mitochondrial gene *MTCO1* (evidence for *cis*-effects: $p=1.4\times10^{-11}$; evidence for G×A effects: $p=1.9\times10^{-12}$). Such genes are likely to harbor regulatory variants close to their structural locations that are involved in differential aging. This empirically determined set of genes influenced by G×A interaction represent excellent candidates for the rapid identification of genetic variation influencing aging.

2756/T

A Novel Bimodal Replication Timing Program In Human Mesenchymal Stem Cells Is Revealed by a High-Throughput Approach to Measure Timing of Replication Using Tiling Arrays. *T.Y Takova[1], C.L Schildkraut[2], R. Desprat[2], Q. Yang[3], R. Green[1], E. Bouhassira[2].* 1) NimbleGen Systems, Madison, WI; 2) Hematology/Cell biology, Albert Einstein College of Medicine, Bronx, NY, USA; 3) Cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA.

Replication-timing programs change with development and differentiation.The significance of these changes is not understood, but different timing programs are believed to be associated with different chromatin structures and different transcriptional programs.We hypothesize that the replication-timing program of stem cells can be used to assess their epigenetic status and their differentiation potential.We describe here a new high-throughput approach to define the temporal order of DNA replication.The method relies on high-resolution custom-made NimbleGen tiling microarrays containing about 400,000 oligonucleotides to measure the small differences in DNA content that are associated with differential timing of replication.We have assessed the DNA replication profile of about 1.5% of the genome the of a mesenchymal stem cell derived from the human embryonic stem cell line H1.Analysis of a two megabase region encompassing the IgH locus revealed that the downstream part of the locus replicates early in S, that the upstream part of the locus replicates late in S and that these two regions were joined by a transition region in which no replication initiation occurs and which is replicated by a single fork that proceeds from early to late in S.Eleven additional large temporal transition regions similar to the one described for the IgH locus were also observed.Total RNA,mRNA and poly-A negative nuclear RNA were hybridized on the same tiling microarrays.Comparison of the timing and transcription data revealed positive correlations, particularly between the amount of primary transcripts and early replication.This is consistent with a remarkable bimodal timing program in which the early replicating genome is enriched in active genes,and the late in inactive genes.Experiments to characterize the replication program in undifferentiated human ES cells and in hematopoietic cells derived from hESCs are in progress.

2757/T

L1 retrotransposition events occur mainly in early embryogenesis. *H. Kano[1], E.M. Ostertag[1], I. Godoy[1], C.E. Courtney[1], T. Merdiushev[2], G.L. Gerton[2], H.H. Kazazian[1].* 1) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Center for Research on Reproduction and Women's Health, University of Pennsylvania School of Medicine, Philadelphia, PA.

L1s are abundant retrotransposons that comprise ~17% of the human genome. Despite having great impact in the genome, little is understood about L1 natural biology. Several studies of L1 transgenic mice and human retrotransposition events have demonstrated that L1 retrotransposition can occur in germ cells, early embryos and/or somatic cells; however, it is still controversial as to when L1 retrotransposition mainly occurs. To characterize the timing of human L1 retrotransposition, we created transgenic mouse and rat models harboring a human L1 element under the control of its endogenous promoter. Offspring of L1 transgene heterozygotes exhibited high retrotransposition activity in both animals. L1 insertions were seen in more than 60% of the transgene containing offspring and in 5-13% of offspring without the transgene. Nearly all the de novo L1 insertions were somatic, because these L1 insertions were rarely inherited by the next generation. Upon studying spermatogenic cell fractions and pre-implantation embryos, abundant L1 RNA from the L1 transgene was seen in both spermatogenic cells and pre-implantation embryos, consistent with relatively unmethylated L1 transgene in these tissues. On the other hand, L1 retrotransposition was much more apparent in embryos (>10 fold) than in spermatogenic cells. Our data suggest that there is a time lag between L1 transcription and integration, and L1 RNA can be carried over after fertilization and integrate into the genome during early embryogenesis. In addition to our transgenic animal data, there is evidence that a human L1 insertion has occurred during early human development, and because of bias of ascertainment it is difficult to determine the timing of the others. We now speculate that most human L1 retrotransposition events occur in early development rather than in germ line, suggesting a significant role of somatic L1 insertion in human development and biology.

2758/T

Weird animal genomes and the evolution of sex chromosomes. *J. Graves.* Research Sch Biol Science, Australian National Univ, Canberra, ACT, Australia.

In humans and other therian mammals, females have two X chromosomes and males a single X and a Y that bears the testis-determining gene SRY. Birds and reptiles have completely different triggers for sex determination; either genes on unrelated sex chromosomes (Z and W in snakes and birds), or environmental triggers like temperature. Or both, as we recently discovered in dragon lizards. X and Y chromosomes evolved from an ordinary pair of autosomes as the Y progressively degraded (the bird Z and W evolved independently from a different autosome pair). Our strategy is to compare the chromosomes, genes and DNA between distantly related mammal groups, as well as birds and reptiles. The genomes of Australia's unique kangaroos and platypus, now being completely sequenced, are particularly valuable because these "alternative mammals" are distant enough to provide informative variation, but not too distant to compare DNA sequences. Kangaroo sex chromosomes reflect the original mammal sex chromosomes and define evolutionary layers in the X. The bizarre multiple platypus sex chromosomes are related to the bird Z, implying that our sex chromosomes are relatively young. The human X contains more than a thousand genes biased toward functions in male reproduction, and intelligence, and often both, perhaps because of positive selection for male-advantage genes on the hemizygous X in males, and selection by females of intelligent mates, acting independently on X-borne genes. The small human Y bears only 45 different protein-coding genes, 27 (mostly testis-specific) in the male-specific region. Most Y genes (even those with functions in sex determination and spermatogenesis) evolved from widely expressed partners on the X. Comparisons between mammals shows that the Y degraded independently in different lineages. For instance, the kangaroo Y contains several novel testis-specific genes with X-borne partners. Degradation is ongoing, and if it continues at the same rate, will wipe out the human Y in 7 million years.

2759/F

Identification of novel interactive partner proteins for PCBP1. *B. He[1,2], L-R. Huo[1,2], N. Zhong[1,2,3].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Peking University Health Science Center, Beijing, China; 3) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

PCBP1 is a family member of heterogeneous nuclear ribonucleoproteins (hnRNPs), which belong to RNA-binding proteins and bear three KH domains. The protein plays a pivotal role in post-transcriptional regulation for RNA metabolism and RNA function in gene expression. We hypothesized that the regulating function of PCBP1 is performed along with other proteins, with which a protein complex may be formed. To test our hypothesis, an approach of protein walking with the yeast two-hybrid system is employed for this study. The PCBP1 is used as the initial "walker" to search for its interactive partner proteins, which were identified with a yeast two-hybrid (Y2H) system and validated by pulling down, co-immunoprecipitation, and co-localization. Eight partners have been identified, which include a previously identified actin and seven novel proteins of MYL6, RECAM1, CSH1, Rab7, p57KIP2, PSG4, and RBMS1. It is likely that these novel interactive partners have mediated PCBP1 functions involved in apoptosis through regulating cell cycle, in cell autophagy through molecular migration and controlling translation.

2760/F

Incorporating Fuzzy theory to the Dynamic Bayesian network modeling of gene expression data. *S. Gao, X. Wang.* medical college of wisconsin, milwaukee, WI.

Dynamic Bayesian networks (DBYN) plus Monte Carlo Markov Chain (MCMC) sampling has become an important approach to reconstruct genetic networks from gene expression data. It is a graphic probabilistic model that allows the incorporation of prior biological knowledge to improve performance. We have recently, for the first time, introduced fuzzy theory-based rules to the MCMC learning of DBYN in order to efficient incorporate prior biological knowledge, which are often incomplete and plagued with quality issues. Our method uses the Bayesian naïve network to estimate the probability of causal relationship between all gene pairs according their PubMed co-citation significance and their GO similarity. A sampling reservoir is then created where the copy number of each candidate gene pair is proportional to this probability. At each simulation iteration, a candidate network structure is generated by randomly picking a gene pair from the reservoir as a new network edge to be operated. We evaluated our new algorithm with both simulated expression data generated by SynTreN and the yeast cell cycle data. When compared with using DBYN alone, the sensitivity is improved by 80% on simulated data and 60% on the yeast data. We have then utilized it in the study of pancreas development, which is important in understanding normal pancreas function and the pathology and treatment of diabetes. A list of 15 genes that are deemed critical to pancreas development/function according to literature and their network structure were manually created. A time-series dataset that profiled mouse pancreas development were downloaded from the RNA Abundance Database (http://www.cbil.upenn.edu/RAD2/ ID 2 & 1790). We found that our new method is able to recover significantly more known gene pair relationships at the same false positive level. Out of the 23 manually curated network edges, it recovered 12. This is in contrast to only 4 by a plain DBYN method. These results demonstrated the advantage of our new DBYN algorithm. In addition, our approach can be extended to incorporate more types of biological data/knowledge as priors, including the DNA-protein and protein-protein interaction information.

2761/F

LGMD2B (Dysferlin-deficiency) shows compensatory upregulation of vesicle trafficking pathway Rab27A and its effector Synaptotagmin like protein 2. *A. Kesari[1], M. Fukuda[2], S. Knoblach[1], EP. Hoffman[1].* 1) Center of Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Developmental Biology and Neurosciences, Tohoku University Miyagi, Japan.

Mutations in the dysferlin gene (DYSF) on chromosome 2p13 cause Limb girdle muscular dystrophy 2B (LGMD2B) and Miyoshi Myopathy (MM). Dysferlin-deficient patients show a relatively acute onset with marked muscle inflammation in late teens. Dysferlin-deficient muscle shows abnormalities of vesicle trafficking. We hypothesized that the late yet acute onset of LGMD2B could be associated with compensatory up-regulation of alternative vesicle traffic pathways, and inflammatory signals. Twenty-five patients showing Dysferlin-deficiency on muscle biopsy were used for mutation screening, and 19 mutation-positive patients identified. Ten of these biopsies were used for genome-wide mRNA profiling, and data compared to a disease control (FKRP mutation-positive subjects [LGMD2I]), and normal volunteer muscle. Two inflammatory-associated genes, Tenascin and Versican, were found highly expressed in Dysferlin-deficient muscle, at both the mRNA and protein levels. These two interacting proteins may respond to the vesicle traffic and monocyte abnormalities, leading to over-aggressive inflammatory cascades. A vesicle trafficking pathway involving two interacting proteins, Synaptotagmin-like protein (Slp2a) and a small GTPase (RAB27A), were highly induced in dysferlin-deficient muscle, while not expressed in normal muscle, Becker muscular dystrophy, or FKRP. We suggest that the C2-containing Slp2a protein shows disease-specific compensatory up-regulation in Dysferlin-deficiency, leading to induction of an alternative vescicle trafficking pathway.

2762/F

COMPUTATIONAL ANALYSIS OF STRUCTURAL AND NON-STRUCTURAL PROTEIN SYNTHESIZED BY CHIKUNGUNYA VIRUS - MOSQUITO BORNE DISEASE AS POTENTIAL TARGET MOLECULES FOR VACCINE. *Mahdieh. Khosroheydari[1], K.R. Rupesh[2].* 1) Medical Genetic Department, Special Medical Center, P.O. Box 15815/3333, Tehran, Iran; 2) Laboratoire de Microbiologie des Environnements Extrêmes, CNRS UMR 6197, IFREMER-Centre de Brest, BP 70, 29280 PLOUZANE, France.

Background & Objective: Chikungunya virus (CHIK) is an alphavirus borne by Aedes mosquitoes that produces a dengue-like illness in humans, characterized by fever, rash, painful arthralgia and arthritis throughout sub-Saharan Africa, Southeast Asia, and the Western Pacific. The recent widespread geographic distribution, recurrent epidemics, and infection of military personnel, travelers, and laboratory staff working with CHIK have indicated the need for more understanding and to have a efficacious vaccine. Results: In our present study we have analyzed the characteristics of structural and non-structural proteins synthesized by CHIK using computational tools and predicted the effective possible candidates for use as potential vaccine. The computational analysis of the non-structural protein revealed that it is 275.65 kDa hydrophilic protein, whose pI is 6.841 while that of the structural protein revealed a 138.88 kDa hydrophilic protein of pI 8.88. The antigenic prediction sites on the non-structural and structural proteins predicted were examined for their use in as vaccine candidates for effective control of the disease. The positions of the alpha helix and b-sheets in the secondary structure of the proteins were predicted which laid the path for 3D structural characterization of the target proteins. On analyzing the hydropathy plot, the structural protein and the non-structural protein was found to be hydrophilic. Using the nucleotide sequence of the proteins, degenerate primers were designed for its use in PCR based diagnostic identification of the CHIK. Conclusion: The primers designed could find its use as a diagnostic tool for identifying CHIK infected patients specifically and sensitively. The predicted antigenic sites on the non-structural and structural proteins could be used as effective vaccine candidate which will be further evaluated for its effectiveness by in vitro and in vivo methods.

2763/F

Gene Regulation Studies of the Friedreich Ataxia Locus Using Genomic Reporter Assays. *N. Puspasari[1,2], P.A. Ioannou[1,2], M.B. Delatycki[1,2], J.P. Sarsero[1].* 1) Bruce Lefroy Centre for Genetic Health Reserach, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia; 2) Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Victoria, Australia.

Friedreich ataxia (FA) is a progressive cardio and neurodegenerative disease caused by a trinucleotide repeat expansion in the first intron of the *FXN* gene, resulting in the insufficiency of frataxin protein production but not its complete loss. As the coding sequence of the *FXN* gene is unaltered, targeted upregulation of gene expression may restore cellular frataxin to therapeutic levels in patients. Unravelling the mechanisms that regulate *FXN* gene expression would therefore lead to a rational approach for the pharmacological restoration of frataxin levels and the therapy of FA. However, no information is currently available about the position of any long-range, *cis*-acting regulatory sequences that regulate human *FXN* gene expression. We have established a system for the bioinformatic identification and experimental verification of regulatory mechanisms that direct the expression of the *FXN* gene. Utilisation of data from the sequence assemblies of the human and other mammalian genomes for cross-species comparative genomics analysis has identified a number of conserved, non-coding regions surrounding the *FXN* gene. To investigate the role of these regions identified in human gene, we have developed a dual-reporter BAC vector that contains an *FXN-EGFP* genomic reporter consisting of the fusion of the EGFP gene to the entire normal genomic human *FXN* locus on a BAC clone. The construct also contains an independently expressed gene encoding DsRed-Express fluorescent protein as an internal control. The roles of the conserved, non-coding sequences is being evaluated by their deletion or modification in the context of the *FXN-EGFP* genomic reporter, The EGFP/DsRed-Express ratio will provide a sensitive and specific assay for detecting the effects of deletions of regulatory regions of the gene while simultaneously allowing for correction of differing transfection efficiencies.

2764/F

Design of primers, Comparative Sequence Analysis, Structure Prediction of ApolipoproteinE protein in Alzheimer's disease. *K.R. Rupesh[1], M. Khosroheydari[2].* 1) Laboratoire de Microbiologie des Environnements Extrêmes, CNRS UMR 6197, IFREMER-Centre de Brest, BP 70, 29280 PLOUZANE, France; 2) Medical Genetic Department, Special Medical Center, P.O. Box 15815/3333, Tehran, Iran.

Background & Objective: Alzheimer's disease (AD), a neurodegenerative disease, is the most common cause of dementia and characterized clinically by progressive intellectual deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. Researchers have identified an increased risk of developing late-onset AD related to the apolipoprotein E (apoE) gene found on chromosome 19. In this study we have compared the ApoE gene its protein in different organisms to understand the mechanism of expression of ApoE. Results: Multiple sequence alignment (MSA) of the apoE gene of Human revealed that it is analogous to all the other 16 organisms whereas the apoE protein is found to be 66% homologous. On detailed analysis of the nucleotide sequence of apoE (NM_000041), it was found that the ORF region was present from 84-1034 position. Specific primers for the apoE coding region in the AD were designed and its specificity was checked using computational tools which resulted in a PCR product size of 1109 bp. The nucleotide and protein sequence of the apoE in AD was studied in detail using the computational tools. The analysis of the apoE protein showed that it was hydrophilic and its pI was predicted. 13 antigenic determinants were found when the apoE protein was analysed for Antigenic prediction sites which could be used as targets for other proteins or drugs to interact with. The positions of the alpha helix and b-sheets in the secondary structure of the proteins were predicted along with 3D structure prediction of apoE. Conclusion: The degenerate primers designed could be used as a diagnostic tool for identifying apoE protein expression in AD patients specifically and sensitively. The predicted antigenic sites on the apoE proteins could be used as an effective target for interaction with candidate drugs for the control of the expression of the apoE in AD patients, this has to be further evaluated using in vitro and in vivo methods.

2765/F

Semax and PGP affect the mRNA expression of neurotrophins and their receptors under the focal cerebral ischemia in rats. *V.G. Dmitrieva[1], L.V. Dergunova[1,2], I.V. Vlasova[1], O.V. Povarova[2], V.I. Skvortsova[2], S.A. Limborska[1,2].* 1) Human Molecular Genetics Dept, Institute of Molecular Genetics RAS, Moscow; 2) Institute of Stroke RSMU, Moscow, Russian Federation.

Neuroprotective polypeptide Semax is used for acute therapy of stroke. The effect of Semax and its C-terminal PGP tripeptide therapy on mRNA expression of neurotrophins Bdnf, Nt-3 and their receptors TrkB, TrkC after 3, 24, and 72 hours of cerebral ischemia was investigated. Focal cerebral ischemia was induced in male Wistar rats by permanent middle cerebral artery occlusion (MCAO). The intraperitoneal injections of either Semax or PGP were done at 15 min, 1, 4 and then after every 4 hour. Real-time RT-PCR has been used to measure changes in mRNA expression of genes investigated in the lesioned cortex of rat brains. Gapdh was used as the internal control. Compare with the increase of Bdnf mRNA expression observed in the ischemic tissue 24 h after MCAO, Semax promotes the increase of Bdnf mRNA level in the ischemic tissue as early as 3 h after occlusion and supports the high level of Bdnf mRNA to the point of 72 h after occlusion. PGP also increases Bdnf mRNA expression 3 h after ischemia but then Bdnf expression returned to control level. The analysis of TrkB mRNA expression in ischemic cortex of rats under the Semax treatment didn't reveal any significant difference compare to control. However under the treatment with PGP the level of TrkB mRNA was increased at 24 h after MCAO. In the damaged cortex the Nt-3 mRNA expression increases after 72 h of MCAO while under the Semax treatment the increase of the level of Nt-3 transcripts was detected at 24 h but then it returned to the control level. The Nt-3 mRNA expression in rat ischemic cortex didn't change under the PGP treatment compare to control. The changes in TrkC mRNA expression in ischemic cortex of rats under the Semax treatment were not observed. However under the treatment with PGP tripeptide the level of TrkC mRNA was increased during fist 24 h after MCAO. Thus in MCAO conditions used Semax increases the expression of neurotrophins Bdnf, Nt-3 whereas PGP mainly affect on the mRNA level of their receptors TrkB, TrkC.

2766/F

Neural Restrictive Silencer Factor (NRSF) and Choline Acethyltransferase (CHAT) expression in cerebral tissue of Alzheimer's disease patients. *R.E. González-Castañeda[1], V.J. Sánchez-González[1], A. Barba-González[1], E. Martínez-Cano[1], S. Sustersick-Castro[1], O. González-Perez[2], K. Solorza-Camacho[1], A. Jimenez-Delgado[1], A. Miranda-Riestra[1], F. Pacheco-Moises[3], V. Loera-Castañeda[4], G. Ortiz[1].* 1) Laboratorio de Enfermedades neurodegenerativas, Centro de Investigación Biomédica de Occidente, Division de Neurociencias, CIBO (IMSS), México; 2) Departamento de Neurociencias, Centro Universitario de Ciencias de la Salud, U de G, México; 3) Departamento de Química, Centro Universitario de Ciencias Exactas e Ingeniería, U de G,Mexico; 4) CIIDIR-IPN, Unidad Durango, México.

Background:Decreased choline acetyltransferase (ChAT) brain levels is one of the main biochemical disorders in Alzheimer's Disease (AD). Recent data show that the ChAT gene can be regulated by a neural restrictive silencer factor (NRSF). Objective. To evaluate ChAT and NRSF genetic and protein expression in frontal, temporal, enthorrinal and parietal cortices of AD patients. Methods. A total of 4 patients with AD and 4 without dementia were studied. Cerebral tissue was obtained and processed by the guanidine isothiocyanate method for RNA extraction. ChAT and NRSF expression was determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting. Results. Global levels of ChAT gene expression were decreased by 39% in AD patients as compared to the control group ($p<0.05$, U test), whereas ChAT protein levels decreased only by 17% ($p=0.02$). Compared to the control group, NRSF gene expression was increased by 86% in the AD group ($p=0.001$). On the other side, NRSF protein levels were increased by 57% ($p>0.05$). Conclusion. Greater NRSF protein levels determine low ChAT gene expression levels in AD patients.

2767/F

PRELIMINARY EVIDENCE OF A NOS2A PROTECTIVE EFFECT IN PATIENTS WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS. *I. Manna[1], M. Liguori[1], P. Valentino[2], F. Condino[1], A. Clodomiro[2], R. Nisticò[2], G. Di Palma[1], A. Quattrone[1,2].* 1) Institute of Neurological Science (ISN) - CNR, Mangone, Cosenza, Italy; 2) Institute of Neurology, University "Magna Græcia", Catanzaro, Italy.

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system, characterised by a chronic inflammatory process. Nitric oxide,was implicated in the inflammatory process and its potential contribution to the development of MS has been extensively tested in humans and animal models. The human gene encoding inducible nitric oxide synthase (NOS2A) is located on chromosome 17q11.2-q12.In order to evaluate the possible implication of NOS2A in the pathogenesis of MS, we performed a case-control study in a selected RRMS population from Southern Italy and in an ethnically matched healthy subjects, by considering two distinct polymorphisms:(CCTTT)n and(AAAT)n. A group of patients with clinically definite MS (n=113) and ethnically matched healthy controls (n=237) were studied. Patients and controls were genotyped for the NOS2A polymorphic markers using a PCR methods. All PCR products were electrophoresed on ABI PRISM 377 and then sized by the GENESCAN TM software. Unpaired t-test was used to compare age at examination between patients and controls. This test was also applied to compare age at disease onset, disease duration and brain volumes (GM, WM). The distribution analysis of the markers frequencies showed that the (CCTTT)14 allele was found in 11.5%; of the RRMS patients and in 25.3%; of the healthy subjects, with a statistically significant difference ($\chi2= 8.843$, $p= 0.003$). Considering the presence/absence of at least one (CCTTT)14 allele, the RRMS patients carrying one (CCTTT)14 allele showed a significant higher age at disease onset ($p= 0.03$) and a higher current disability score ($p= 0.027$) than the other patients, whereas no significant differences were observed for the other studied clinical or MRI features. In the our group of RRMS patients and healthy controls, we found a significant different distribution of the (CCTTT)n marker located in the NOS2A gene. Further studies in different populations are needed to better investigate the role of the NOS2A gene in MS.

2768/F

Epistatic interaction between REST and BDNF is associated with cognitive functioning. *F. Miyajima[1], J. Quinn[2], N. Pendleton[3], M. Horan[3], W. Ollier[1], A. Payton[1].* 1) CIGMR, University of Manchester, Manchester, UK; 2) Neurotransmitter Biology Group, University of Liverpool, Liverpool, UK; 3) Clinical Gerontology, University of Manchester, Manchester, UK.

INTRODUCTION: Brain-derived neurotrophic factor (BDNF) is a pleiotrophic protein involved in neuronal proliferation, differentiation, synaptic plasticity and survival. Independent studies investigating association between the Val66Met and cognitive function have reported conflicting findings which may reflect additional polymorphic regulatory factors other than the Val66Met polymorphism which contribute towards overall gene expression. One of these factors is the RE1-silencing transcription factor (REST) which down-regulates BDNF expression. METHODS: We have looked for possible associations between several polymorphisms within the REST gene and cognitive abilities using a cohort of 746 community-dwelling older volunteers who have been followed-up for changes in cognitive performance at five year intervals for up to twenty years. RESULTS: We have identified a 3-locus haplotype located within the proline-rich domain not yet characterised. This contained a hexadecapeptide polymorphic motif with either four or five copies. Volunteers homozygous for the five repeat allele scored lower on all cognitive tests, reaching significance for the two tests of fluid intelligence (Heim part1, $p= 0.025$ and Heim part2, $p=0.032$). Interaction analysis between the BDNF Val66Met and the REST VNTR revealed positive associations with all cognitive tests, except semantic memory. When the combined analysis was covariated with the general common factor, a significant global p-value was obtained following 10,000 shuffled permutations ($p=0.0002$). In contrast to the cross-sectional analysis, no significant results were observed in the longitudinal assessment. We are pursuing further confirmation of these findings in a sample of 1,400 subjects using a string of multidisciplinary approaches. CONCLUSION: Our results suggest that investigation of these polymorphisms in combination may reduce inconsistencies currently plaguing the literature, highlighting how a full appreciation of epigenetics is missing from our understanding.

2769/F

Neurotrophins and their receptors: mRNA expression in ischemic rat brain under the treatment with neuropeptide Semax and its C-terminal tripeptide PGP. *V.V. Stavchansky[1], L.V. Dergunova[1,2], A.B. Botsina[2], T.V. Tvorogova[2], V.I. Skvortsova[2], S.A. Limborska[2].* 1) Human Molecular Genetics Dept, Institute of Molecular Genetics RAS, Moscow, Russian Federation; 2) Institute of Stroke RSMU, Moscow, Russian Federation.

To elucidate the effect of neuroprotective polypeptide Semax containing the fragment of adrenocorticotropic hormone - ACTH(4-10) and its C-terminal fragment Pro-Gly-Pro (PGP) on expression of neurotrophins Bdnf, Nt-3 and its receptors TrkB, TrkC and p75 after global cerebral ischemia the profile of its mRNA expression in rat cerebellum and forebrain cortex were analyzed. The study was carried out on 2-3-month-old male Wistar rats (n=85). After 15 minutes of irreversible bilateral common carotid artery occlusion the animals were exposed to intraperitoneal injection of either Semax, PGP or saline 1 hour, 4 hours and 8 hours after the occlusion. Animals were decapitated 30minutes, 1hour, 2, 4, 8, 12 and 24 hours after operation. Intact and sham-operated animals were used as control groups. The mRNA expression of neurotrophins and its receptors was assessed by relative quantification in real-time RT-PCR. Gapdh was used as the reference gene. The most appreciable effect of Semax was revealed in the analysis of p75 mRNA expression in forebrain cortex; the level of p75 transcripts was decreased at 8 h and 24 h after operation compare to animals with ischemia treated with saline. Some considerable effects of the PGP treatment were observed. The level of TrkB transcripts was increased 30 minutes and 1 hour after occlusion while the increase of Nt-3 and TrkC mRNA expression was observed 24 hours after operation. It could be suggested that neuroprotective effect of Semax and PGP is possibly mediated by neurotrophins and its receptors.

2770/F

A Frequent TGFβRII PolyA tract mutation that downregulates the TGF-β signal pathway results in inactivation of CDK2-AP1 expression in human MSI CRC. *Z. Yuan[1], J. Shin[2], K. Fordyce[3], P. Sreeramoju[3], T. Kent[4], J. Kim[3], V. Wang[3], K. Sacchini[5], TK. Weber[1,2].* 1) Molecular Genetics; 2) Surgery,Einstein College of Medicine,NY; 3) IBM; 4) Surgery,U.of Pittsburgh,PA; 5) Irvington High School,NY.

Background:A frequent(~90%)frameshift mutation in the PolyA tract of the type II transforming growth factorβreceptor(TGFβRII)has been reported only in MSI colorectal cancer(-CRC)inhibiting the growth suppressive effect of the binding of its ligand,TGF-β.Recently,we reported significant decreased expression of the growth suppressor gene Cyclin Dependent Kinase2-Associated Protein1(CDK2-AP1)in 85%of MSI CRC and its association with decreased apoptosis and increased S-phase.This study tests the hypothesis that inactivation of CDK2-AP1 in MSI CRC results from the absence of growth-inhibitory signal via TGFβRII due to the inactivating polyA tract mutation.Methods:We utilized a transient transfection of RNAi to target TGFβRII in the CRC cell lines SW620(Wild type:WT)and Dld1(mutant receptor:MT).We induced over-expression of TGFβRII by transfection of the WT receptor into these lines.A reporter construct(p3Tu-Lux)of TGF-βsignal was cotransfected into the same lines to assess response.mRNA and protein of CDK2-AP1 were measured by real-time PCR and Western blot assays.The effect of modulating the TGF-βsignal on cell proliferation,apoptosis,and differentiation was measured with an MTT,FACS,and Matrigel assay.Results:The methods demonstrated the transfection of RNAi targeting the WTTGFβRIIin SW620 resulted in a substantial decrease in CDK2-AP1 expression.Conversely introduction of WT TGFβRIIinto the mutant Dld1 cell line resulted in a significant increase in CDK2-AP1 expression and its association with decreased proliferation,increased apoptosis and cell differentiation.Discussion:The results support our hypothesis that down-regulation of CDK2-AP1 in MSI is the result of impaired TGF-βsignaling due to inactivating mutations of the PolyA tract in TGFβRII and the broader concept of different pathways of malignant transformation and progression in MSI vs MSS which suggests further study of TGFβRII modulation of CDK2-AP1 is indicated.

2771/F

Dysfunction of the GH/IGF pathway and human longevity. *G. Atzmon[1], M. Cho[1], T. Budagov[1], D. Hwang[2], B. Liu[2], N. Barzilai[1], P. Cohen[2], Y. Suh[1].* 1) Department of Medicine, Albert Einstein College of Medicine, Bronx, NY,NY; 2) Mattel Children's Hospital and David Geffen School of Medicine at UCLA, LA, CA.

The role of altered GH/IGF signaling in lifespan extension is well established in lower species but has not been shown in humans. To investigate the role of GH/IGF axis in human longevity we analyzed chemotypic, phenotypic and genetic variations in the GH/IGF axis molecules in a cohort of Ashkenazi centenarian (n=180), their offspring (n=147)and unrelated matched control (n=221). Female offspring of centenarians were shorter and had higher serum IGF-1 levels than control females ($p<0.01$), a gender-specific response similar to a report in heterozygote IGF1R-KO mice. We thus comprehensively screened genomic DNA for all possible genetic variants throughout the coding exons of the IGF1R gene. We discovered 2 novel non-synonymous mutations in the IGF1R in female centenarians with short stature and/or elevated IGF-1 that were not seen in controls, as well as 3 novel synonymous mutations detected only in centenarians. We also studied the Exon 3 deletion polymorphism of GHR gene (d3GHR), and demonstrated that the frequencies of the d3GHR homozygote were significantly higher in male centenarians (14%) and in their male offspring (12%) as compared to male controls (4%). The d3GHR carriers male centenarians were 3 inches shorter than centenarians carrying the wt GHR variant and had lower serum IGF-1 ($p=0.03$). To assess the functional consequences of the genetic variations identified in this study, we analyzed immortalized lymphocytes. Lymphocytes from the D3GHR carriers displayed significantly slower growth rates and lower activation of ERK at baseline, but higher growth and activation of ERK in response to GH treatment, as compared to wild type carriers ($p<0.01$). Furthermore, lymphocytes from the IGF1R mutation carriers showed significantly lower level of both basal and IGF-induced activation of AKT, and reduced levels of IGF1R protein. These findings provide the first genetic, functional, sexual dimorphism, and phenotypic links between the GH/IGF pathway and aging in humans, supporting the role of this conserve pathway in exceptional longevity.

2772/F

Human miR-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3'UTR - a mechanism for functional SNPs related to phenotypes. *C. Borel[1], P. Sethupathy[2], M. Gagnebin[1], C. Gehrig[1], G.R. Grant[2], S. Deutsch[1], T.S. Elton[3], A.G. Hatzigeorgiou[2], S.E. Antonarakis[1].* 1) Dept Genetic Medicine, Univ Geneva Medical Sch, Geneva, Switzerland; 2) Penn Center for Bioinformatics, School of Medicine, University of Pennsylvania, Philadelphia; 3) Davis Heart and Lung Research Institute, The Ohio State University, Columbus.

Animal microRNAs (miRNAs) regulate gene expression through base pairing to their targets within the 3' UTR of protein coding genes. Single Nucleotide Polymorphisms (SNPs) located within such target sites can affect miRNA regulation. We mapped annotated SNPs onto a collection of experimentally supported human miRNA targets. Out of the 143 experimentally supported human target sites, nine contain twelve SNPs. We further experimentally investigated one of these target sites for hsa-miR-155, within the 3' UTR of the human AGTR1 gene that contains SNP rs5186. Using reporter silencing assays, we show that hsa-miR-155 downregulates the expression of only the 1166A, and not the 1166C allele, of rs5186. Remarkably, the 1166C allele has been associated with hypertension in many studies. Thus the 1166C allele may be functionally associated with hypertension by abrogating regulation by hsa-miR-155, thereby elevating AGTR1 levels. Since hsa-miR-155 is on chromosome 21, we hypothesize that the observed lower blood pressure in trisomy 21 is partially caused by the over-expression of hsa-miR-155 leading to allele-specific under-expression of AGTR1. Indeed we have shown in fibroblasts from monozygotic twins discordant for Trisomy 21 that AGTR1 protein is lower in trisomy 21.

2773/F

Nonhuman basepaired insertiones in cDNA of fibroblast cells. *Z.V. Vassileva(Nickl).* Zwetelina Vassileva, Biology, Dragan Tzankov Str. 8, St. Kliment Ochridksi University Sofia, Zwetelina Vassileva(Nickl).

Nonhuman base-paired insertions in cDNA of fibroblast cells. Despite of the research activities in the last years there are still many uncertain open questions about the expression regulation during the mRNA processing of some human genes. Analysis of some genes shows irregularities and variations in those sequence structure and composition between the different developmental stages and pathological changes in different tissues. By now some of those irregularities are connected to some known functional mechanisms of microRNAs. Similar activities may occur also in NBIs contained genes. Analysis of some human gene sequences consisting of NBIs would be an advice for analysis of certain connections to other genes families. This would be also a helpful hint for analysis of genes development. One of the above mentioned sequences was amplified in fibroblast cells of human adult smooth vascular tissue. Analyses of all transcripts are made primarily by the use of NCBI Genbank resources.

2774/F

Two polymorphisms in ugt1a1 5'-flanking region: their transcriptional regulation and association with coronary heart disease. *J. Zhang[1], Y. Wang[2], J. Dai[1], L. Zhang[1], M. He[1], J. Zhang[2], W. Sun[2], W. Huang[2], J. Jin[1], L. Jin[1,3].* 1) MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) CAS-MPG Partner Institute of Computational Biology, Shanghai Institutes of Biology Sciences, Chinese Academy of Sciences, Shanghai, China.

The antioxidant ability enables bilirubin to protect against coronary heart disease (CHD). UGT1A1 is the main and speed-limiting gene catalyzing bilirubin metabolism. The transcriptional regulation of ugt1a1 plays a key role in controlling UGT1A1 expression. Hence, we investigated the transcriptional effects of two polymorphisms, $(TA)_n$ short tandem repeat (STR) polymorphism and a single nucleotide polymorphism (SNP) at -3,264 site in the 5'-flanking region of ugt1a1 by dual-luciferase reporter assay. We found that both polymorphisms significantly affect the transcription of the gene but only (TA)n polymorphism had significant effect on bilirubin level. We further studied such transcriptional effects on serum total bilirubin level and CHD occurrence in a Chinese population. Although bilirubin level is an important factor in CHD occurrence, (TA)n showed no association with CHD. The observed disruption of causative relationship indicates the complexity of the etiology of complex diseases.

2775/F

Sequencing of PKHD1 in Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF). *D. Adams[1], H. Edwards[1], A. Garcia[1], E. Font-Montgomery[1], M. Huizing[1], P. Choyke[3], T. Heller[5], P. Mohan[6], K. Daryanani[7], L. Guay-Woodford[4], W. Gahl[1], M. Gunay-Aygun[1,2].* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI; 2) Intramural Office of Rare Diseases, NIH; 3) NCI, NIH; 4) Univ. of Alabama, Birmingham AL; 5) NIDDK, NIH; 6) CNMC, Wash., DC; 7) NIH Clinical Center.

ARPKD/CHF, a form of PKD with onset primarily in childhood, is typically associated with CHF complicated with portal hypertension (PH). ARPKD/CHF results from mutations in PKHD1, one of the largest genes in the human genome. PKHD1 exhibits a complex splicing pattern. The longest open reading frame, composed of 66 exons, encodes fibrocystin, a 4074 amino acid protein located on the primary cilia-basal body/centriole complex. PKHD1 also has 19 alternate exons. Although the diagnosis of ARPKD/CHF is still made clinically in most patients, confirmation of diagnosis with DNA analysis is increasingly employed, especially in atypical patients and for prenatal diagnosis. The current mutation detection rate ranges from 75-85%. To date, more than 300 PKHD1 mutations throughout the gene have been reported. As part of an ongoing NIH natural history study on ARPKD/CHF and other ciliopathies (www.clinicaltrials.gov, trial NCT00068224), we have sequenced the PKHD1 gene in a total of 66 patients, including 45 clinically typical ARPKD/CHF, 17 atypical/unknown PKD/CHF, 3 CHF/PH associated with ADPKD, and 1 Caroli's disease. The pathogenicity of the missense mutations was evaluated using existing databases, intraspecies sequence conservation, and the conservation of amino acid chemistry. In the 84 typical ARPKD/CHF proband alleles, 70 potentially pathogenic mutations were detected. Sixteen of these had not been previously reported. In the 34 atypical/unknown alleles, 10 potentially pathogenic mutations were detected. No pathogenic PKHD1 mutations were found in the 6 alleles of patients with CHF and PH associated with ADPKD or in the 2 with Caroli's disease. We continue to sequence DNA from more ARPKD/CHF and related ciliopathy patients, enrolling new patients in an effort to improve diagnostic accuracy and better characterizing these disorders.

2776/F

Characterization of deletions in the *SPAST* gene. *C. Beetz[1], C. Oubrayme[1], C. Depienne[2], S. Zuchner[3], E. Reid[4], R. Schüle[5], M. Auer-Grumbach[6], S. Klebe[7], J. Schickel[1], A. Brice[2], M. Pericak-Vance[4], L. Schöls[5], T. Deufel[1].* 1) Institut f. Klinische Chemie, Uniklinikum Jena, Jena, Germany; 2) INSERM U679, Groupe Hospitalier Pitie-Salpetriere, Paris, France; 3) Miami Institute of Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 4) Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK; 5) Hertie-Institut f. Klinische Hirnforschung, Uniklinikum, Tübingen, Germany; 6) Zentrum f. Medizinische Forschung, Medizinische Universität Graz, Graz, Austria; 7) Klinik für Neurologie, Uniklinikum Schleswig-Holstein, Kiel, Germany.

Not much is known about the mechanisms underlying large genomic deletions as they are not easily detected and as only few have been characterized in detail. We here present a comprehensive analysis of *SPAST* deletions which we previously showed to account for >10% of cases of hereditary spastic paraplegia. A total of 55 of these aberrations have been identified to date. They affect 25 different (combinations of) exons. Of the 110 breakpoints, 75 reside in *SPAST* introns, while 16 and 19 lie upstream and downstream of the gene, respectively. In contrast to a number of other deletion-prone disease genes, there is no evidence for a breakpoint hotspot. Instead, the number of breakpoints correlates with intron size ($R^2=0.60$) and only weakly with the density of Alu elements ($R^2=0.09$). Sequencing of the junction in 19 intragenic events reveals non-homologous recombination as the mutational mechanism in 10 cases, homologous recombination between repetitive sequences in 7 cases, and complex insertion-deletion events in 2 cases. The latter figure is likely to be higher as extensive screening by long range PCR failed in an additional 5 cases. The apparent lack of a predominant mutational mechanisms is, again, in contrast to the few other genes for which a similar amount of sequence data is available. We expect our world-wide analysis of a comparatively rare disease gene to stimulate similar efforts for other genes. A broader range of this kind of studies may, eventually, lead to a better understanding of the causative events underlying large disease-causing deletions.

2777/F

Co-transcription of genes into single transcripts: another regulatory mechanism for gene expression in vertebrates. *T.D. Taylor, T.P. Srivastava, V.K. Sharma, Y. Nishida, T. Fujikake, T. Takeda, R. Ozawa, M. Mushiake, R. Okumura, Y. Sakaki.* Computational and Experimental Systems Biology Group, RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan.

Co-transcription of two distinct genes (child genes) into a single transcript (conjoined gene) has not been well explored. Either it is a somewhat rare phenomenon, or the current methods of genome annotation are not sensitive enough to identify such genes. Towards this we have designed a new computational algorithm "Conjoin" for the identification of conjoined genes in any genome given its messenger RNA or EST information. Applying *Conjoin* to the human genome, we have identified nearly 900 conjoined genes of variable lengths, some with multiple isoforms. We have so far confirmed the existence of 195 of these conjoined genes using RT-PCR and sequencing. In view of the fact that we observed several cases of conjoined genes occurring in the human genome, it appears that these conjoined transcripts are arising out of novel functional requirements and are not merely artifacts of transcription. However, the underlying mechanism controlling the formation of such conjoined genes in human and other vertebrate genomes remains to be explored. In order to confirm the presence of conjoined genes in other vertebrates, we implemented the *Conjoin* algorithm on both the mouse and chimpanzee genomes. It is remarkable to observe that the number of conjoined genes in mouse is far less than that in human, even though there is roughly the same amount of mRNA/EST data available. Thus it appears that the conjoined genes might be performing some novel functions and are contributing to human complexity as compared to other lower organisms. Therefore, we carried out a detailed functional analysis of the human conjoined and participating child genes. Further, in order to explore the intrinsic mechanisms of this process, the 5' and 3' flanking regions of the child genes were analyzed to search for the presence of any alternate or common regulatory elements that might be controlling the formation of conjoined genes.

2778/F

Identification of a novel ZIC3 isoform and mutation screening in patients with congenital heart malformations. *J.E.J. Bedard[1], S. Fernbach[2], J.W. Belmont[2], S.M. Ware[1].* 1) Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Department of Pediatrics, Cincinnati, OH; 2) Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX.

Congenital heart malformations are the most common birth defect and cause significant morbidity and mortality. Patients with heterotaxy have characteristic cardiovascular malformations, abnormal arrangement of visceral organs, and midline patterning defects due to abnormal left-right patterning during early embryogenesis. Loss of function of the transcription factor ZIC3 causes X-linked heterotaxy and isolated congenital heart malformations, and represents one of the few known monogenic causes of congenital heart disease. Although the birth prevalence of heterotaxy spectrum malformations is significantly higher in males, we have previously demonstrated that this gender bias is not accounted for by mutations in ZIC3. The current investigation identifies an alternatively spliced ZIC3 mRNA (ZIC3-B) with a previously unrecognized exon, suggesting a possible novel genetic cause of X-linked heterotaxy. Characterization of the ZIC3 isoforms indicates that exons 3 and 4 are alternatively spliced and share less than 35% identity, suggesting that exon 4 evolved independently and did not arise by a duplication and divergence of exon 3. Exon 4 is highly conserved across species and results in an isoform with a distinct C-terminus of the protein while maintaining the zinc finger DNA binding domain, protein interaction domains and nuclear localization and export signals. Expression analysis of ZIC3-B indicates that it is expressed in murine embryos at critical stages of cardiac development, suggesting it as a possible cause of heterotaxy and cardiovascular malformations. In adult tissues, its expression pattern overlaps with that of ZIC3-A. To further investigate the role of ZIC3-B in cardiac development, 109 male heterotaxy cases (5 familial and 104 sporadic) were screened. No mutations were identified in ZIC3-B, suggesting that this novel isoform is not a major contributor to heterotaxy spectrum cardiovascular abnormalities.

2779/F

Mapping Promoter of Endothelial Lipase Gene and Functional Studies of Two SNPs in the Regulatory Regions. *D. Slavov, H. Razzaghi.* Department of Medicine, University of Colorado, Aurora, CO.

A low concentration of high-density lipoprotein cholesterol (HDL-C) is a significant risk factor for atherosclerosis, leading to coronary heart disease. Understanding the mechanism by which genetic factors influence HDL-C levels are therefore very important. Endothelial lipase (LIPG) plays a major role in HDL metabolism. We investigated the role of possible regulatory elements within 5' and 3' regions flanking LIPG gene using luciferase assay. Fragments spanning 1409 bp upstream of ATG codon and 2388 bp downstream of stop codon were cloned with luciferase reporter gene. Serial deletions of 300bp and 60bp were made in order to study different regions of promoter. The results indicate that the region -61 to -120 plays an essential role for promoter activity. It contains the critical CCAAT element and the consensus sequence for Oct1 binding site. The region -1 to -60 contains elements that resemble the consensus Inr site and TATA box. However this region by itself has no promoter activity. DNA sequences upstream of position -120 cause variations in promoter activity, suggesting presence of additional regulatory elements. The nature of these elements is currently under determination by EMSA and mass spectrometry. Two SNPs in LIPG regulatory regions (position -384 A/C and +2237 A/G) have been previously reported to have an association with HDL-C levels in Asian population. We evaluated the role of these SNPs in cellular model. Each one alone showed an increase in luciferase expression. When both SNPs were combined in the same construct, the effect on expression was higher, indicating that they work in synergy manner. The allele frequencies, A=1.00 for -384 A/C and G=0.32 for +2237 A/G in our high and low HDL groups of Whites and Hispanics were different than the reported allele frequencies, A=0.88 for -384 A/C and G=0.64 for +2237 A/G in Asian population. We did not find a significant association of +2237 A/G SNP with either high or low HDL group. In summary we reported a functional map of regulatory regions of the LIPG gene and characterization of two of its SNPs.

2780/F

Pre-mutation alleles in myotonic dystrophy type 2. *L.L. Bachinski[1], T. Czernuszewicz[1], L.S. Ramagli[1], T. Suominen[3], C.A. Thornton[2], B. Udd[3], M.J. Siciliano[1], R. Krahe[1].* 1) Dept. of Cancer Genetics,Univ.of Texas MD.Anderson Cancer Center, Houston,TX; 2) Dept. of Neurology,Univ.of Rochester Medical Center, Rochester,NY; 3) Dept. of Neurology, Tampere Univ. Hospital, Finland.

Myotonic Dystrophy types 1 and 2 are neuromuscular disorders with multi-system involvement, caused by expansion of microsatellite repeats. For DM1 there is a reservoir of pre-mutation alleles in the population. However, there have been no reports of pre-mutation alleles for DM2 and the minimum size of a pathogenic expansion is not known. The DM2 expansion is part of the complex polymorphic motif (TG)12-26_(TCTG)7-12_(CCTG)3-9_(G/TCTG)0-4_(CCTG)4-15. Expansions are as large as 40 kb with the CCTG motif uninterrupted. Reported normal alleles have repeat track lengths of up to 176 bp or 26 CCTG motifs with one or more interruptions. The smallest reported DM2 expansion had an uninterrupted (CCTG)75. Because of their large size, PCR across DM2 expansions is not possible and few disease alleles have been sequenced. To address questions of the presence of pre-mutation alleles in the population, the smallest pathogenic allele size, and the possible role of the DM2 repeat as a modifier in other neuromuscular diseases, we cloned and sequenced a number of unusually large alleles, along with typical ones, from both normal and disease populations. We identified one DM2 patient whose expanded allele contained an uninterrupted (CCTG) track of only 55 repeats. We found one patient with diagnosis of DM2 and a large expansion by Southern in addition to two consistently amplifiable alleles, differing by 4 bp (possibly mosaic). For him we genotyped 276 clones and saw 45 different alleles, suggesting instability. All of the 16 clones sequenced had uninterrupted CCTG tracks and, except for the length of the CCTG motif, appeared identical. Small-pool PCR found 45% novel alleles, confirming this instability (p=3.99 E-07). A number of other large alleles with track lengths of 170 - 222 bp were also sequenced with up to (CCTG)32 and 1-4 interruptions. We conclude that large unstable alleles exist and may represent a pre-mutation allele pool. Also, the minimum pathogenic allele can contain as few as 55 (CCTG) motifs.

2781/F

Identification of small molecules promoting the translation of FMR1 mRNA with expanded CGG repeats. *Y. Qin, P. Jin.* Department of Human Molecular Genetics, Emory University School of Medicine, Atlanta, GA 30322.

Fragile X Syndrome, a common form of inherited mental retardation, is mainly caused by the expansion of CGG triplet repeats within the 5' untranslated region (5'-UTR) of the fragile X mental retardation-1 (FMR1) gene. The loss of functional fragile X mental retardation protein (FMRP) is responsible for fragile X clinical phenotypes. Previously it has been shown that FMR1 mRNA with expanded CGG repeats produces less or no FMRP due expanded CGG repeats. It has been proposed that expanded CGG repeat RNA forms secondary structure and impede the 40S ribosome migration along the 5'-UTR. To further understand the molecular mechanism of this translational suppression, we have taken a chemical biology approach. Here we have established a high-throughput assay that utilizes slot blot to monitor the level of FMRP in cells. A cell line carrying an unmethylated full-mutation CGG repeat that produces little FMRP protein was used for chemical screen with a collection of 2,000 FDA-approved, biologically active and structurally diverse compounds. The identified compounds in the initial screen were further confirmed using western blots and additional cell lines. We have identified and confirmed seven compounds that could promote the translation of FMR1 mRNA with expanded CGG repeats but do not change the level of FMR1 mRNA. Interestingly these compounds do not alter the translation of FMR1 mRNA with normal CGG repeat. We hypothesize that small molecules could alter the secondary structure of expanded CGG repeat RNA and promote the translation initiation of FMR1 mRNA. The elucidation of the action mechanism of these small molecules will be helpful to further understand the translation suppression of FMR1 mRNA with expanded CGG repeats.

2782/F

Characterization of a balanced translocation breakpoint to within the FOXP2 gene in a two-generation family with language impairment. *J.B. Bjork[1], J.B. Tomblin[2], C.A. Williams[3], S.R. Patil[1], M.R. O'Brien[2], J.C. Murray[1].* 1) Dept Pediatrics, U of Iowa, Iowa City, IA; 2) Dept Speech Pathology and Audiology, U of Iowa, Iowa City, IA; 3) Dept Pediatrics, U of Florida, Gainesville, FL.

We have previously reported on a balanced 7;13 chromosomal translocation within the forkhead transcription factor gene, FOXP2, in a mother-daughter pair. Both individuals present with a developmental language disorder that persists despite adequate intelligence and opportunity for language learning. BAC and fosmid clones were utilized in fluorescent in-situ hybridization (FISH) analysis on G-banded metaphase chromosome spreads to map the breakpoint. Long-range PCR was used to amplify a segment of DNA across the breakpoint on the der(7) and der(13) chromosomes. Sequence analysis revealed the translocation breakpoint to be within intron 9-10 of FOXP2 on chromosome 7, and within intron 7-8 of RFC3 on chromosome 13. A frameshift mutation is anticipated for each fusion protein transcribed from the FOXP2 gene variants, FOXP2-RFC3 and RFC3-FOXP2, resulting in the premature truncation of gene transcripts. It is hypothesized that this truncation yields an unstable cytoplasmic protein product, similar to that described in a family with a R328X mutation in FOXP2 (MacDermot et al., 2005; Vernes et al., 2006). It is not yet known whether these products are in fact present in this affected mother and daughter. However, it is probable that haploinsufficiency of the FOXP2 protein cosegregates with language difficulties. In addition, it is hypothesized that the proximity of the RFC3 gene to NBEA, a gene implicated in autism, may further influence the phenotype of this family.

2783/F

INTRACELLULAR TRAFFICKING ANALYSIS OF C111Y AND C111S MUTATIONS IDENTIFIED IN FACTOR IX FROM MEXICAN PATIENTS WITH SEVERE HEMOPHILIA B. ANTECEDENTS. *J. Mantilla[1,3], N. Enjolras[2], C. Négrier[2], A.R. Jaloma-Cruz[1,3].* 1) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México; 2) Laboratoire d'Hémobiologie, Faculté de Médecine RTH Laennec, Lyon, France; 3) División de Genética, CIBO, IMSS, Guadalajara, Jalisco, México.

ANTECEDENTS. We studied two mutations at 17,747 nucleotide in the second-like epidermal growth factor (EGF2), of factor IX gene (FIX), to identify their effect in the structure-function relationship of the protein by the study of their intracellular trafficking. MATERIALS AND METHODS. C111 wild-type and the mutations C111S and C111Y were inserted by directed-site mutagenesis into an expression vector (pcDNA 3.1®) containing the FIX wild-type (wt) gene. Transfection on Cos-7 cells by Fugene6® after 48hrs was tested with a control plasmid containing the green fluorescence protein (pGFP) with a good efficiency (64.5%) evaluated by a flux-cytometry. The intracellular FIX amounts and secretion were quantified by ELISA assay. Transfected cells were incubated in presence of inhibitors like Brefeldin A, which blocks protein transport from endoplasmic reticulum (ER) to the Golgi; N-Acetyl-Leu-Leu-Norleucinal (ALLN) and Clasto-lactacystin beta-lactone, proteasomal inhibitors, and NH4Cl and Leupeptin, lysosomal inhibitors. RESULTS. Respect to FIX wt, the mutations showed a decreased FIX secretion (20%) and intracellular accumulation of 140% (C111Y) and 160% (C111S). The effects of the inhibitors caused a higher intracellular accumulation of the mutants which led a degradation mainly in lysosomes (NH4Cl) and secondly in proteasomes (ALLN). By the effect of Brefeldin A on C111S we can assume an adequate transport from ER to Golgi, opposite to C111Y which seems to be blocked at ER and to have elevated degradation in proteasomes (ALLN effect). CONCLUSIONS. The disruption of the disulfide bond in the mutants have an important effect on the native folding of FIX protein, evident by the effects on its transport through ER and the degradation mechanisms, with a predominant degradation at proteasomes when the ER transport is blocked and a higher degradation at lysosomes when the transport from ER to Golgi complex is adequate.

2784/F

Interaction of Down syndrome-related gene product SIM2 and circadian rhythm protein BMAL1. *Y. Shimizu[1], A. Yamaki[1], M. Ikeda[2], J. Kudoh[3], N. Shimizu[4].* 1) Dept Medical Genetics, Kyorin Univ Fac Health Sci, Hachioji, Tokyo, Japan; 2) Res Center for Genomic Med, Saitama Med School, Saitama, Japan; 3) Dept Mol Biol, Keio Univ School of Med , Shinjuku, Tokyo, Japan; 4) GSP Center, The Leading Institute of Keio Univ, Tsukuba, Japan.

Human *SIM2* gene locates on Down syndrome chromosomal region, 21q22.2, and the protein product belongs to the family of bHLH (basic helix-loop-helix)/PAS (Per-Arnt-Sim) transcription factors. It has been shown that SIM2 protein forms heterodimer with ARNT or ARNT2 to inhibit the expression of target genes and that BMAL1 forms heterodimer with CLOCK to activate the transcription of target genes, such as *PER1*.

To find new partner of SIM2 heterodimer, we tested the possibility of interaction with BMAL1. After the transient expression of SIM2 and BMAL1 in HEK293 cells, SIM2 was immunoprecipitated with BMAL1 using the tag peptide antibodies. Experiments using a series of deletion constructs of SIM2 revealed that the region of nuclear localization signal was required for the interaction of SIM2 and BMAL1.

In the promoter region of *PER1*, there are three E-boxes as cis-elements for BMAL1/CLOCK heterodimer to increase Luciferase activity. We found that SIM1 or SIM2 alone enhanced the promoter activity and BMAL1 affected more positively in the case of SIM1, but not in the case of SIM2. Furthermore, we examined the effect of BMAL1 on *SIM2* promoter activity. BMAL1/CLOCK inhibited by 40% and the addition of CRY1 inhibited by 60%. The transcription factors related to the circadian rhythm, such as BMAL1, CLOCK and CRY1, may play significant roles in the regulation of SIM2 transcription.

2785/F

Common disease related genetic variations associate with the level of expression of DCIR mRNA isoforms. *M. Ronninger, C. Eklöw, J.C. Lorentzen, L. Klareskog, L. Padyukov.* Medicin, Karolinska Institutet and Hospital, Stockholm, Sweden.

To identify possible regulatory regions in a gene showing association to rheumatoid arthritis we analyzed mRNA expression pattern of DCIR in interferon-gamma treated leukocytes together with fine mapping across the locus. This also included validation and recognition of expressed transcripts since four known variants have to date been found for DCIR. Controls (44) and patients (44) with rheumatoid arthritis (RA) included in our study were genotyped for 21 common SNPs in DCIR and flanking chromosome region. mRNA expression of individual isoforms was determined by transcript specific quantitative PCR. In addition, semi-quantitative PCR with primers based on flanking exons was performed to obtain expression of all isoforms in one reaction. Our data show that IFN-g down regulates DCIR expression in PBMCs and the average expression of isoform DCIR_v1 and DCIR_v4 is significantly lower after stimulation in patients (non-stimulated vs. stimulated, $p < 0.0001$ for DCIR_v1, $p < 0.005$ for DCIR_v4, Wilcoxon Signed Rank test). Expression of mRNA DCIR isoforms showed strong association with common variations in the recombination block which corresponds to the area between upstream and promoter region and the third exon of DCIR. This association was represented by significant different level of DCIR_v4 expression in both patients and controls ($p < 0.001$ and $p < 0.01$ respectively, Kruskal-Wallis test for rs2024301). In addition to the four known forms of DCIR, a novel transcript was detected with the sequence lacking third and fourth exons. This data illustrates the influence that common genetic variations may have regarding the expression of the DCIR gene, variations that can act through transcription regulation mechanisms. This could result in different functional activity due to the change in level of expression of the receptor isoforms.

2786/F

A high resolution expression atlas of Retinitis Pigmentosa genes in the human and mouse retinas. *D. Trifunovic[1], M. Karali[1], D. Camposampiero[2], D. Ponzin[2], V. Marigo[3], S. Banfi[1].* 1) Tigem, Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Fondazione Banca degli Occhi del Veneto, Venice; 3) Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Retinitis Pigmentosa (RP) is one of the leading causes of visual handicap in the world population and is characterized by high genetic heterogeneity. The study of the disease mechanisms and the development of efficient therapeutic approaches so far have mostly relied on the availability of animal models for this condition. Nevertheless, little information is available about the RNA expression profiles of the RP genes in the human retina. To overcome this lack of information, we generated an expression atlas of 34 known RP genes in human and murine retinas by RNA *in situ* hybridization. The vast majority of the genes analyzed displayed similar patterns between human and mouse retina. Interestingly, four genes belonging to the visual cycle cascade, namely RGR, RPE65, RLBP1 and LRAT, were differently distributed in human and murine retina. We show that these four genes are expressed by cones in the human retina, suggesting that visual cycle processing occurs also in cones in a mechanism alternative to the one that takes place in the RPE. The generation of this atlas may shed new light on the function of RP genes and their putative role in disease pathogenesis.

2787/F

***Odz4*: Understanding the role of highly conserved elements in gene expression.** *M.J. Cramer[1], M.J. Justice[2], A.C. Lossie[1].* 1) Animal Science, Purdue University, West Lafayette, IN; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Odz4 is an important gene in mammalian development. Within the gene *Odz4*, there are six known embryonic lethal mouse mutants, which are characterized by abnormal mesoderm development, abnormal somite formation and defects in maternal blood flow to the embryo. Of these six mutants only one mutated allele has been found by conventional sequencing methods. The expression of *Odz4* is complicated and not well understood. In the embryo and adult mouse, there is a high incidence of alternatively spliced and tissue specific transcripts. These transcripts have the potential to generate at least 5 different protein isoforms. Recent studies have proven that non-coding RNAs can employ mRNAs to direct tissue and temporal gene expression. Our goal is to determine which of these alternatively spliced transcripts are important during development. We hypothesize that non-coding RNAs will play a major role in embryonic development and *Odz4* expression. We have identified 93 highly conserved non-coding elements (HCEs) in the 1.3Mb region surrounding *Odz4* that are >200bp in length and at least 75% identical between mouse and human. To date, the identity of these homologous regions has not been explored. Using reverse transcription (RT-PCR) we were able to identify that 73 of the 93 HCEs screened are expressed. RT-PCR between exon 1 and each HCE and each HCE and exon 6 was performed to identify which of these expressed HCEs are new exons of *Odz4*. The results indicate that there are many more exons in this gene than previous thought. Currently, 48 out of 93 HCEs are new exons of *Odz4*, while 20 out of 93 HCEs are not new exons. Our future research includes sequencing each of the newly identified exons to potentially identify the remaining mutations. We also intend to investigate the identity of the expressed HCEs that are not new exons of *Odz4*. It is very likely that they could be cis-regulatory elements, non-coding RNAs or they could play a role in the alternative splicing of *Odz4*.

2788/F

Localization Of The *Cis*-enhancer Element For Mouse *Col10a1* Expression In Hypertrophic Chondrocytes *In Vivo*. *Q. Zheng[1, 6], B. Keller[1, 4], G. Zhou[3], D. Napierala[2], Y. Chen[2], B. Zabel[4], A. Parker[5], B. Lee[1, 2].* 1) Molec & Human Genetics, Baylor College Med, Houston, TX; 2) Howard Hughes Medical Inst., Baylor College Med, Houston, TX; 3) Dept. of Orthopedics, Case Western Reserve University, Cleveland, OH; 4) Center of Pediatrics and Adolescence Medicine, University Hospital of Freiburg, D-79106, Germany; 5) Respiratory and Inflammation Res. Area, Astrazeneca, Cheshire U.K; 6) Dept. of Anatomy and Cell Biology, Rush University Medical Center, Chicago, IL.

We and others had previously shown that 4kb or 4.6kb *Col10a1* promoter containing Runx2 or AP-1 (Activator Protein-1) elements contribute to its hypertrophic chondrocyte-specific expression *in vivo* (Zheng et al., 2003; Gebhard et al., 2004). These data suggest that the *Col10a1* distal promoter (-4.4 to -3.8 kb) harbors a critical enhancer that mediates its tissue specificity. To further localize the tissue-specific enhancer element, we have generated series of transgenic reporter mice containing 600, 300 or 150 base pairs of DNA derived from this region upstream of the *Col10a1* basal promoter driving *LacZ* gene. We identify a 150bp *Col10a1* promoter element (-4.3 to -4.15 kb) that is sufficient to direct its tissue-specific expression *in vivo*. *In silico* analysis of this region identified several putative transcription factor binding sites including two potential AP-1 sites within 5'- and 3'- ends, respectively. Interestingly, transgenic mice using reporter constructs deleted for these two putative AP-1 elements still showed tissue-specific reporter activity. Electrophoretic mobility shift assays using oligonucleotide probes derived from this region and MCT cell nuclear extracts identified DNA/protein complexes that were enriched from cells stimulated to hypertrophy. Moreover, these elements mediated increased reporter activity on transfection into MCT cells. These data identify a minimal cis enhancer required for tissue specific *Col10a1* expression *in vivo* and putative DNA/protein complexes that may contribute to the regulation of chondrocyte hypertrophy.

2789/F

Investigation of rare alleles in MODY genes and their implication in controlling the level of fasting blood glucose. *B. Oh[1], H.R. Han[1], M.J. Go[1], Y.J. Kim[1], S.J. Yang[2], J.E. Lee[3], H.R. Kim[1].* 1) Center for Genome Science, National Institute of Health, Seoul, Korea; 2) Macrogen Co., Seoul, Korea; 3) DNA Link, Inc., Seoul, Korea.

Since the prevalence of complex traits such as hypertension and diabetes is high, according to the hypothesis of common disease common allele genetic variations such as SNP having the high frequency in the population have been favored to search the genetic cause of the common diseases. However recently researches have been published to support that rare alleles contribute the expression of complex traits such as HLD or LDL level of blood. In this study rare alleles were identified by resequencing 6 MODY genes, HNF1α (MODY1), GCK (MODY2), HNF1α (MODY3), HNF1β (MODY4), IPF1 (MODY5), NeuroD1 (MODY6), in 120 unrelated Koreans, 60 each in the group of low fasting blood glucose level and in the group of high level. Total 126 alleles were discovered and 46 among them were novel, however only 7 nonsynonymous alleles were discovered from these genes, suggesting that these metabolism-related genes are highly conserved in a repect of protein structure in the population. In addition to the nonsynonymous alleles 41 alleles located upstream of the initiation site were discovered and 9 alleles were selected from these nonsynonymous and promoter alleles for the further study. The selected alleles were genotyped in 7400 individuals collected from the prospective cohort in Korea in order to investigate their relationship to the level of fasting blood glucose by regression analysis. In addition to the analysis the distributions of fasting blood glucose level in carriers were compared with those of total population.

2790/F

Natural gene expression variation in Down syndrome modulates the outcome of gene dosage imbalance. *P. Prandini[1], S. Deutsch[1], R. Lyle[1,7], M. Gagnebin[1], C. Delucinge Vivier[2], M. Delorenzi[3,8], C. Gehrig[1], P. Descombes[2], S. Sherman[4], F. Dagna Bricarelli[5], C. Baldo[5], A. Novelli[6], B. Dallapiccola[6,9], S.E. Antonarakis[1].* 1) University of Geneva,CH; 2) NCCR Genomic Platform, University of Geneva,CH; 3) Swiss Institute of Bioinformatics (SIB), Lausanne,CH; 4) Emory University School of Medicine, Atlanta,USA; 5) Genetics Laboratory, Galliera Hospital, Genoa,IT; 6) IRCCS-CSS, San Giovanni Rotondo and CSS-Mendel Institute, Rome,IT; 7) Ullevål University Hospital, Oslo, NO; 8) Swiss Institute of Experimental Cancer Research (ISREC), Epalinges,CH; 9) University of Rome "La Sapienza", Rome, IT.

Down syndrome (DS) is characterized by extensive phenotypic variability with most traits occurring in only a fraction of affected individuals. Substantial gene expression variation is present among normal individuals and this variation has a strong genetic component. Since DS is caused by genomic dosage imbalance, we hypothesize that gene expression variation of human chromosome 21 (HSA21) genes in DS individuals has an impact on the phenotypic variability among affected. We studied gene expression variation in 14 lymphoblastoid (LCLs) and 17 fibroblast cell lines from DS individuals and an equal number of controls. Gene expression was assayed using qRT-PCR on 100 and 106 HSA21, and 23 and 26 non-HSA21 genes in each cell type respectively. Gene expression variation in DS and normal samples was evaluated using the Kolmogorov-Smirnov test. According to the degree of overlap in expression levels, we classified all genes into 3 groups: (A) non-overlapping; (B) partially overlapping; (C) extensively overlapping expression distributions between normal and DS samples. We hypothesize that in each cell type, group A genes are the most dosage sensitive and likely involved in the constant DS traits; those in group B might be involved in variable DS traits, whereas those in group C are not dosage sensitive and less likely to participate in DS pathological phenotypes. This study provides the first extensive data set on HSA21 gene expression variation in DS and underscores its role in modulating the outcome of gene dosage imbalance.

2791/F

Applications of next generation sequencing using stepwise cycled ligation. *G. Costa, C. Lee, L. Apone, J. Stuart, J. Warner, R. David, A. Sheridan, S. Ranade, J. Ichikawa, K. McKernan.* Genetic Analysis, Applied Biosystems, Beverly, MA.

The SOLiD sequencing system uses stepwise cycled ligation and is being developed for high throughput DNA sequencing. In this novel system, short fragment DNA populations are amplified onto 1-micron beads, enriched and randomly deposited at high density onto glass slides. The DNA bead arrays are then placed into a dual automated flow cell where 4-color, fluorescently-labeled octameric probes are delivered serially and serve to interrogate known template positions on DNA strands. Current enhancements to the SOLiD system have included the development of library construction methods that afford genome sequencing of short fragment (1 x <50 bp) and mate-paired (2 x 25 bp) DNA libraries. Consistent improvements have been made in total sequence throughput by enhanced read length and base calls, higher bead density and optimized ligation biochemistry. Taken together, recent improvements have demonstrated performance of >2 Gb per single tag (fragment library) and up to 4 Gb per dual tag (mate-pair library) per instrument run. Results presented will highlight a number of collaborative projects directed at the sequencing of microbial, fungal and human genomes. Sequencing applications have included directed resequencing of human cancer-based amplicon libraries, whole genome sequencing of microbial and fungal genomes, and transcriptome analyses.

2792/F

High resolution genome-wide copy number analysis by copy number inferring tool (CNIT) and its usage in data from DNA pooling. *C.H. Lin[1], M.C. Huang[2], L.H. Li[3], J.Y. Wu[3], Y.T. Chen[3], C.S.J. Fann[1,3].* 1) Institute of Genome Sciences, Yang-Ming University, Taipei, Taiwan; 2) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Recently copy number variation (CNV) has been found as one of the important genomic structure variants in human population, and some of them are related to specific traits and diseases. Single nucleotide polymorphism (SNP) microarrays provide high resolution tool to analyze human genomes. Although many programs were designed to analyze data from Affymetrix SNP microarrays, they all have high false-positive rates in CN analysis. Copy number analysis tool (CNAT) 4.0 was a newly-developed program with large improvement in CN estimation, but small amplifications and deletions were lost when using genomic smoothing procedure. In this report, we propose Copy Number Inferring Tool (CNIT) algorithm for 100K and 500K SNP microarrays to investigate CNVs at the 29.6 and 7 kb resolution, respectively. CNIT estimated SNP allelic and total CN with reliable p-value. In addition, hidden Markov model (HMM) method was applied to predict CN-changed regions by considering contiguous SNPs. Based on the CN analysis of twenty-three unrelated Taiwanese and thirty HapMap CEPH trios, CNIT showed higher accuracy and power than other programs. The false-positive and false-negative rates of CNIT were 0.001 and 0.0016, respectively. CNIT showed better sensitivity in detection of small amplifications and deletions. Furthermore, DNA pooling of ten normal unrelated individuals was applied to 100K SNP microarray and analyzed by CNIT. Three common CNVs were successfully identified and validated by qPCR experiments. CNIT has high power to detect CN-changed SNPs and DNA segments without smoothing, and is applicable in CNV finding, disease gene mapping and pharmacogenetic studies. SNP microarray coupled with CNIT provided a high resolution tool to investigate subtle chromosomal structures in disease or trait studies of interest. In addition, DNA pooling was performed to identify common CNVRs, suggesting that DNA pooling is a feasible way in CNV studies.

2793/F

Comparative molecular and functional studies of proline and hydroxyproline oxidases. *A.S. Willis[1], C.-A.A Hu[2], G. Steel[1], W.-W. Lin[3], C. Obie[1], H. Levy[4], D. Valle[1].* 1) Inst Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Dept Biochem and Mol Biol, Univ New Mexico Sch Med, Albuquerque, NM; 3) Dept Psychiatry, Tri-Service General Hospital and National Defense Medical Sch, Taipei, Taiwan; 4) Dept Pediatrics, Harvard Medical Sch, Boston, MA.

PRODH (22q11) encodes proline oxidase (POX) and is of interest as the gene responsible for hyperprolinemia type 1 (HP1) and is also involved in schizophrenia and cancer. A similar gene, *PRODH2*, located at 19q13.1 is a candidate structural gene for hydroxyproline oxidase (OHPOX). We utilized RT-PCR, 5' RACE, and expression studies to refine the exon-intron structure of *PRODH2* and determine the function of its protein product. We found the *PRODH2* transcript to be shorter that the cDNA sequence in the public databases (NM_021232) lacking 157 bases at the 5' end, corresponding to the curated exon 1, in either liver or kidney, tissues where this gene is highly expressed. The ORF begins in the curated exon 2 and encodes a 461 amino acid protein with predicted molecular weight of 51 kDa. We expressed this cDNA in CHO cells and found that the *PRODH2* enzyme catalyzes the oxidation of hydroxyproline rather than proline. In addition, we studied a patient with hyperhydroxyprolinemia and found her to be a compound heterozygote for two mutations in *PRODH2* (a slice site change and a 1 bp deletion in the coding sequence). To ask what determines the substrate specificity of POX and OHPOX, we used comparative genomics. We found that all but 2 of 18 active site residues of POX that are conserved to *E. coli* are identical in OHPOX. To investigate the significance of these 2 residues, we designed a residue switching experiment where the residues in OHPOX were changed to those found in POX and vice versa and expressed these recombinant proteins in stably transfected CHO cells. In initial assays, we find that C279Y and S409Y in OHPOX confer low but detectable POX activity. The converse experiment is in progress. We conclude that *PRODH2* (19q13.1) encodes OHPOX and is responsible for hyperhydroxyprolinemia and are defining active site residues that determine the substrate specificities for these 2 closely related imino acid oxidases.

2794/F

A common polymorphism in the CARD domain of RIG-I modifies the innate immune response of human dendritic cells. *J. Hu, A. Voho, Y. Ding, A. Ganee, M. Kumar, A. Pendleton, J.G Wetmur.* Microbiology, Mount Sinai School of Medicine, New York, NY.

Infection of human dendritic cells (DCs) by negative-stranded RNA viruses leads to activation of the RNA helicase RIG-I, exposing its CARD domain. The CARD domain binds to MAVS and initiates downstream signaling resulting in the induced transcription of the interferon beta gene (*IFNB1*). Interferon beta expression is a crucial step in both induction of innate immunity and in the DC maturation response leading to induction of adaptive immunity. Transcription of the RIG-I gene (*DDX58*) is itself induced by interferon beta signaling through the type-1 interferon receptor. A common and potentially functional SNP, rs10813831 (A/G), encodes a Arg7Cys polymorphism in the RIG-I CARD domain. qRT-PCR analysis of the total RNA extracted from 130 DC samples infected by Newcastle disease virus revealed a significant association of Arg7Cys with increased *IFNB1* transcription (p=0.05 for heterozygous and p=0.019 for homozygous variants; p_{trend}=0.021) and *DDX58* transcription (p=0.067 for heterozygous and p=0.034 for homozygous variants; p_{trend}=0.023), particularly in homozygous variants. RNA allelic imbalance (AI) analysis on these infected DC samples showed that the Arg7Cys polymorphism was not associated with AI (p=0.845), ruling out linkage disequilibrium and demonstrating that the observed association between Arg7Cys and transcription originated from the structural change of RIG-I CARD domain, possibly affecting RIG-I folding or interaction with MAVS. In a separate experiment, single-cell level transcription of *IFNB1* and *DDX58* in individual DCs was examined by nested multiplex real time qPCR. This single cell analysis revealed a much stronger association between *IFNB1* and *DDX58* transcription in the presence than in the absence of blocking antibodies (R^2=0.60 vs. 0.28). Taken together, these data indicate that the innate immune response to viral infection in human DCs is strongly dependent on the basal level of *DDX58* transcription and is modified by a functional polymorphism in its CARD domain. Support: NIAID HHSN266200500021C and U19AI06231.

2795/F

Role of DNA-protein interactions in central nervous system signaling: the interaction between serotonergic and glucocorticoid signaling systems in HPA axis regulation. *V.R. Falkenberg, S.D. Vernon, E. Aslakson, M.S. Rajeevan.* Centers for Disease Control and Prevention, Atlanta, GA USA 30333.

The central nervous system (CNS) functions through coordinated interaction between several signaling systems. Serotonergic and glucocorticoid signaling systems play pivotal roles in regulation of the hypothalamic pituitary adrenal (HPA) axis with negative feedback regulation exerted by glucocorticoid receptor (GR). Computational models indicate an influence of pituitary GR expression on HPA axis homeostatic set points. The HPA axis is also regulated by serotonin (5-hydroxytryptamine [5-HT]) through complex and unclear interactions. In this study, experimental and bioinformatic approaches are used to determine if DNA-protein interactions provide a molecular link for the interaction between various CNS signaling systems. Glucocorticoid responsive elements (GREs) are identified in the promoters of a number of 5-HT receptor genes. These GREs include sites for GR, the progesterone receptor (PR), and the androgen receptor (AR). 5-HT receptors with putative GREs specific for the GR include *HTR1D, HTR1F, HTR3A* and *HTR6,* and those 5-HT receptors containing GREs with slightly higher affinities for AR or PR include *HTR1D, HTR1F, HTR6* and *HTR2A.* A number of 5-HT receptors (*HTR1A, HTR1B, HTR2B, HTR2C, HTR3D, HTR3E, HTR4, HTR5A*, and *HTR7*) do not contain any putative GREs. EMSA is used to experimentally verify the predicted DNA-protein interactions. GRE binding (GR, AR, or PR) was verified for *HTR1D, HTR1F, HTR6* and *HTR2A* demonstrating that DNA-protein interactions exist between the serotonergic and glucocorticoid signaling systems. Computational models of the HPA axis are being generated to reflect this molecular connection and to determine the impact on HPA axis homeostasis. Studies are in progress to characterize the functional significance of these interactions in the transcriptional control of genes involved in the regulation of the HPA axis. Identification of these molecular links is important for modeling systemic feed-forward and feed-back mechanisms between the systems. These results are indispensable to understanding the functioning of inaccessible organs like the human brain.

2796/F

***Caenorhabditis elegans*: a model to further dissect features of Bardet-Biedl Syndrome.** *C. Mok[1,3], M. Zhen[3], E. Héon[1,2].* 1) Program of Genetics & Genome Biol, Sick Kids Hosp, Toronto, ON, Canada; 2) Department of Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, ON, Canada; 3) Mount Sinai Hospital Research Institute, Toronto, ON, Canada.

Bardet-Biedl syndrome (BBS) is an autosomal recessive, genetically heterogeneous, pleiotropic disorder. Cardinal features include photoreceptor degeneration, obesity, digit anomalies, kidney anomalies, cognitive impairment and hypogonadism. BBS genes have recently been linked to ciliary proteins and the intraflagellar transport (IFT) system. C. elegans bbs-7 and -8 mutants have decreased ciliary axoneme length and chemotaxis defects (Blacque et al., 2004). Neurosensory defects in C. elegans have been associated with decreased body length (Fujiwara et al., 2002) and increased fat accumulation (Mak et al., 2006). We hypothesize that C. elegans bbs mutants will share specific phenotypes related to various ciliated-neurons. The following strains were used: N2 (Bristol), *bbs-1(ok1111)I*, *bbs-5(gk507)III*, *bbs-7(ok1351)III*, *bbs-7(n1606)III*, *bbs-8(nx77)V*, *bbs-9(gk471)I*, *che-3(ok1574)I*, *che-3(e1124)I*, *sma-1(ru18)V*. Transgenic rescue strains of *bbs-1*, *-7*, and *-8* mutants were created by co-injection of wild type *bbs* genes along with a transcriptional podr-1::GFP fusion. Measurements were completed on all strains listed at the L4 stage as well as 24 and 72 hours post-L4 stages. DiI staining was completed on mixed populations of all strains. Worms were mounted and observed for DiI uptake the following day. Measurements showed a statistically significant loss of 15-20%; in mean body length of *bbs* mutants that was abrogated in transgenic rescue lines. DiI staining identified *bbs* mutants as having a dyf phenotype that was abrogated in transgenic rescue lines. Body length and DiI results indicate that *bbs-5(gk507)III* may be a hypomorphic allele as it did not appear to have severe body length defects and was normal for DiI uptake. Mutations in *C. elegans bbs* orthologs lead to a marked decrease in mean body length for *bbs* knockout mutants and DiI uptake defects. Body length measurements correlate strongly with DiI defects. *C. elegans* can be used to identify possible pathways involved in BBS.

2797/F

An intronic SNP affects the HTR2a gene expression in the human brain and outcome of antidepressant treatment. *S.H. Hashemi, T. Arentzen, S. Haugbol, D. Erritzoe, V. Frokjaer, J. Madsen, G.M. Knudsen.* Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, Rigshospitalet, Copenhagen University Hospital, Section 9201, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.

BACKGROUND: The single nucleotide polymorphism (SNP) rs7997012, a result of a transition mutation from a G to an A in the second intron of the gene encoding the serotonin 2a receptor (HTR2a), was recently found to be associated with lower risk of no response to antidepressant treatment (McMahon et al, Am J Hum Genet. 2006.78(5):804-14). The biological impact of this polymorphism is, however, unknown. MATERIAL AND METHODS: To functionally characterize rs7997012 a cohort of 97 unrelated healthy Caucasian volunteers (39 women and 58 men; age range: 18.47 - 79.62 years, mean age 41.45 years, SD = 17.06) underwent positron emission tomography scanning with [18F]-altanserin for assessment of the cerebral HTR2a-binding and was subjected to 5'-exonuclease Taqman SNP genotyping by applying specific primers/probes. RESULTS: The distribution of the three genotypes (AA, AG, GG) were in Hardy-Weinberg equilibrium (Chi-square = 0.66, df=2, P= 0.72). The frequencies of AA, AG and GG were 14.4%, 52.6%, and 33.0%, respectively which were similar to those of Caucasians reported by HapMap (Chi-square=1.03, df=2, P=0.60). A reduction of HTR2a receptor binding in neocortex (RBN) was found to be age-dependent (slope: -0.01002 ± 0.004198, p= 0.019). In a linear regression analysis with age as covariate the polymorphism was found to impact RBN significantly in an additive fashion (GG>AG>AA) (df=1, p=0.03). CONCLUSION: The ancestral G-allele of rs7997012 is associated with higher HTR2a-binding in neocortex and the transition mutation generating the A-allele may lead to reduction of the receptor gene expression at both mRNA and protein levels. We speculate that this reduction in cortical HTR2a receptor density is related to better outcome after antidepressive treatment.

2798/F

Role of promoter polymorphism in the MIF gene in the pathogenesis of Severe Malarial Anemia. *G.A. Awandare[1], C. Ouma[1,2], G. Davenport[1], J.M. Ong'echa[2], R.E. Ferrell[1], R. Bucala[3], J.J. Martinson[1], D.J. Perkins[1].* 1) University of Pittsburgh, Graduate School of Public Health; 2) KEMRI, Kisumu, Kenya; 3) Yale University School of Medicine.

Severe malarial anemia (SMA) is one of the major causes of childhood mortality in regions of sub-Saharan Africa where *Plasmodium falciparum* malaria is holoendemic. The molecular causes of SMA are largely undefined, but dysregulation in host-derived inflammatory mediators influence its severity. The cytokine Macrophage Migration Inhibitory Factor (MIF) is an important regulator of innate inflammatory responses that suppresses erythropoiesis and promotes pathogenesis of SMA in murine models. We investigated the role of MIF in childhood malaria by examining peripheral blood MIF production in children residing in a holoendemic region of western Kenya.

We had previously shown that circulating MIF levels, and peripheral blood mononuclear cell (PBMC) MIF production, progressively declined with increases in anemia severity and in levels of monocytes containing the malarial by-product hemozoin. However, MIF levels were not significantly associated with reticulocyte production in children with acute malaria, and adding exogenous MIF, or blocking endogenous MIF, *in vitro* did not significantly affect erythropoiesis. Experiments in malaria-naïve individuals showed that the effects of hemozoin on MIF production in cultured PBMC were influenced by genetic differences. Analysis of SNPs in the MIF gene promoter revealed that the CC genotype of the C-173G SNP was associated with an increased risk of High Density Parasitemia compared to the GG genotype. Here, we present the results of a more systematic survey of genetic variation in the MIF promoter, including an upstream CATT tetranucleotide STR locus located at position -794, and show that individuals with the $CATT_6$/-173G haplotype were significantly protected from SMA while those with $CATT_{7\text{-}8}$/-173C haplotypes were at an increased risk of developing SMA. SMA is associated with decreased MIF production, and individuals with high MIF-producing genetic variants are less susceptible to severe malaria.

2799/F

Apolipoprotein E Proximal, Promoter and Distal SNPs and Associations with Cerebrospinal Fluid Apolipoprotein E Protein Levels. *L.M. Bekris, N.M. Galloway, S. Millard, D. Tsuang, E. Peskind, C.E. Yu.* Medicine, Univ Washington, Seattle, WA.

Apolipoprotein-E (ApoE) is involved in lipid transport. The APOE ε4 polymorphism is associated with an increased risk of Alzheimer's disease. Associations between APOE regulatory SNPs and ApoE expression in human populations independent of APOE ε4 genotype has been difficult to establish and may be in part due to strong linkage disequilibrium (LD) in the APOE gene region. The aim of this investigation was to generate hypotheses regarding the regulatory potential of SNPs in a large 70 kb region surrounding the APOE gene, while taking into account the strong LD in the region, as well as age, gender and APOE ε4. Cerebrospinal Fluid (CSF) was collected from control subjects 21-87 years of age (n=148). CSF ApoE levels were measured and genotypes were determined for several SNPs (n=22) surrounding the APOE gene. SNPs genotyped include; promoter SNPs, APOE proximal SNPs, and APOE distal enhancer SNPs (ME1, HCR2, BCR). Backward linear regression models were performed to evaluate the influence of these SNPs on CSF ApoE levels while taking into account age, gender, APOE ε4 and correlation between SNPs (LD). The results indicate that CSF ApoE levels increase significantly with age and are influenced by a subset of APOE proximal SNPs (within the TOMM40 gene), APOE promoter SNPs, and distal enhancer SNPs. R2 values indicate that these SNPs are not in strong LD with each other or with APOE ε4. In support of these results, the distal hepatic control region SNP (HCR2) did not show an influence on CSF ApoE levels which would be expected given that hepatic ApoE should not contribute to CSF ApoE levels. In summary, utilizing backward linear regression models to investigate the influence of APOE proximal, promoter and distal SNPs on CSF ApoE levels, we found an influence by a subset of proximal, promoter and distal SNPs, on CSF ApoE levels, that vary in their contribution to CSF ApoE levels according to age, gender and the presence of APOE ε4. A combined influence by these SNPs on CSF ApoE levels implicates APOE regulatory haplotypes as playing a role in ApoE expression. *APOE.*

2800/F

Functional polymorphisms of FPR1 gene and aggressive periodontitis in Japanese. *T. Gunji[1,2], Y. Onouchi[2], T. Nagasawa[1], H. Watanabe[1], H. Kobayashi[1], S. Arakawa[1], K. Noguchi[1], A. Hata[2], Y. Izumi[1], I. Ishikawa[3].* 1) Periodontology, Department of Hard Tissue Engineering, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; 2) Laboratory for Gastrointestinal Disease, SNP Research Center, RIKEN, Yokohama Institute, Yokohama, Japan; 3) Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan.

Aggressive periodontitis (AgP) is a severe type of periodontitis with possible familial aggregation that causes rapid alveolar bone destruction in individuals without any systemic disorders. Formyl peptide receptor 1 (FPR1) is a chemotactic G protein-coupled receptor that is expressed on the surface of polymorphonuclear neutrophils (PMNs) and other phagocytes. FPR1 of PMNs in AgP patients is reported to be defective and a role of FPR1 Single Nucleotide Polymorphisms (SNPs) in AgP progression was suggested, but these results have not yet been fully confirmed. The purpose of this study is to investigate the possible role of FPR1 in AgP in Japanese.

To examine whether polymorphisms in *FPR1* gene are associated with AgP, we performed an association study with 38 AgP patients and 373 controls using 30 variations identified by sequencing the 21.1 kb gene region. Three SNPs showed a significant association with AgP (chi squared test, $p < 0.05$). Among them, the susceptible allele of the SNP which was located in approximately 10kb upstream of the gene decreased activity of transcriptional regulation in *vitro*. Haplotype association analysis with this SNP and a non synonymous SNP in Exon 2 (Asn > Lys) revealed that one haplotype was significantly represented in AgP patients ($p = 0.035$).

In conclusion, our result suggested that structurally and transcriptionaly altered FPR1 function might be relevant to the risk for AgP in Japanese.

2801/F

A regulatory code controlling gene expression during heart development. *F.E. Arimura[1], I. Ovcharenko[2], M.A. Nobrega[1].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Computational Biology Branch, National Center for Biotechnology Information, NIH, Bethesda, MD.

Identifying biologically active transcriptional noncoding elements in mammalian genomes and understanding the combinations of sequence signatures that are necessary and sufficient to confer quantitative, temporal- and tissue-specific expression in vertebrates represents one of the greatest challenges in genome biology. It is unclear if groups of genes that are co-expressed in a tissue or coordinately respond to certain stimuli share a cis-regulatory code underlying this concerted expression. To investigate whether the complex orchestration of gene expression during heart development is controlled by a common regulatory code, we combined whole genome alignments, gene expression profiling in developing hearts and pattern analysis of DNA binding motifs to identify putative heart-specific regulatory elements in the mouse genome. A list of 450 putative heart enhancers sharing common DNA motifs was generated, and we tested 27 of these elements, randomly selected, in a newly developed zebrafish transgenic reporter assay. A total of 11 elements (40.7%) demonstrated heart-specific expression in developing fish embryos. To assess the false discovery rate of this strategy, 52 elements in the vicinity of genes expressed during heart development were also tested, and none drove reporter expression in the developing fish. We also tested in zebrafish 17 mammalian heart enhancers previously identified using transgenic mice. 13 (76.5%) of these elements reproduced the expression pattern in fish hearts, demonstrating the feasibility of using zebrafish transgenics to test mammalian sequences. Together, these results indicate that our predicted heart enhancers likely share a common code of DNA binding motifs. Further experimentation is under way to improve the predictive power of the method and refinement of the specific DNA motifs.

2802/F

A Cross Talk between SMRT and Jag2 in Multiple Myeloma. *p. ghoshal[1], c. houde[1], a. Szafranek[1], a. Nganga[1], t. Johnson[1], a. Bigelow[1], j. Moran-Guiati[1], j. Dolce[1], d. Smiraglia[1], a. Chanan-Khan[2], I. Coignet.[1].* 1) cancer genetics, Roswell park cancer institute, Buffalo, NY; 2) Department of Medicine,Roswell park cancer institute, Buffalo, NY.

Multiple myeloma (MM), affects terminally differentiated B cells. In the bone marrow, the myeloma cells and stromal cells secrete cytokines, which support the growth and survival of the myeloma cells and lead to osteolytic complications associated with MM. It is now well recognized that interleukin-6 (IL-6) is a major cytokine, regulated by the NOTCH gene, promotes the proliferation of malignant plasma cells in MM. Previously published work suggested that JAG2 localized in 14q32.3 might be the ligand of interest for NOTCH in the MM context.JAG2 is also down-regulated during normal bone marrow differentiation. We hypothesized that over-expression of JAG2 in myeloma cells induce production of IL-6 in stromal cells, and subsequent binding with NOTCH receptor which enhances the proliferation of myeloma cells. JAG2 has been shown to be over-expressed in all cell lines and patient samples studied (n= 5), both at the RNA and protein levels. To explore the reason for JAG2 overexpression, we assessed the modification of the JAG2 promoter region in both MM cell lines and patient samples by studying both methylation and histone acetylation levels. It appears that difference in H4 acetylation level might play a critical role in MM. Acetylation state of histones can be regulated by the recruitment of histone deacetylases (HDAC). HDACs are typically recruited to promoter regions through interaction with nuclear co-repressors such as SMRT. The cell lines and patient samples studied presented significantly reduced levels of SMRT. So based on these observations we can propose a model that partial down regulation of SMRT recruits less active HDAC3 (confirmed by immunoprecipitation), as a result the deactylation process of histones in promoter region has been impaired and the cells lost their control on transcriptional regulation of Jag2. This is the first report of SMRT alteration and its direct involvement in MM pathogenesis and might open a new area for potential therapeutic approaches in the treatment of MM.

2803/F

Characterization of cis-regulatory elements in the Alpha-synuclein gene. *B. Schuele, L. Sterling, J.W. Langston.* Parkinson's Institute, Sunnyvale, CA.

Purpose: To evaluate evolutionary conserved regions within the Alpha-synuclein (SNCA) gene as cis-acting regulatory elements in vitro. Background: A microsatellite repeat NACP-Rep1, 9.8kb upstream of the SNCA gene, has been shown to modulate the expression of the SNCA gene and two of the five alleles of this repeat have been shown to be associated with Parkinson's disease (PD), one is protective and one is causative. Based on these findings and the observation that SNPs in other regions of the SNCA gene show highly significant p-values in case-control studies of PD, we hypothesized that other cis-regulatory elements in the SNCA genomic region regulate expression of SNCA and can be causative for PD if disrupted or impaired. Methods: Pair-wise comparison of a 206kb genomic region encompassing the SNCA gene including a 44.5kb upstream and 50kb downstream intergenic region revealed 32 conserved DNA sequences between human and mouse. These regions had >75% sequence identity and were at least 100bp in length. All elements were cloned into pGL3 promoter vector (Promega). In dual luciferase reporter assays, constructs were co-transfected with pRL-TK renilla plasmid into SK-N-SH neuroblastoma cell lines. Assays were run in quadruplicates and three independent experiments. All data were normalized to the pGL3 promoter plasmid. Results: Overall 11 of 32 elements exhibited either an enhancement or reduction of the expression of the reporter gene. Three elements upstream of the SNCA gene displayed an approx. 1.5 fold ($p<0.009$) increase in expression. Of the intronic regions, three showed a 1.5 fold increase and two others indicated a 2 and 2.5 fold increase in expression ($p<0.002$). Two elements downstream of the SNCA gene showed 1.5 fold and 2.5 fold increase ($p<0.0009$). One element downstream of SNCA had a reduced expression of the reporter gene of 0.35 fold ($p<0.0009$) of normal activity that was also confirmed in a pGL3 control (containing promoter and enhancer) vector. Conclusion: Our results demonstrate that the SNCA gene contains cis-regulatory regions that might regulate the expression of SNCA. Further studies are ongoing to test that these regions specifically modulate the transcription and expression of the SNCA gene.

2804/F

Haplotypes in the promoter of KIF5B affect promoter activity in a cell type specific manner. *A.M. Stütz[1], T. Rice[2], D.C. Rao[2], C. Bouchard[1], T. Rankinen[1].* 1) Pennington Biomedical Research Center, Baton Rouge, LA; 2) Washington University School of Medicine, St Louis, MO.

KIF5B was identified as a candidate gene for the training response in submaximal exercise stroke volume in the HERITAGE Family Study. Significant associations were found for several KIF5B SNPs, one of them in the putative promoter. A 2 kb region surrounding the KIF5B promoter was sequenced in 95 White individuals, revealing 10 SNPs of which four are novel. Haplotype analysis revealed seven major haplotypes that were all cloned as 1,794 bp promoter fragments into a luciferase reporter vector. Strong promoter activity was found in the three tested cell lines HEK293, C2C12 and RH-30, at 103, 598 and 773 times higher than the empty vector, respectively (four experiments per cell line, each in triplicate). For all cell lines, a significant difference in promoter activity between haplotype constructs was observed ($p<0.0001$ for HEK and C2C12, $p=0.011$ for RH-30). In comparison to the haplotype containing all the common alleles, all three haplotypes that contained the rare allele at rs211302 and rs211300 showed greater promoter activity in HEK cells ($p<0.0001$, $p=0.0003$ and $p<0.0001$). The haplotype that was defined by a unique rare allele at -444 showed higher promoter activity than all other haplotype constructs ($p<0.0001$) and a 25.0 % increase compared to the common haplotype. In C2C12 cells, three haplotype constructs again showed different activity compared to the common haplotype ($p=0.02$, $p=0.005$ and $p<0.0001$ respectively). However, unlike the HEK cells, the activity was consistently lower in the C2C12 cells. A similar trend, although less pronounced, was observed in the second muscle-like cell line RH-30. Changes in the transcription factor binding profile between haplotypes (predicted using Alibaba, Match and Consite programs) may explain the differences in promoter activity. Additionally, changes in GC content and creation of CpG dinucleotides in the very GC rich, TATA-box less KIF5B promoter may also contribute. In summary, KIF5B promoter haplotypes showed cell type specific differences in promoter activity and may thus alter the function of the gene in tissue-specific manner.

2805/F

Whole Transcriptome Amplification from Degraded Transcripts. *B. Ward, K. Heuermann.* Research and Development, Sigma-Aldrich Corporation, St. Louis, MO.

The possibility of obtaining gene expression profiles from fixed samples of diseased tissues is an extraordinary opportunity for the medical research community. Due to the methods used for tissue fixation and questionable care for nucleic acid stabilization, such samples often have insufficient quantities of intact transcripts to allow for expression analysis. The TransPlex whole transcriptome amplification (WTA) system provides a means to amplify damaged and intact RNAs from limited quantities of total RNA. The method comprises preparation of a reverse transcription-mediated cDNA library absent of 3' bias, followed by limited PCR amplification. The method produces microgram quantities of amplification product that is representative of the pre-amplification transcriptome. Quantitative PCR and dual-color microarray analysis of amplified versus unamplified RNA reveals that representation of differential gene expression between RNA sources are maintained during amplification. Studies using degraded RNA have demonstrated that WTA with TransPlex enables PCR detection of template molecules that could not be detected in the original, unamplified samples. In conclusion, TransPlex WTA is able to provide microgram quantities of amplified cDNAs from samples containing limited quantity and/or quality RNA.

2806/F

MicroRNA Transcriptome Of Mouse Retina & Functional Characterization of a Sensory Organ Specific miRNA Cluster. *S. Xu[1], P. Witmer[2,3], S. Lumayag[1], B. Kovacs[1], D. Valle[3].* 1) Dept of Ophthal/Neurol Sci, Rush U Med Ctr, Chicago, IL; 2) Predoc Training Prog in Human Genet; 3) McKusick-Nathans Inst of Genet Med, Johns Hopkins U. Baltimore, MD.

To begin to understand the functions of miRNAs in retina, we compared miRNA profiles in adult mouse retina, brain and heart by microarray analysis & showed that at least 78 miRNAs are expressed in adult mouse retina, of which 15 are confirmed retina specific or preferentially expressed. Among these, we identified a polycistronic, sensory organ-specific paralogous miRNA cluster including miR-96, miR-182 & miR-183 on mouse chr6qA3 with conservation of synteny to human chr7q32.2. In situ hybridization showed that they are expressed in photoreceptors, bipolar & amacrine cells. qRT-PCR showed they have a similar expression pattern with abundance increasing postnatally & peaking in adult, suggesting that they may play important roles in the differentiation during development & the maintenance of the phenotypes & functions of adult retina. Target prediction & in vitro functional studies showed that MITF, which is required for the establishment & maintenance of retinal pigmented epithelium (RPE), is a direct target of miR-96 & miR-182, suggesting that these miRNAs may contribute to the establishment & maintenance of the neuroretinal identity. We also performed miRNA profiling with retinal RNA of noon (ZT5) & midnight (ZT17) & identified 12 miRNAs, including miR-182 with diurnal expression. Target prediction & in vitro functional studies showed that miR-96 & miR-182 directly downregulate adenyl cyclase VI (ADCY6), an important regulator of AANAT, a key enzyme in melatonin synthesis. qRT-PCR showed that Adcy6 is expressed in retina with a circadian pattern with an apex at ZT9 & a nadir at ZT17, inverse to that of miRs-96/182 with an apex at ZT13 & a nadir at ZT5 but about 4 hours out of register, supporting that these miRNAs may be involved in circadian regulation partly through regulating Adcy6 expression. Additional target prediction suggests that the miR-183/96/182 cluster may play important roles in modulating the circadian clock machinery through multiple genes in the clock network.

2807/F

Unstable siRNA duplex is a prerequisite for accurate prediction of siRNA efficiency - Proposal of a new parameter based on the linear regression model -. *K. Ohno[1], M. Ichihara[2].* 1) Division of Neurogenetics and Bioinformatics, Center for Neurological Diseasses and Cancer, Nagoya Univ Grad Sch Med, Nagoya, Japan; 2) Department of Biomedical Sciences, College of Life and Health Sciences, Chubu University, Aichi, Japan.

Short interfering RNA (siRNA) is an essential tool to analyze gene function. Some siRNAs efficiently knock down the gene expression, but some cannot. The siRNA efficiency is determined by the position of siRNA on the target gene. Several different algorithms have been developed to design the most efficient siRNAs, but none can predict the siRNA efficiency with near 100% accuracy. We therefore usually have to synthesize two or more different siRNAs for each gene. We here developed a new siRNA-designing parameter, the i-Score, by applying the linear regression model on 2431 siRNAs reported by Huesken et al. (*Nat Biotechnol*, 23, 995, 2005). We validated the prediction accuracy of the i-Score using 419 siRNAs reported by others. We also compared the i-Score with other algorithms reported by Huesken, Reynolds, Ui-Tei, Amarzguioui, Katoh, Hsieh, and Takasaki. Among these, the BioPredSi by Huesken et al. was the most efficient predictor of siRNA, and the i-Socre was as good as the BioPredSi. An advantage of the i-Score over the BioPredSi, which employs the neural network modeling, is that we can visually inspect which nucleotides at which positions are beneficial or deleterious. We also found that the stability of the siRNA double helix is an important parameter that determines the siRNA prediction accuracy. When the siRNA double helix is unstable, we can readily predict its knock-down efficiency, whereas when it is stable, we cannot accurately predict it. To our surprise, this is true for other parameters and for most siRNA datasets. We also developed the SELL/pDaul system with which we can test siRNA efficiency of any artificial sequence, and validated some siRNAs with it.

2808/F

Characterization of splicing variants in NRG3, a positional candidate for schizophrenia. *L. Zhang[1], N. Feng[1], R. Wang[2], N. Cheng[2], S. Almashanu[1], A. Pulver[1,2], D. Valle[1], D. Avramopoulos[1,2].* 1) IGM, JHMI; 2) Psychiatry, JHMI, Baltimore, MD.

The neuregulins (NRGs) are a protein family containing an epidermal growth factor (EGF)-like motif that activates ErbB receptors. Neuregulin 1 (NRG1) performs a wide range of functions in the developing nervous system and has been implicated in schizophrenia (SZ). NRG3 has structural similarity with NRG1 but its expression is more specific to the CNS. Additionally NRG3, maps to10q22, a region suggested to harbor a SZ susceptibility gene by our previous linkage study in Ashkenazi Jewish families and an independent study in a Han Chinese population. NRG1 has been reported to undergo extensive alternative splicing giving rise to multiple isoforms, however very few studies have examined NRG3 for alternative splicing. We have systematically screened NRG3 for alternative transcripts using RT-PCR, cloning and sequencing methods. We found that NRG3, like NRG1, has more than 10 alternative exons and 20 splicing forms as well as multiple transcription start sites producing different N-terminal sequences. Splicing variants lacking the EGF domain were identified in human and confirmed in mouse brain. A novel start site of NRG3 transcription was identified, that leads several brain transcripts encoding a very short extracellular N-terminal sequence of 10 amino acids without an EGF domain. Interestingly we found that this start site is included in a small deletion that occurs with a frequency of 1.5%. Using quantitative PCR we determined that the abundance of the identified alternative transcripts is relatively low in total RNA, each being 20 to 100 fold less abundant than the annotated 9-exon transcript. We conclude that NRG3 undergoes extensive alternative splicing but in total RNA from adult brain the annotated transcript is markedly more abundant than others. The importance and relevance to disease of an identified small deletion that interferes with at least one of these transcripts remains is currently the subject of further study.

2809/F

Characterization and expression of cytoplasmic actins(β & γ)in LLC-PK1-CL4 cells to elucidate the role of γ-actin mutations causing non-syndromic hearing loss. *S. Korrapati[1], M. Zhu[2], K. Friderici[3].* 1) Genetics Program; 2) Cell & Molecular Biology Program; 3) Microbiology & Molecular Genetics-Michigan State University,East Lansing,MI48824.

Mutations in the γ isoform of actin cause non-syndromic,post-lingual,autosomal-dominant,-progressive sensorineural hearing loss.Localization studies of the actins in the guinea pig and chicken hair cells reveal differential spatial arrangements of the two cytoplasmic actin isoforms (β,γ).Our hypothesis is that γ-actin is involved in specific functions in the inner ear.Stereocilia are derivatives of actin-filled microvilli found on the surface of many cell types.-The pig proximal kidney epithelial cell line,LLC-PK1-CL4(CL4),lacks endogenous actin bundling protein espin,but form long spiky microvilli when transfected with espin.Our goals for this project are to study the function of the actin isoforms using CL4 cells as a model system and to investigate the role of γ-actin and the mutants in the repair of the microvilli.This study,we hope,will mimic the condition of the hair cells of individuals suffering from age-related hearing loss since aging hair cells constantly undergo damage and repair.Using CL4 cells and confocal imaging,the results obtained so far reiterate the observations(in other cell types)-that the cytoplasmic actins are differentially distributed within the cell.β-actin is located primarily at the periphery of cells while γ-actin is abundant in the perinuclear space and cytoplasm of the cell.Exogenous expression of β and γ-actin shows distribution of the actins to all the relevant actin structures in the cell-periphery,stress fibers and perinuclear space.In response to exogenous espin expression,espin and filamentous actin co-localize in the microvilli.Co-immunoprecipitation assay confirmed espin-γ-actin interaction.Co-transfection of espin and the mutants resulted in each of the mutants co-localizing with filamentous actin in the microvilli.-Cytochalasin D,a potent inhibitor of actin polymerization,disrupted actin microfilaments in the microvilli following 13hrs of treatment.At a concentration of 100nm,this effect was reversible as the microvilli recovered by 4.5 hrs post drug removal.

2810/F

Identification of long-range interactions between the *FOXL2* core promoter and three *cis*-regulatory elements. *D. Beysen[1], J. Dostie[2], A. De Paepe[1], J. Dekker[2], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, USA.

Recently, defects in long-range transcriptional regulatory elements have emerged as potential mechanisms underlying human developmental genetic disorders. One of them, the blepharophimosis syndrome (BPES), an autosomal dominant condition affecting eyelid and ovary development, is caused by mutations in the forkhead transcription factor gene *FOXL2*. *FOXL2* expression has been shown to occur in a spatiotemporally specific manner. The need for a strict regulation of *FOXL2* expression was emphasized by the recent identification of deletions upstream and downstream of the transcription unit of *FOXL2* as underlying cause of BPES. We demonstrated that these rearrangements remove several conserved nongenic sequences (CNGs) harbouring potential long-range *cis*-regulatory elements.

Here, we used the chromosome conformation capture (3C) method to identify long-range interactions of *cis*-regulatory elements with the *FOXL2* promoter in two adult *FOXL2* expressing cell systems. We found that in adult ovarian granulosa cells and adult fibroblasts three long-range *cis*-regulatory sequences located 177 kb, 283 kb and 360 kb upstream of *FOXL2* come in close vicinity to the *FOXL2* core promoter. Interestingly, 3C analysis in a human adult fibroblast cell line derived from a BPES patient with a heterozygous deletion of the region encompassing the regulatory element at 283 kb, revealed decrease of interaction of the deleted element and the *FOXL2* core promoter and the two other regulatory elements. Interestingly, the element at 283 kb corresponds to a sequence deleted in the Polled Intersex (PIS) goat, which is an animal model for BPES. In conclusion, we hypothesize that the interaction between the *cis*-regulatory element located at 283 kb and the *FOXL2* core promoter is essential to establish efficient transcriptional regulation of *FOXL2* expression.

2811/F

Demonstration of PCBP1 in regulation of fundamental cellular metabolism. *L. Huo[1,2], N. Zhong[1,2,3].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Peking University Health Science Center, Beijing; 3) New York State Institute for basic Research in Developmental Disabilities.

PCBP1 is known to participate in biological regulation of RNAs transcription, pre-mRNAs processing and maturating, and mRNAs export through its RNA binding functions. To further investigate the transcript targets of PCBP1, we have studied gene expression profiles by knocking down endogenous PCBP1 transcript and by over expression of exogenous PCBP1 in neuroblastoma SH-SY5Y cell lines. Either an shRNA specifically targets endogenous PCBP1 was constructed into a lentiviral plasmid, packaged, and transducted, or a full length of PCBP1 cDNA was expressed with a plasmid of pcDNA6. Gene expression profiles of global RNAs were analyzed using Agilent oligochip. Our results showed that the expression level of a total number of 1,606 transcripts has been significantly altered when endogenous PCBP1 were knocked down. This alteration includes 974 down-regulated and 732 up-regulated transcripts. Compared to this alteration, there were 679 transcripts up-regulated and 58 down-regulated after exogenous PCBP1 was over-expressed in SY5Y cells. Functional analyses showed that the PCBP1 targeted transcripts are involved in a certain number of cellular pathways, which include in TGH beta signaling pathway, hypertrophy model pathway, cell cycle related pathway, integrin-mediated cell adhesion pathway, translational factors related pathway, apoptosis related pathway, GI3 signaling pathway, etc. Our study provided evidence for the first time of identifying PCBP1 targeted global cellular transcripts in different metabolic pathways.

2812/F

Transcriptional regulation of 14-3-3. *A. Kasinski[1,2], H. Fu[1].* 1) Department of Pharmacology, Emory University, Atlanta, GA; 2) Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA.

The seven isoforms of 14-3-3 play an intricate role in the signaling events leading to cell survival, cell-cycle progression, oncogenesis, and apoptosis. The activity of many proteins involved in proliferation and apoptosis is attenuated by the binding of 14-3-3. The binding typically sequesters the client protein away from its site of activation rendering it inactive. Since 14-3-3 proteins inhibit death promoting proteins, and in contrast act to induce functions of proteins involved in growth and proliferation it is not surprising that upregulation of 14-3-3 proteins has correlated with tumor progression. The 14-3-3 isoforms exhibit a remarkable degree of amino-acid sequence conservation, between species and isoforms, however they differ substantially at the nucleotide level. Minimal work has been reported on the transcriptional regulation of the 14-3-3 genes; however, the link between increased 14-3-3 protein levels and oncogenesis has made it a compelling area. Genetic data from all isoforms has been compiled and evaluated. A more detailed analysis of zeta was performed due to its role in tumor progression. Four independent splice variants of zeta were identified, two of which are novel to this study. Their transcriptional profiles have been confirmed. Data conclusively show that the variants are regulated independent from each other and in a tissue specific fashion. Additionally, luciferase reporter assays addressed the fundamental regions within the promoter of 14-3-3 zeta that are necessary and sufficient for expression from each of the four variants. The zeta variants were also found to be regulated by a putative CpG island. Elevated protein levels of this highly conserved gene family have been implicated in tumor progression, however the cause for this elevation has been left undiscovered. Due to the high degree of functional and structural similarity of the isoforms, targeting them individually at the protein level is quite trying. Identification of mechanisms that control transcription of these genes will help to identify independent molecular targets that can be used to modulate the 14-3-3 genes in an isoform specific manner.

2813/F

The quantification of the allelic variations of gene expression in peptidylarginine deiminase type 4 (PADI4). *A. Suzuki[1], Y. Kochi[1], K. Kobayashi[1], R. Yamada[1, 2], M. Mori[3], K. Yamamoto[1, 3].* 1) SNP Research Ctr, RIKEN, Yokohama City, Kanagawa, Japan; 2) Human Genome Center, The Institute of Medical Science, The University of Tokyo,Minato-ku, Tokyo, Japan; 3) Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Multiple studies have shown that human single nucleotide polymorphisms (SNPs) affect gene regulation, resulting allelic imbalances of gene expression. The finding of these regulatory SNPs leads to understanding phenotypic diversity and the identification of alleles that modify disease risks. Therefore, it is important to quantify the allelic variations of gene expression in vivo for identification of regulatory SNPs. Monitoring of these variations is possible in tissues and cells of heterozygous individuals using informative markers within the genes. For measurement of these variations, we performed TaqMan real-time RT-PCR system using cDNA from tissues or cell lines of heterozygous individuals. In this study, we examined allele-specific gene expression in PADI4, which was reported to be associated with rheumatoid arthritis (RA), and of which allelic imbalances of the gene expression were observed. We used TaqMan probes, rs11203366 (G/A) as a marker located in exonic region of PADI4. We measured the signal intensity using cDNA and DNA from heterozygous individuals, and we calculated allele-specific gene expression ratio. We also indicated that two major haplotypes with 4SNPs in PADI4 exonic region including rs11203366 were different stability in vitro and in vivo. We performed the gene expression analysis using cDNA from peripheral blood leukocyte of each genotype individuals and showed that PADI4 expression actually was different in peripheral blood leukocytes with each genotype. These data supported the hypothesis that expression levels of PADI4 are associated with RA susceptibility1, 2 . 1) Nat. Genet. 2003, 34:395-402, 2) Nat. Genet. 2003, 37:478-485.

2814/F

A novel retrotransposon that was recently inserted into exon 67 of the dystrophin gene. *H. Awano, M. Yagi, Y. Okizuka, Z. Zhang, Y. Takeshima, M. Matsuo.* Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan.

<Background> Retrotransposons are transposable elements that can spread autonomously on the human genome. L1 and Alu elements are well-known retrotransposons in the human and have been shown to cause diseases. Here, we identified a novel retrotransposon that was inserted into the dystrophin gene of a Duchene muscular dystrophy (DMD) patient. <Case> The proband is a six years old Japanese boy. He had no family history of neuromuscular diseases. At three years old high CKnemia (14780IU/L) was found. At four years old muscle biopsy was performed to disclose no dystrophin staining and he was diagnosed as DMD. <Results and Discussion> The patient was found to carry an insertion of approximately 320bp in exon 67 of the dystrophin gene, and mRNA analysis disclosed exon 67 skipping, thereby producing out-of-frame dystrophin mRNA. The identified insertion sequence consisted of long poly T tract, 212bp unknown sequence encoding a poly adenylation signal and target site duplications (TSDs). Though these findings strongly suggested retrotransposon However, the inserted unknown sequence was found not homologous to any known retrotransposons like L1 or Alu element. Instead, homology search disclosed one perfectly homologous sequence in chromosome 11q, suggesting the origin of the insertion segment. In fact, the homologous sequence encodes a poly adenylation signal but no poly A sequence. These results suggested that mRNA transcribed from the sequence in chromosome 11q was reverse-transcribed and integrated into exon 67 of the dystrophin gene. Remarkably, his parents had no insertion mutation in exon 67 of the dystrophin gene, so the insertion event was decided to have occurred at his generation, indicating recent event of retrotransposition. Therefore, the inserted sequence was concluded as a novel retrotransposon.

SPEAKER AND AUTHOR INDEX

This index includes an alphabetical listing of all speakers, presenting authors, and co-authors for the 2007 ASHG meeting.
Session numbers follow speaker names; abstract numbers follow author names.
Presenting author names are noted with an asterisk.

B

C

D

E

F

G

H

I

J

K

L

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

PERMUTED TITLE INDEX

This index was produced by a computerized permutation process that creates an entry for every significant term in the title of an abstract and of selected special/invited presentations. These appear alphabetically in bold type, followed by the text to provide subject context. If space permits, the ending word in each title is followed by a slash and then by the preceding word or words. Abstract numbers appear at the end of each index line.

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

NUMERIC

SPEAKER AND AUTHOR DISCLOSURES

The American Society of Human Genetics (ASHG) and The Federation of American Societies for Experimental Biology (FASEB) require that audiences at educational programs sponsored by ASHG and FASEB be informed of a presenter's (i.e., speaker's, faculty member's, author's, and contributor's) academic and professional affiliations, and the disclosure of the existence of any significant financial interest or other relationship a presenter has with the manufacturer(s) of any commercial product(s) discussed in an educational presentation. This policy allows the listener/attendee to be fully knowledgeable in evaluating the information being presented.

Disclosure includes any relationship that may bias one's presentation or which, if known, could give the perception of bias. These situations may include, but are not limited to:

1. stock options or bond holdings in a for-profit corporation or self-directed pension plan
2. research grants, scholarships, or fellowships
3. employment (full or part-time)
4. ownership, partnership, or equity
5. consulting fees or other remuneration
6. receipt of substantial in-kind or donated goods or services
7. non-remunerative positions of influence such as officer, board member, trustee, or public spokesperson
8. receipt of royalties
9. speakers' bureau
10. inventor/patent owner
11. advisor
12. collaboration
13. company owner
14. receipt of travel grants/honoraria

Following is an alphabetical list of invited session speakers and author of abstracts who have disclosed one or more such relationships, and names of companies with which those relationships exist. The number following each company name represents the specific relationship from the list above. Session and/or abstract program numbers appear in parentheses. If a presenter is not listed, there are no relationships to disclose. Additional information may be obtained from FASEB by contacting Sara Hamilton at FasebCME@faseb.org

Albano, L. M. J., Genzyme Corporation - 2 (1448)
Auray-Blais, C., Genzyme Corporation - 2, 14 (50)
Barba, M. A., Shire Human Genetic Therapeutics - 14; Genzyme Corporation - 14 (1453)
Basehore, M. J., Sequenom, Inc. - 2 (2410)
Berrettini, W., GlaxoSmithKline - 2, 5 (1904)
Bibikova, M., Illumina, Inc. - 1, 3 (708)
Bodamer, O., Genzyme - 2, 5, 7 (1495)
Bouffard, P., 454 Life Sciences - 3 (2699)
Boyd, V., Applied Biosystems - 3 (711)
Brown, S., Susan G. Komen Breast Cancer Foundation Grant - 2 (787)
Burke, H., Komen Breast Cancer Foundation Grant - 2 (356)
Bustamante, C. D., Michele Cargill - 3; Eric Wang - 3 (1307)
Canick, J. A., Beckman Coulter Diagnostics - 5; Individual Patent - 8, 10 (Session 49)
Cardon, L., Illumina - 11; Astra-Zeneca - 5 (Session 47)
Casal, M. L., Apoxis, SA Switzerland - 12 (2298)
Case, L., Genzyme - 11 (1990)
Cheng, Y., Merck - 2 (1800)
Chim, S. S. C., Sequenom, Inc. - 4, 8, 12; Core Healthcare - 8; The Chinese University of Hong Kong - 10 (2412)
Cho, M. H., Idaho Technology - 6 (2709)
Constantino, J. N., Western Psychological Services - 8 (Session 48)
Curran, J. E., ChemGenex Pharmaceuticals - 1, 2, 3, 5, 7, 11, 12 (140)
Dapprich, J., Generation Biotech - 3, 4, 10, 13 (1199)
de Silva, D., Idaho Technology - 3 (2700)
Dickson, P., BioMarin Pharmaceutical - 2, 5; Genzyme - 5 (2294)
Edwards, J., Applied Biosystems - 12 (717)
Elstein, D., Neurotrax Corp - 3 (1501)
Fan, J., Illumina, Inc. - 1, 3 (694)
Fu, H., CCRI - 3; OSU - 3 (2289)
Garcia, A. R., Shire Human Genetic Therapies - 1, 3 (2236)
Giampietro, P., Scoliosis Research Society - 2 (242)
Giannini, E. H., Genzyme Corporation - 5 (1446)
Goldgar, D., University of Utah/Myriad Genetics - 8, 10 (5)
Goldman, S., Quest Diagnostics - 3, 10 (1436)
Greene, C. L., HRSA - 2 (Session 25)
Gudbjartsson, D., deCODE Genetics - 1, 3 (143)
Gulcher, J., Decode Genetics - 1, 3, 4; Illumina - 1, 3 (2523)
Gundry, C. N., Idaho Technology - 1, 3, 4, 8 (2711)
Gunn, S., Combimatrix Molecular Diagnostics - 3 (304)
Gustafson, S., Mt. Sinai School of Medicine, Porphyria DNA Testing Laboratory - 2, 3 (1484)
Habibian, R., Genzyme Genetics - 1, 3 (614)
Haider, M. Z., Kuwait University, Kuwait - 3 (2493)
Hajianpour, A., Genzyme Genetics - 1, 3 (2320)
Hantash, F. M., Quest Diagnostics Nichols Institute - 1. 3 (1254)
Harmatz, P., BioMarin Pharmaceutical, Inc. - 2, 5 (268)
Hartshorne, T., Applied Biosystems - 3, 1 (2715)
Haskins, M., BioMarin Pharmaceutical, Inc - 1, 2, 3 (2234)
Hatfield, J. L., OU Genetics Laboratory - 5 (788)
Heald, B., Signature Genomics Laboratory - 3 (566)
Heaton, C., Vanda Pharmaceuticals, Inc. - 3 (1039)
Helgadottir, A., deCODE genetics - 1 (138)
Ho, J., Roche Molecular Systems - 12 (2379)
Homer, N., University of California, Los Angeles - 2 (2161)
Honisch, C., Sequenom, Inc. - 3 (2696)
Hough, C., Idaho Technology, Inc. - 3; Idaho Technology, Inc. - 1 (720)
Huang, M. Y., National Science Council - 2 (2733)
Hughes, A., Queen's University, Belfast - 10 (2467)
Hughes, D. A., Shire HGT - 2, 9 (2279)
Hujsak, P., Autogenomics - 3 (1054)
Hyland, F. C. L., Applied Biosystems - 3, 1, 10 (2134)
Isasi, R., International Stem Cell Forum - 2 (2191)
Isler, J. A., Wyeth Research - 1, 3, 10 (1056)
Jalali, G., MLPA - 12 (1625)
Janisch, K. M., Burroughs Wellcome - 2 (651)
Janssens, T., Roche Applied Science - 12 (2713)
Jin, P., SiGen Pharmaceuticals, Inc. - 4, 10, 13 (Session 26)
Kampmann, C., Shire HGT - 2, 5, 11 (2280)
Keutzer, J., Genzyme Corporation - 1, 3 (1449)
Khanna, R., Amicus Therapeutics - 3 (2250)
Kishnani, P. S., Genzyme Corporation - 2, 5, 6, 7 (Session 67); Genzyme Corporation - 2, 5 (2232)
Knoll, J. H. M., Phylogenetix Laboratories - 13 (1632)
Kotte, A., Psynomics, Inc. - 1 (175)
Laipis, P., BioMarin Pharmaceutical, Inc. - 2 (2237)
Lavedan, C., Vanda Pharmaceuticals, Inc. - 3 (1035)
Levy, B., Affymetrix - 5 (1616)
Licamele, L., Vanda Pharmaceuticals, Inc. - 3 (1036)
Lo, D., Sequenom - 1, 5, 8, 12; Institut Jacques Boy - 8; Plasmagene - 1, 8 (Session 49)
Lu, Y., Shire HGT - 3 (2272)
Lupski, J. R., Athena Diagnostics - 10, 5, 8, 9; Baylor College of Medicine - 3, 10 (Session 52)
Lynch, K., Myriad Genetic Laboratories - 1, 3 (Session 50)
Lynn, T., Quest Diagnostics - 3 (1437)
Mahboubi, P., Sequenom - 3 (2417)
Marsden, D. L., Genzyme - 1, 3 (2238)
McCarthy, L. C., GlaxoSmithKline - 1, 3 (1045)
McLaughlin, S. F., Applied Biosystems - 3 (2620)
Mehta, A., Shire - 2, 5, 7, 9 (1485)
Miebach, E., Shire Company - 2 (2239)
Mons, B., Knewco Inc. - 4 (1991)
Mooser, V., GlaxoSmithKline - 3 (2462)
Muenzer, J., Shire HGT - 2, 5, 11 (2281)
Newkirk, H. L., Children's Mercy Hospital - 10 (2642)
Nicolino, M., Genzyme Corporation - 7, 11 (2242)
Ntouroupi, F., Ikonisys, Inc. - 1, 2, 3 (388)
Ober, C., Genentech - 5 (Session 23)
Oeth, P., Sequenom, Inc. - 1, 3 (2665)
Olckers, A., DNAbiotec (Pty) Ltd - 4 (2450)
Ostrander, E. A., Mars Foods - 10, 12 (Session 24); American Kennel Club - 11 (1283)
Palmer, R., Genzyme Corporation - 1, 3 (1454)
Peiffer, D. A., Illumina, Inc. - 3 (2515)
Peters, M. A., Roseatta Biosoftware - 1, 3 (1909)
Pettersson, F., GlaxoSmithKline - 12 (1381)
Pine, C. W., Amicus Therapeutics - 1, 3 (2254)
Plenge, R. M., Biogen Idec - 2 (2478)
Pouresmaeili, F., Taleghani Hospital, Endocrinology Research Center, Tehran, Iran - 2 (1675)
Prasad, S., Genzyme Corporation - 3 (1480)
Ramakrishnan, R., Fluidigm Corp - 3 (2666)
Ramaswami, U., Shire HGT - 2, 5, 9 (2291)
Randolph, L., BioMarin Pharmaceutical Inc - 11 (2230)
Remmers, E. F., Biogen Idec, Inc - 5; Genentech, Inc. - 5; Celera Diagnostics - 5; Roche Pharmaceuticals - 5 (2606)
Reynolds, J. D., Affymetrix, Inc - 3 (2637)
Richard, G., GeneDx (BRLI) - 3, 1 (Session 3)
Rieder, M. J., Illumina - 1 (90)
Rubinstein, W. S., Myriad Genetic Laboratories, Inc. - 9 (419)
Saitta, S. C., Affymetrix - 11 (518)
Salonen, J. T., Oy Jurilab Ltd - CSO (260)
Schrodi, S. J., Celera - 1, 3, 5 (2479)
Schwartz, I., Genzyme - 2; BioMarin - 2; Shire - 2, 5 (1451)
Schwartz, S., Affymetrix - 6 (84)
Seppo, A., Ikonisys, Inc. - 1, 3 (2422)
Sheen, C. R., Bayer Healthcare - 2 (1246)
Slack, F., Asuragen - 2, 12 (Session 26)
Smith, R. L. L., Stiefel - 2 (2345)
Song, S., Agilent Technologies - 3; Sigma-Aldrich - 3 (699)
Sozer, A. C., Sozer, Niezgoda and Associates, LLC - 3, 4 (Session 4)
Spence, W., Myriad Genetic Laboratories, Inc. - 1 (377)
Stefansson, H., deCODE genetics - 1, 5 (105)
Steinthorsdottir, V., decode genetics - 3 (2358)
Sulem, P., deCODE Genetics - 1, 3 (253)
Taylor, K. E., Genentech - 12 (2484)
Terry, P. F., Genomic Health, Inc. - 1, 3, 4, 13 (2195)
Titlow, M., Genzyme Corporation - 1, 3 (1452)
Tosi, J. T., Burroughs Wellcome - 2 (2618)
Towers, G. W., DNAbiotec (Pty) Ltd - 3 (2451)
Tsalenko, A., Agilent Technologies - 1, 3 (2516)
Tylki-Szymanska, A., Genzyme Corporation - 1, 2, 3, 5 (1505)
Van den Veyver, I. B., Baylor College of Medicine, Department of Molecular and Human Genetics - 3 (Session 49)
van der Merwe, A., DNAbiotec (Pty) Ltd - 3 (2452)
Veltman, J. A., Affymetrix Inc - 12 (1623)
Vertemati, T., Shire Human Genetic Therapies, Inc. - 12 (1483)
Viskochil, D., Shire Human Genetic Therapies, Inc. - 9 (2268)
Volpi, S., Vanda Pharmaceuticals, Inc. - 3 (1037)
Wakui, K., FALCO biosystems Ltd - 12 (1645)
Walz, G., Novartis - 5 (Session 27)
Weinreb, N., Genzyme Corporation - 2, 5, 9 (2244)
Wolfgang, C., Vanda Pharmaceuticals, Inc. - 3 (1040)
Wraith, J. E., Shire - 2, 5 (1488)
Wu, X., Chromotrax, Inc. - 3 (1655)
Wustman, B. A., Amicus Therapeutics - 1, 3 (2256)
Yau, C., Illumina, Inc. - 12 (2630)
Yeakley, J., Illumina, Inc. - 3 (2692)
Zahorchak, R. J., Partnership for Biotechnology Research - 5; Open Biostystems, Inc. - 12 (835)
Zhang, J., Canadian Institutes of Health Research - 2 (2383)
Zhang, Z., Applied Biosystems - 3 (2645)
Zimran, A., Shire HGT - 5 (2245)
Zuchner, S., Athena Diagnostics, Inc. - 5, 8 (97)

Advertisements appear in the following order:

Advertiser	*Booth Number*
AAAS/SCIENCE	422
Applied Imaging Corporation	1030, 1032
Combimatrix Molecular Diagnostics	504
Deerac Fluidics	226
Elsevier	737
GE Healthcare	537, 539
Genzyme Corporation	630, 632, 729, 731
Idaho Technology, Inc.	626
Molecular Devices, now part of MDS Analytical Technologies	1020, 1022
Nature Publishing Group	538, 540
Oxford Gene Technology	1125
Roche Applied Science	716, 718, 720
Shire	617, 619, 621
Signature Genomic Laboratories, LLC	916, 943, 945
Whatman, Inc.	831
Xceed Molecular	427
SEQUENOM, Inc.	208, 210, 307, 309
Affymetrix, Inc.	715, 717