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Dejan B.
Budimirović, MD

Svetski dan zdravlja
MALI UJED, VELIKI RIZIK

SLOVO ZAKONA O NASILJU U PORODICI

Specijalni dodatak
ACTA SCIENTIAE
Medicine World Wide

Serbian Diaspora Medical Conference 2014.

Specijalni dodatak

Kennedy Krieger Institute

Dr Dejan B. Budimirović

Medical Director,

Akademska posvećenost dr Dejana Budimirovića i njegovi napori u tom pravcu dosežu daleko. Kao jedan od najboljih studenata medicine, tokom svog obrazovanja bio je dobitnik na desetine nagrada i priznanja. Student generacije, nagrađen je da završi svoj lekarski staž na Univerzitetskim klinikama Medicinskog fakulteta u Beogradu. Ranih 90. godina javno je promovisao naučno potkrepljene studije zdrave ishrane u cilju prevencije kardiovaskularnih i malignih bolesti. Ovi pionirski koraci u smislu obrazovanja šire populacije u našoj sredini su kasnije, pokazalo se, stvorili mogućnost i za saradnju sa institucijama u SAD

Ekskluzivno

AUTIZAM
- neistraženo polje izazova

Dr Dejan Budimirović je medicinski direktor *Fragile X* Klinike i glavni lekar u nedavno oformljenom istraživačkom Centru za kliničko ispitivanje novih lekova u *Kennedy Krieger* Institutu. On je dugogodišnji ordinirajući prvenstveno dečji neuropsihijatar u istom institutu i puno radno vreme redovni član nastavnog osoblja u zvanju asistent profesora na *Johns Hopkins University School of Medicine*. Dr Budimirović je sa odličnim ocenama položio specijalizaciju iz psihijatrije odraslih osoba, a posebno je iskusan u dugogodišnjem kliničkom radu sa decom i adolescentima, što je potvrdio i sa ponovljenim najvišim ocenama u sveobuhvatnoj proveri znanja iz tih oblasti. Kao takav stručnjak, već 14 godina je priznati član američke licencne Komore za psihijatriju i neurologiju i nagrađivan je od strane svojih profesora i kolega za svoje terapeutske kliničke sposobnosti i profesionalizam.

Dr Budimirović je prvo stekao diplomu škole za medicinske tehničare/sestre u Šapcu, pa onda diplomu Medicinskog fakulteta Univerziteta u Beogradu, gde je diplomirao magna cum laude i nagrađen kao najbolji student u svojoj generaciji (Klasa 1987). Svoje medicinsko specijalističko obrazovanje je proširio i usavršio na Harvardu, a posle toga na, takođe privatnom, Njujork univerzitetu.

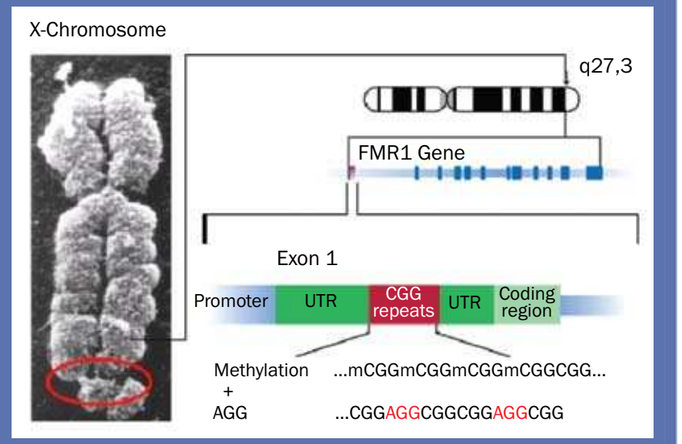
Nakon obaveznog pripravničkog staža, dr Budimirović je izabrao oblast neuronauka, koja se veoma brzo razvijala u to vreme, i angažovao ga je Univerzitetski klinički centar, Katedra za psihijatriju. Stekao je izuzetno bogato kliničko iskustvo na dvogodišnjoj specijalizaciji iz ključnih oblasti opšte psihijatrije, koje je upotpunjeno završetkom postdiplomskih studija iz biološke psihijatrije sa najvišim ocenama.

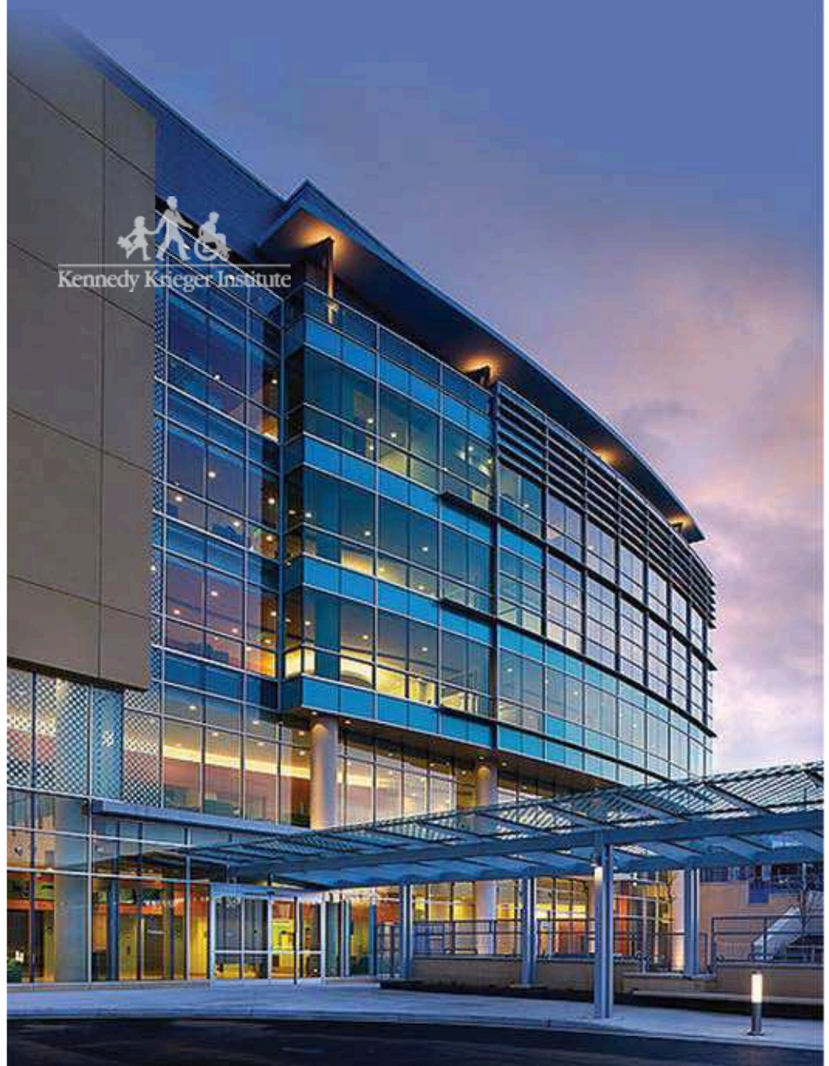
Uspešan i vredan stručnjak - rado je primljen u SAD

Nakon preseljenja sa porodicom u SAD, 1994. godine, dr Budimirovićevi izuzetni uspesi su prepoznati, pa je prihvaćen na Katedri za opštu psihijatriju Medicinskog fakulteta na Harvardu, a zatim jako uspešno i kreativno završava subspecijalizaciju na Katedri dečje i adolescentne psihijatrije Medicinskog fakulteta Univerziteta u Njujorku. Ova praksa mu je omogućila da proširi svoje iskustvo i znanje u kliničkoj psihijatriji i dodatno je stimulisala njegovu istraživačku inicijativu i sposobnost za zajedničke projekte.

Na kraju te subspecijalizacije, za takve postignute rezultate, dr Budimiroviću je dodeljena plaketa za izvanredne terapeutske sposobnosti i profesionalnost u radu sa pacijentima. Ovo priznanje je time veće jer se ne dodeljuje svake godine, a potiče od nastavnog osoblja i kolega sa Odseka za dečju i adolescentnu psihijatriju njujorškog Centra za studije dece i već čuvene *Bellevue* Gradske bolnice, i *Lenoks Hill* bolnice.

Taj tek otvoreni Centar, drugi te vrste po redu u SAD, izuzetno se brzo i uspešno širio kao jedan od nadolazećih ''zvezda vodilja''. Uz to, takav uspeh dr Budimirovića je kulminirao sa njegovim prvim akademskim imenovanjem na Univerzitetu Jejl, gde je bio postavljen za asistenta profesora. To zvanje se daje kandidatima čiji rezultati obećavaju najviša moguća akademska dostignuća u svojoj branši. Tokom svog četvorogodišnjeg mandata na Jejlu, kao specijalista psihijatar, dr





Budimirović se uspešno brinuo o nekoliko stotina pacijenata, prvenstveno omladine, ali i pacijenata uz opšte i gerijatrijske psihijatrije.

Svoje zvanje je i opravdao tako što se izborio da bude glavni istraživač na jednom projektu iz oblasti poremećaja raspoloženja, koji je finansirala uticajna privatna fondacija. On je, takođe, pokazao liderske veštine, i izabran je za jednog od direktora adolescentne psihijatrije Univerzitetske bolnice na Jejlu. Posle jednog prelaznog perioda na Univerzitetskoj bolnici, Dečje odeljenje na Univerzitetu *Stoni Brook*, gde se dr Budimirovićevo kliničko iskustvo dalje upotpunilo sa posvećenim vođenjem samo dečje populacije, u novembru 2004, dr Budimirović je angažovan i primljen u nastavno osoblje u zvanju asistenta profesora na Univerzitetu Johns Hopkins, i specijaliste dečje i adolescentne psihijatrije u Institutu Kenedi Kriger.

Naučno-istraživački projekti na polju autizma

Dokazujući svoje sposobnosti, dr Dejan Budimirović je tokom svih godina svoje karijere učestvovao u različitim naučno-istraživačkim projektima. Sa njegovim učešćem kao člana istraživačkog tima na projektima finansiranim od privatnih fondacija, Vlade SAD – Nacionalnog instituta za zdravlje (NIH), tokom rada na univerzitetima *Jejl*, *Stoni Brook*, i *Johns Hopkins*, dr Budimirović je učvrstio svoje naučno - istraživačke temelje.

Od dolaska na svetski poznati Institut Kenedi Kriger u oblasti razvojnih poremećaja, uključujući i autizam, dr Budimirović je doprineo proširenju kliničke aktivnosti u *Fragile X* klinici (osobe sa ''nestabilnim'' dugim krakom na X hromozomu). U 2006, klinika je postala jedna od članica osnivača ''*Fragile X* klinika i Istraživačkog konzorcijuma'', koji sada broji 26 članova u SAD i Kanadi. Počev od 2008. pa sve do danas, Centar za kontrolu i prevenciju bolesti Vlade SAD finansira neke od istraživačkih aktivnosti ovog konzorcijuma, u cilju boljeg razumevanja osoba sa nestabilnim dugim krakom na X hromozomu, koje često imaju i autizam!

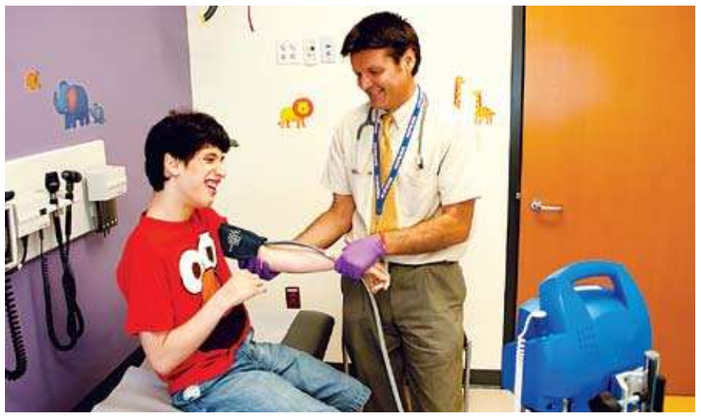
Tokom poslednjih godina, dr Budimirović je objavio nekoliko publikacija u časopisima sa značajnim

impakt faktorom. Jedna od njegovih publikacija je originalni rad na glavnim faktorima socijalnih deficita kod osoba sa FXS i autizma (2006). Ovaj originalni rad bio je osnova za publikacije i nekoliko podnetih naučno-istraživačkih radova u nekoliko finansijskih agencija, prvenstveno NIH. Sa razvojem i početkom korišćenja novih lekova u ovoj oblasti, njegove kolege sve više citiraju ovaj njegov originalni rad. Njegovi rezultati su nedavno ponovljeni u 10 puta većem uzorku u studiji najviše lokacija u kojoj je učestvovala i njegova klinika, što dokazuje kvalitet njegovog istraživačkog rada na duge staze.

Sistematskom primenom različitih metoda, dr Budimirović će u toku istraživačke aktivnosti nastaviti da doprinosi na tom terenu.

Pored toga, dr Budimirović je i glavni istraživač u tri projekta: Fragile X klinička istraživanja i Konzorcijum registar i baza podataka, Fragile X klinika Farmakološka baza podataka, Prvi znaci Fragile X sindroma, međunarodnog projekta koji ima za cilj mnogo raniju kliničku identifikaciju FXS, radi što ranije genetske dijagnostike FXS, i tako podgrupe autizma.

On je, takođe, glavni istraživač na nedavno podnetom velikom pro-



jektu koji koristi novu tehnologiju testiranja FXS, koja ima za cilj da „iscedi još više soka iz FMR1 gena (FXS) i tako unapredi razumevanje i autizma. Jedan nedavno podneti projekat iz oblasti FXS, koji je uključio četiri različita univerziteta iz dve američke države, takođe je angažovao dr Budimirovića zbog njegove visoke stručnosti u ovoj oblasti.

Fragilni X gen ima važnu ulogu ''kontrolora'' sinteze proteina na mestima gde se dve nervne ćelije ''poljube'' (tzv. synapsa).

Jedan gen, puno više markera. Povećanje broja CGG nukleotidnih ponavljanja (slova azbuke DNK) u području FMR1 genu daje izgled X hromozoma kao da će se ''prekinuti'' (crveni ovalni krug). Ovaj region je odgovoran za sve kliničke forme uzrokovane promenama (mutacija) ovog gena.

Ovi markeri su CGG ponavljanja, AGG ponavljanja (novi test da se prati stepen rizika da se pogorša u sledećoj generaciji, vidi dole), i metilacija (mehanizam koji umrtvi aktivnost ovoga gena, pa nema njegovog proteina – FMRP).

Različite grupe mutacija

Tačnije, postoje dve velike grupe mutacija sa ''nestabilnim – proširenim'' dugim krakom na X hromozomu:

a) mnogo teža klinička forma, sa potpuno nestabilnim, tzv. fragilni X sindrom (FXS), koji je vodeći poznati uzrok autizama izazvan pojedinačno izmenjenim poznatim genima;

b) u prvim decenijama života mnogo blaža klinička forma, tzv. delimično izmenjen fragilni X gen, koji je u manjoj meri povezan sa autizmom, a mnogo više sa povećanim rizikom za ranu menopauzu kod žena i jedne posebne vrste rane demencije kod muškaraca. Važno je istaći i to da je delimično izmenjen fragilni X gen 10 puta češći, i takvih slučajeva nosilaca u SAD se smatra da ima jedan milion! Iako najčešće ne znaju da su nosioci ove genske promene, buduće majke

Deo predavanja dr Dejana Budimirovića u školi "Dr Milan Petrović" u Novom Sadu, u okviru nedavno održane Četvrte konferencije srpske medicinske dijaspore

– Osobe sa autizmom imaju problem u učenju, počev od najranijeg usvajanja osnovnih znanja i navika, pa do pisanja, usvajanja znanja uopšte i drugih veština. Smetnje su najviše izražene u domenu komunikacije, u razumevanju i prihvatanju pravila koja zahtevaju određene socijalne situacije. Glavni razlog zašto se polje neuro nauke okreće prema FXS kao "modelu za studiranje autizma", bar jedne grupe, jeste da su istraživači ustanovili da Fragilni X gen utiče, ''ostavlja otiske prstiju'' na polovinu od mnogobrojnih gena u autizmu!

nose 50:50 rizik da dobiju muško potomstvo sa FXS, koji se ubrzo po rođenju često manifestuje sa teškim poremećajima psihomotornog razvoja.

Dr Budimirović je i dalje posvećen tom cilju, uključujući dalje unapređenje ''jezgrovnog'' tretmana ovog poremećaja. Kao direktor Klinike za fragilni X poremećaj i autizam na Kennedy Krieger institutu, u okviru Medicinskog fakulteta i bolničkog kompleksa Johns Hopkins u Baltimoru, zahvaljujući dugogodišnjem uspešnom radu ovog lekara, otvoren je prošle godine Centar za istraživanje ovih, i drugih, lekova na institutu. Ova klinika je deo jednog zajedničkog napora na desetine klinika u SAD i svetu da to provere u kliničkim studijama, u nadi da će moći mnogo bolje da pomognu obolelima.

Klinika je izabrana kao jedna od dve lokacije za klinička ispitivanja za koja je dr Budimirović glavni saradnik-istraživač:

1. Faza III, ključno kliničko ispitivanje STKS209 (Arbaclofen-GABA-B stimulator, radi kao kočnica) za lečenje izražene socijalne povučenosti kod odraslih, adolescenata i dece sa FXS, pod pokroviteljstvom Seaside terapije, Inc. Ova istraživanja su bila proširena i sledi nastavak ove studije.

2. Faza II, kliničko ispitivanje GluR5 antagonista (NP 27936), radi tako da olabavi pedalu za

gas (glutamate), i koristi se za lečenje socijalnog povlačenja kod odraslih sa FXS, a pod pokroviteljstvom Hoffmann-La Roche Ltd. U okviru svoje nastavne aktivnosti, dr Budimirović predaje studentima na Johns Hopkins University Homevood kampusu, studentima medicine i specijalizantima. On je, takođe, mentor i gostujući naučnik

u Austriji na zajedničkim projektima.

Dr Budimirović je član američkog Odbora za psihijatriju i neurologiju odraslih, dece i adolescenata, a ponovo je sertifikovan za dečju i adolescentnu psihijatriju. Aktivan je član Američke akademije za dečju i adolescentnu psihijatriju, bivši član Američke psihijatrijske asocijacije tokom 14 godina i Američkog medicinskog udruženja. Kao medicinski direktor Fragile X klinike, dr Budimirović je, takođe, član FX konzorcijuma, u nastojanju da unapredi raniju dijagnostiku i lečenje za fragil X u vezi istraživanja i kliničke primene. Dr Budimirović je aktivno učestvovao na Devetim danima zastupanja fragilnog X, održanim u Američkom kongresu.

–Ono što se, sa sigurnošću, zna u okviru ovog poremećaja – jeste da postoje medikamenti koji su u završnoj fazi testiranja, koji mogu pomoći osobama sa autizmom. Istraživanja idu u tom pravcu da se sa primenom ovih lekova počne i kod osoba sa autizmom nepoznate etiologije, da bi se procenio njihov efekat. Od velikog je značaja naglasiti da je dejstvo ovih lekova usmereno ka suštinskom rešavanju problema – rekao je dr Budimirović.

–Autistični spektar je termin koji se danas koristi kako bi se ukazalo na postojanje velikog broja različitih oblika ispoljavanja autizma, da bi se sve te razlike obuhvatile jednim pojmom. Autizam, ogroman i rastući problem javnog zdravlja u SAD, i šire, predstavlja hitan medicinski slučaj

Ono što se, sa sigurnošću, zna u okviru ovog poremećaja – jeste da postoje medikamenti koji su u završnoj fazi testiranja, koji mogu pomoći osobama sa autizmom

(1:38 ukupan broj slučajeva, 1:110 dijagnostikovano, sa 3,2 miliona dolara cenom koštanja za života). Taj težak psihomotorni poremećaj počinje u najranijem detinjstvu, i nije jedna bolest već grupa bolesti različitog porekla koja se slično manifestuje. Te mnogobrojne grupe imaju sličnosti i različitosti. I pored velikog ulaganja u istraživanja u genetiku u autizmu, ne zna se šta je tačno uzrok toga. Smatra se da je preko 100 gena izmenjeno u autizmu. Nema leka, ali postoji tretman. Ono što se smatra velikim napretkom u nauci u ovoj oblasti jeste područje nestabilnog X hromozoma. Tu se jasno zna šta je jezgrovni uzrok autizma: pojednostavljeno rečeno,"previše aktivna pedala za gas-transmiter u mozgu glutamat i/ili nedovoljno aktivne kočnice-transmiter GABA". Postoje i specifični lekovi za taj poremećaj koji to jasno koriguju na životinjskom modelu, i oni su u završnoj fazi testiranja na ljudima. Kao što je navedeno, fragilni X gen utiče, „ostavlja otiske prstiju", na polovini od mnogobrojnih gena u autizmu! U tome je ključna nada, i obećavajući rezultati, da će tretman autizma u fragilnom x sindromu biti od koristi i u autizmu drugog porekla. U kojoj su fazi sve ove studije? Figurativno, ovo kao da ''gradimo most po kome upravo sada i hodamo''. Dakle, građenje mosta napreduje, ali se mora ići polako i sigurno, bezbedno.

– Treba podsetiti da se autizam javlja i kod jednog od 50-80 novorođene dece, što svakako nije broj za zanemarivanje. Koliko sam ja obavešten, broj evidentiranih osoba sa autizmom u Srbiji je oko 3.000. Taj broj je sigurno veći jer značajan broj osoba sa autističnim problemima, nažalost, nije ni dijagnostikovan.

– Moja zapažanja sa susreta sa kolegama i običnim ljudima tokom boravka u Srbiji za vreme trajanja Konferencije srpske medicinske dijaspore – ukazuju na činjenicu da se sada sve više govori o problemu autizma na ovim prostorima. U Americi je tako već dosta dugo. Razlozi su vrlo veliki i važni i neophodno je da se javno mnenje što bolje upozna sa ovom problematikom. I podržavanje postojećih kao i dalje proširenje istraživanja je neophodno da bi se ovaj, sada već svetski, problem što bolje razumeo, i da bi pomoć bila svrsishodnija i ovim osobama i njihovim porodicama.

Pripremila:
Dr Božana Noskov-Peregi

Klinički istraživački centar na Kennedy Krieger Institutu/ Johns Hopkins

716 Severna Široka Ulica
Baltimor, država Merilend, SAD
Telefon: (443) 923-2634 begin_of_the_skype_highlighting (443) 923-2634 FREE end_of_the_skype_highlighting
Mejl: budimirovic@kennedykrieger.org

Kennedy Krieger Institute

Dr Dejan B. Budimirović, MD,

Medical Director, Fragile X Clinic, Johns Hopkins Medical Institutions

Assistant Professor of Psychiatry & Behavioral Sciences, Johns Hopkins School of Medicine

Acta SCientiae

Autism in Fragile X Syndrome

1. What is Fragile X Syndrome and what areAutism Spectrum Disorder?

Fragile X syndrome (FXS) is the leading known single gene cause of autism spectrum disorder (ASD). FXS is caused by a full-mutation of the *FMR1* gene, which results in a deficit of its encoded protein: the Fragile X Mental Retardation Protein (FMRP). FMRP normally acts as a "brake" in the process of protein synthesis, particularly in response to synaptic activity.FXS is a medical diagnosis, or more precisely, a genetic diagnosis, whereas ASD is a purely behaviorally-defined diagnosis, representing a group of disorders with varied and yet incompletely elucidated etiology but with a common set of clinical manifestations.

The just released Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-5)groups the different disorders with autistic symptoms, represented in DSM-IV by the more severe form or autistic disorder (i.e., Kanner type), the less severe pervasive developmental disorder (PDD-NOS), and the higher intellectual functioning Asperger disorder as a single diagnosis of autism spectrum disorder (ASD). DSM-5 defines ASD as an entity with two sets or domains of behavioral manifestations: i) deficits in socialcommunication and interaction and ii) presence of restricted interests and repetitive and stereotyped behaviors. All three criteria of the coresocialdomain and at least two out of the four of the restricted/repetitive behaviors are needed for the diagnosis of ASD.To better delineate the range of manifestations by ASD, a set of specifiersthat include cognitive, behavioral, and medical factors.

Over the past decade, many studies, using the DSM-IV criteria, have attempted to define the relationship between the genetic diagnosis of FXS and the behavioral symptoms commonly seen in patients with FXS. These studies have found that the vast majority of males with FXS have autistic features. Moreover, approximately 46-67% of males and 20% of femaleswith FXS meet criteria for ASD. Of these, 18-36% of males have the more severe autistic disorder. On the other hand, 1-2% of children with ASD is identified as having *FMR1* full mutation or FXS in their genetic diagnostic workup.Genetic and neurobiologic research suggeststhat ASD is the common clinical expression of arange of disorders with overlappingmolecular and cellular features. Currently, FXS is considered one of the syndromic types of ASD although*FMR1* testing is also recommended for individuals without any physical phenotype.

2. Why is studying syndromic ASD in FXS increasingly important for the field of ASD?

Autism spectrum disorderis an enormous and growing public health challenge.Nationally, there are one million individuals with ASDs, which represents an urgent, unmet medical need (i.e., ~1:50 prevalence, 1:110 diagnosed, with $3.2 M lifetime cost).This is a lifelong and often severe neurobehavioral syndrome that manifests with a widespectrum of behavioral symptoms, severity, and co-occurring conditions.Such complexity has hindered the understanding of core underlying neural mechanisms of the ASDsand the development of new therapies targeting these causes. Thus, treatments that target the core deficits in this convoluted disorder are lacking. Since ASD are highly heterogeneous genetically, reliable homogeneous genetic models for its study are being sought.

As the expansion-type mutationis the cause of FXS in more than 99% of cases, in that sense, FXS is biologically a highly homogeneoussingle gene disorder that accounts for 2-6% of cases of ASDs. Hence, their association with FXS, which can be considered a genetic cause of ASD or one of the "autisms,"allows for the opportunity to refine a behavioral phenotype against the established genetic background of FXS. Indeed, the underlying complex pathophysiology of FXS provides important links to the behavioralexpressions of FXS+ASD and other ASD. The translation of treatments targeted at FXS pathophysiology in animal models has led to clinical trials in humans of promising targeted medications for FXS+ASD and FXS-only, without ASD (FXS-O), and possibly other ASD (ASD-O). Thus, headlines such as 'New Roadmap to Unlock the Mystery of Autism: Is Fragile X the Key?' reflect this meaningful progress in the field.

The behavioral phenotype of FXS includes many typical "autistic" features such as deficits in social interaction (i.e., poor eye contact, peer relationships) and communication (i.e., language), motor stereotypies, perseverative behavior, social withdrawal behaviors and self-injurious behavior (i.e., hand biting). As these typical "autistic" features overlap with ASD-O, clinicians ought to understand and appreciate the similarities and differences between FXS+ASD and ASD-O. Nevertheless, because of a significant phenotypic overlap between these two conditions, it can be challenging to identify these differences, which is important in order to better guide its management effort. Together, understanding what is known about FXS+ASD can in particular help advance the development of appropriate therapies (i.e., biological, behavioral, educational) not only in FXS but potentially also in ASD-O. Although rare, FXS is the most prevalent monogenetic cause of intellectual disability and autism. Moreover, due to its high penetrance and homogeneity, FXS is the most desirable genetic model of intellectual disability and ASD, and as such is well-studied.

3. What clinical features differentiate individuals with FXS-ASD and FXS-O?

In general, these children are similar but also different. If the FXS phenotype includes many typical DSM-defined autistic features, what differentiates children with FXS-Ofrom FXS+ASD?In general, while boyswith FXS-O typically have intellectual deficits, hyperarousal and social anxiety, the core deficits in FXS+ASD are due to impairment incomplexsocial interactions. Characteristics *common* to both entities include (a) social interaction deficits, (b) poor eye contact, (c) motorand communication-language deficits, and (d) repetitive behaviors.

Social Interaction Deficit. Children with FXS+ASD have deficits in complex social interaction skills (i.e., peer relationships, social-emotional reciprocity) with frequent social withdrawal behaviors (i.e., social avoidance and social indifference).Social avoidance and failure to recognize social cues are more prominent features in individuals with FXS+ASD than in those with FXS-O. These impairments in the social domain are expressed on a continuum in FXS regardless of the comorbidity (i.e., anxiety). Moreover, as social avoidance and anxiety are generally common in individuals with FXS, these features may not reliably distinguish those with FXS-O from those with FXS+ASD. Similarly, impairments in friendship are present in all individuals with FXS regardless of their ASD status, which could be attributed to anxiety symptoms, communication difficulties, and cognitive delays..

Earlier studies comparing discrete social behaviors between people with FXS-ASD and ASD-O have found that those with FXS-ASD show an 'intermediate' level of social impairment but higher rates of repetitive behavior.A recent studyhas examined the patterns of repetitive and social behavior in two groups of young boys with FXS+ASDand ASDsO. Thestudy found that the behavioral phenotypes of these two groups are:

(i) most*similar (overlap)*with respect to lower-order(motoric) restricted, repetitivebehaviors (i.e., stereotypy, self-injury), and *social approach (initiation)*but that they

(ii) contrast in more complex forms of repetitive behaviors (i.e., significantly lower in FXS+ASD) and some *social response* behaviors (i.e., people with FXS+ASD appear more attuned to social cues than people with ASD-O).

Furthermore, a lack of social initiative alone does not necessarily imply the absence of social awareness orsocial interest. For those with FXS+ASD, social initiation deficits may reflect the social anxiety, which is common to children with FXS. In contrast, social deficits in ASDwithout FXS are likely to originate from a failure to attend to social information and to a general social indifference, thus precluding appropriate social behavior.

(b)Poor Eye Contact. Eye contact is a fundamental component of human social interactions. Though "poor" eye contact is symptomatic of both FXS+ASD and FXS-O, the quality of eye contact deficits is substantially different. Individuals with FXS only directly avoid eye contact, looking away in another direction to cope with their emotional discomfort driven by underlying social anxiety. On the other hand, individualswith FXS+ASD do not recognize social cues such as eye gaze as a source of information or interaction. Thus, *eye gaze avoidance* in FXS is probably unrelated to ASD because only

*persistence*of eye gaze avoidance after a social challenge is linked to other DSM diagnostic criteria and the diagnosis of FXS+ASD.

Further illustrating these differences, individuals with FXSmay seek human interaction, but the social anxiety consistent with FXS often significantly gets in the way. On the other hand, individuals with FXS+ASDare largely unaware of the function others serve in relation to themselves; hence they rarely seek social interaction.In practical terms, insisting on eye contact with those who have FXS-Ooften leads to greatly heightened social anxiety and may initially worsen cortisol dysregulation. Insisting on eye contact with those who have FXS+ASDmay have some merit (i.e., ABA trains them to take in key social information). At times, teachers and caregivers may say that they do not need to know about FXSsince they have training to work with children with ASD. However, interventions appropriate for children with FXS-O may be quite opposite from those for ASD. Not all children with FXS have autism, but may still have social anxiety, sensory processing problems, and/or repetitive behaviors that require interventions, which may be different for the child with an ASD.

(c) Motor and communication deficits. Nearly all parents report delays in early motor and communication (i.e., receptive-expressive language skills) in individuals with FXS. In FXS-O, males have moderate to severe deficiencies with a greater impairment in expressive language compared to receptive language skills. In contrast, in FXS+ASDthere are greater impairments in receptive language skills, and fine and gross motor abilities than those with FXS-O. Additionally, some studies have found greater deficits (lower scores)in motor abilities in individuals with FXS+ASD than individuals with ASD-O.Furthermore, differences in profiles of other skills (i.e., imitation) may be helpful in a clinical evaluation Imitation is a pivotal developmental skill that is generally a strength for those with FXS-Oand typically impaired in individuals with ASD.

(d)Repetitive behaviors. Both individuals with FXS-O and with FXS+ASD have repetitive behaviors, such as stereotypical object manipulationand motor stereotypies (hand-flapping, body-stiffening, and rubbing or wiping the head with a hand) Those with FXS-O tend to exhibit more repetitive behaviors due to excitement, anxiety, or difficulty "stopping" or inhibiting their behavior, while individuals with FXS+ASD appear to engage in those behaviors for less specific and more varied reasons (i.e., vestibular stimulation results in a lot of spinning and jumping). As of yet, the precise pattern of repetitive behavior associated withASD in FXS remains unclear.

4. What are challenges in characterizing social interaction disorders in FXS: ASD-Anxiety link?

In FXS, the behavioral manifestations of anxiety and hyperarousal complicate the ability to diagnose ASD. According to the National Parent Survey, anxiety is the second most common behavioral abnormality in FXS individuals older than 6 years of age. Another recent studyfound a greater percentage of individuals with FXS meeting criteria for a variety of anxiety disorders than in other intellectual disability groups or in the general population.One of anxiety's variants, social anxiety, is adisorder characterized by avoidance in social situations and are not simply secondary to cognitive deficits.An epidemiological report notes that social anxiety is the most common comorbidity in ASD-O.

5. Why is a reliable early diagnosis of ASD in FXS important?

Persistent abnormalities in social interaction could be a sign of an emergingASD. Children with FXS are often first diagnosed with ASD,as theseneurobehavioral features are the most prominent symptoms; then FXS is identified as part of a medical/genetic evaluation. As in the general population, a diagnosis of an ASD in FXS is based on history and a behavioral observation, usually complemented by standardized questionnaires, DSM-based clinical impression, rating scales, and observational measures, such as the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS). Autistic behaviors are more severe in those with FXS+ASD than in those with FXS-O. As detailed above, it is important to consider social anxiety disorder alone or with ASD in the differential diagnosis of social withdrawal in a child with FXS.

*Individuals with FXS+A*SD tend to have the following characteristics: (i) lower language skills, particularly receptive skills, (ii) more impaired nonverbal cognition, (iii) lower adaptive skills, (iv) more complex socialdeficits, and (v) more severe overall behavioralproblems than those without ASD. Consequently, from educational and vocational viewpoints, individuals with FXS+ASD face more severe challenges.

Similar to the general population, early identification of ASD in FXS is important because it can lead to earlier and more intense interventions. The guidelines of the American Academy of Pediatrics (AAP) regarding developmental screening would allow early identification of children with FXS. The Academy recommends the use of standardized developmental surveillance and screening tests administered during well-child visits (the 9-, 18-, 24- or 30-month). Additionally,it recommends that an ASD specific screening tool be administered at 18 and 24 months. Moreover, a developmental assessment should also be performed any time a concern is raised.Guidance statements from professional organizations also emphasize the need for fragile X testing in individuals with ASD. Yet, general clinical practice and available literature reveal that, for different reasons, only 30% to 40% of individuals with ASD get actually tested for *FMR1* mutations.

6. What treatment strategies are used to helpindividuals with FXS and ASD?

A wide range of maladaptive behaviors is common in children with ASD in FXS. These behaviors cause enormous academic and adaptive behavior impairments beyond intellectual deficit. Psychotropic medications are often necessary to target different symptom clusterssuch as ADHD symptoms, anxiety, and aggressive behavior, but are not specific to FXS. Medication may be necessary to support therapeutic services (i.e., speech-language, occupational, behavioral, educational) and to allow a child to learn in the least restrictive environment.

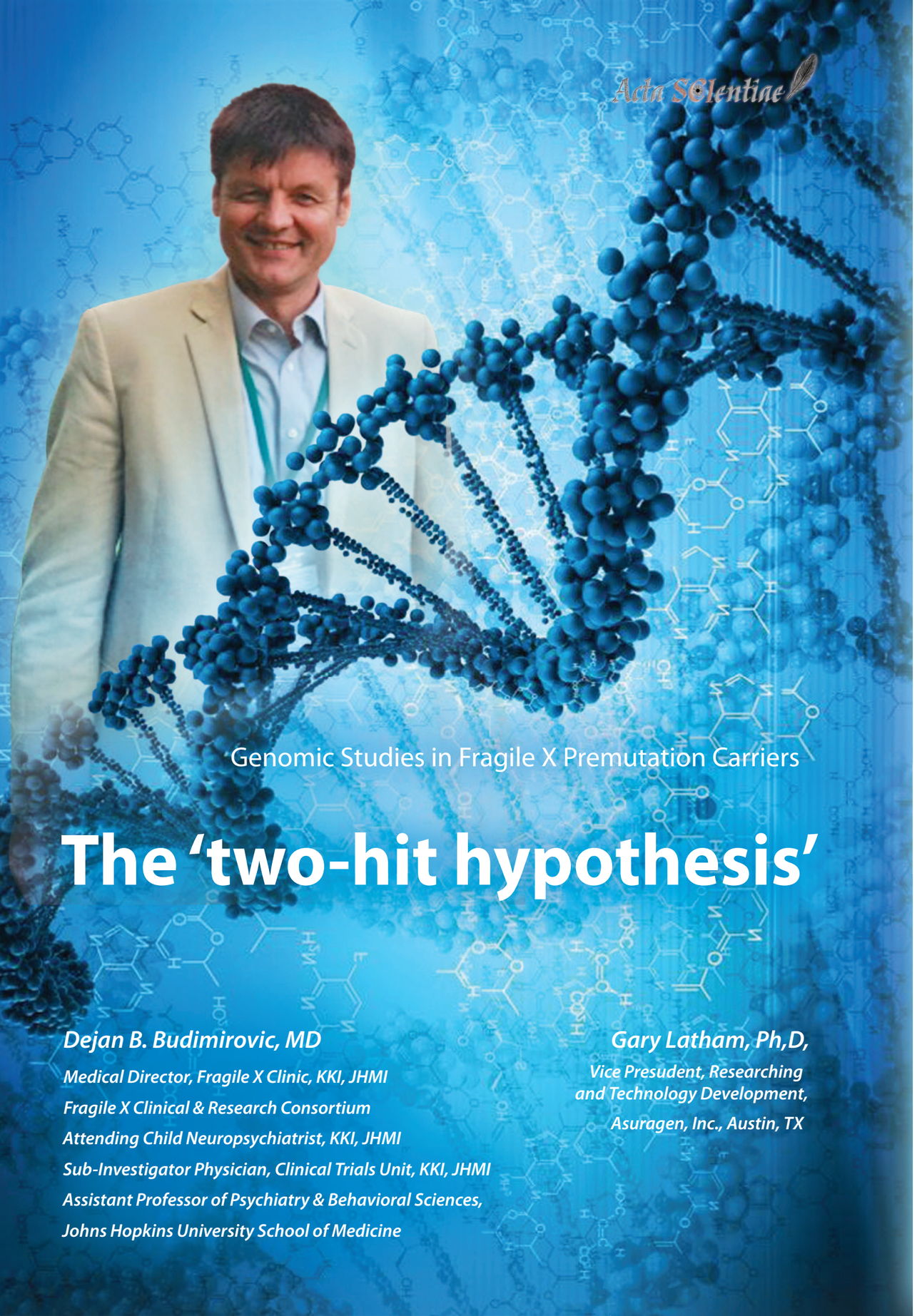
Many of the behavioral issues in FXS are related to anxiety and hyperarousal and associated challenges in managingthem.In younger males with FXS-ASD, sensory processing problems and hyperarousal are particularly common; their treatment includes occupational therapy and alpha adrenergic agonists.

For children with FXS-ASD, intense interventions that target communication and socialization skills are appropriate. Because of their relatively lower cognitive level, educational strategies for children with FXS-ASD may need to focus on functional skills as much as on academics.The crucial point for teachers, therapists, and others involved in the support of people with FXS (with or without ASD) is to utilize existing knowledge about behavior and learning styles of people with FXS in an "individualized" manner in order to better customize these educational, behavioral and other strategies.

While the aforementioned 'targeted symptoms' approach of treating associated symptoms with psychotropic medications is helpful, it is inadequate, as it does not address the underlying cause. To date, there have been no adequately controlledstudieof any of the symptomaticpharmacological treatments commonly prescribed for FXS. Nevertheless, we are closer to FXS-specific treatments today than ever before.Targeted molecular therapeutics has shown favorable results in early clinical trials for patients with FXS. Moreover, some related therapies have been successfully extended to those with ASDs, but clearly larger studies are needed to replicate these initial findings. Furthermore, even when these novel targeted treatments are available forgeneral use in treating FXS, it is likely that psychiatric comorbidity will still require administration of conventional psychotropic medications. Hopefully, even if theseearly therapeutic strategies are only somewhat successful, they ought to help guide further treatment development and further enhance our understanding of FXS and its clinical and neurobiological relationship to ASDs.

7. Why does studying ASD in FXS hold much promise for the future as well?

Distinguishing psychiatric diseases by their symptoms has long been difficult. For example, autism was called childhood schizophrenia until the 1970s, and there was a very high rate of undiagnosed FXS in individuals with ASD and/or ASDwere misdiagnosed as intellectual disableduntil the 1990s.In the meantime, evidence has accumulated that the diagnosis of FXS increases the probability that an individual will also meet the criteria for an ASD. Moreover, recent discoveries suggest a genetic basis that points to a specific signaling system underlying five types of mental and developmental disorders, including autism and schizophrenia. This indicates that lesions in a single neural signaling pathway may result in a variety of neurodevelopmental syndromes and clinical outcomes, with the clinical expression certainly dependent on genetic and environmental interactions. FXS is a biologically homogeneous single gene disorder whose 'translational model' has enabled enormous progress in fragile X targeted therapeutics.A strong value of the homogeneity of FXS is also that it offers an ideal model for identifying the neurobiological mechanisms associated with the subpopulation of individuals with FXS-ASD (i.e., highly enlarged caudate and small amygdala in youngsters with FXS-ASD- versus modest caudate and amygdala enlargement in age-matched ones with ASD-O). Furthermore, converging evidence of both animal and human studies emphasize that genetic background isa critical factor in FXS, which supports the notion of aninteraction between *FMR1* and 'modifier' backgroundgenes in the pathogenesis of ASD in FXS. Studies using high resolution analyses of the*FMR1*gene, protein assays for FMRP expression, as well as genetic screening panels (e.g. microarrays, NextGen sequencing, and epigenetic screens) will enable an understanding of the full value of *FMR1*gene and its encoded protein (FMRP)as a diagnostic and potential therapeutic marker for ASD and permit further refinement and understanding of the molecular basis of ASD in FXS.



Genomic Studies in Fragile X Premutation Carriers

The ‘two-hit hypothesis’

Dejan B. Budimirovic, MD
Medical Director, Fragile X Clinic, KKI, JHMI
Fragile X Clinical & Research Consortium
Attending Child Neuropsychiatrist, KKI, JHMI
Sub-Investigator Physician, Clinical Trials Unit, KKI, JHMI
Assistant Professor of Psychiatry & Behavioral Sciences,
Johns Hopkins University School of Medicine

Gary Latham, Ph,D,
Vice Presudent, Researching and Technology Development,
Asuragen, Inc., Austin, TX

Fragile X–associated disorders (FXD) result from an expansion mutation of a CGG polymorphism in the first exon of the (5’UTR) regulatory region of the FMR1 gene. When the normal number (4-45) of CGG repeats increases to >200 (full mutation), a hypermethylation-mediated ‘shut-down’ of FMR1 gene leads to a deficit in its encoded protein, fragile X mental retardation protein (FMRP). The deficit in this RNA-binding dendritic protein results in the symptoms of fragile X syndrome (FXS) (Kaufmann and Reiss, 1999; Sherman, 2002; Darnell et al., 2011). In contrast, intermediate level expansions (55–200 CGG repeats), which are termed permutations, are not associated with FXS (Kaufmann and Reiss, 1999] but with a carrier status or other clinical phenotypes (e.g., mild cognitive/behavioral problems, fragile X-associated primary ovarian insufficiency syndrome (FX-POI), Fragile X Tremor Ataxia Syndrome (FX-TAS) [Hagerman & Hagerman, 2004; Oostra & Willemsen, 2009). It is noteworthy that these problems occur through a different mechanism from the FMRP deficiency in FXS. Regardless, all these disorders (i.e., FXS, FX-POI, FX-TAS) fall under an umbrella of FXD (Boyle & Kaufmann, 2010).

The FMR1 premutation is common in the general population (1 in 130-250 in females and 1 in 250-810 males; reviewed in Tassone et al 2012), and the phenotypic manifestations of carriers may impact more than 1 million individuals in the US alone. In contrast to FXS, these premutation carriers show lower rates of autism spectrum disorder (ASD) (Chonchiaya et al 2012; Farzin et al., 2006) than in FXS. There are a variety of potential ‘phenotypic modifier’ factors that could explain variable penetrance in FMR1 premutation. These may include interactions with other genes within the X-chromosome (Lubs et al., 2012) or other regions of the genome (i.e., CNVs) (Girirajan &Eichler, 2010), epigenetic differences or/and environmental factors. The rapid progress in high-resolution genome scanning technologies has allowed identification of common CNVs and linkage of the same genomic lesions with apparently very diverse phenotypes.

Case example: A 8 year-old female with mild ASD, anxiety, abnormalities of the attentional network, and mild-moderate intellectual disability (ID), whose an extensive metabolic-genetic work up, including an unremarkable SNP array, has not explained the ID and ASD. Nevertheless, 3 potentially relevant X-linked ID synapse-related hits among below mentioned genes detailed on page 4 (NLG4, DLG3, and CASK) may explain the degree of ID, and possibly ASD, in this low level (CGG 58 repeats) fragile X female carrier. While all these aforementioned genes affect a wide range of functions, and the cause of the clinical overlap is not clear, a synergistic synaptic pathology might impact the severity of the phenotype. Furthermore, it is likely that regulation of expression of disrupted genes may explain the phenotypic variability in premutation carriers through additive effects, in which a change in the ‘at-risk allele’ leads to a phenotype-determining feature (“Second Hit hypothesis”).

The ‘two-hit hypothesis,’ which was initially applied to large CNVs (i.e., 16p12.1 microdeletion), could also capture a smaller CNV or a single-nucleotide change affecting a phenotypically related gene or ‘at-risk’ allele derived from a parent. As this ‘second-hit” can result in additive or epistatic effect, thus changing the ‘at-risk allele’ to a phenotype determining feature, the

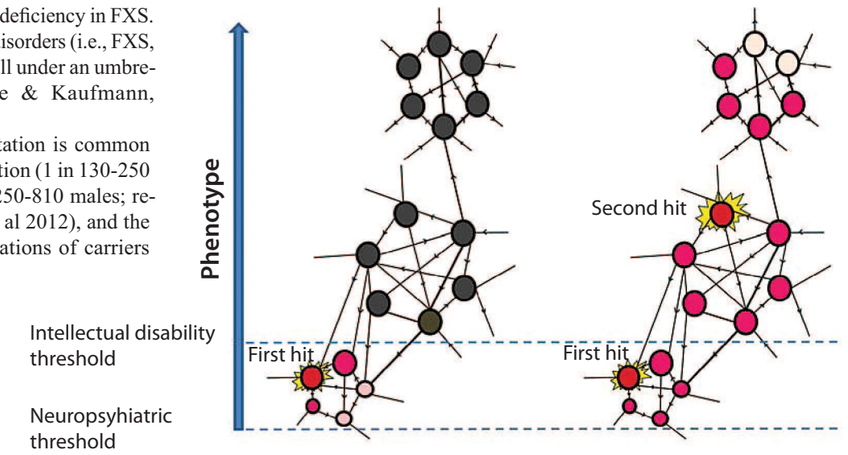


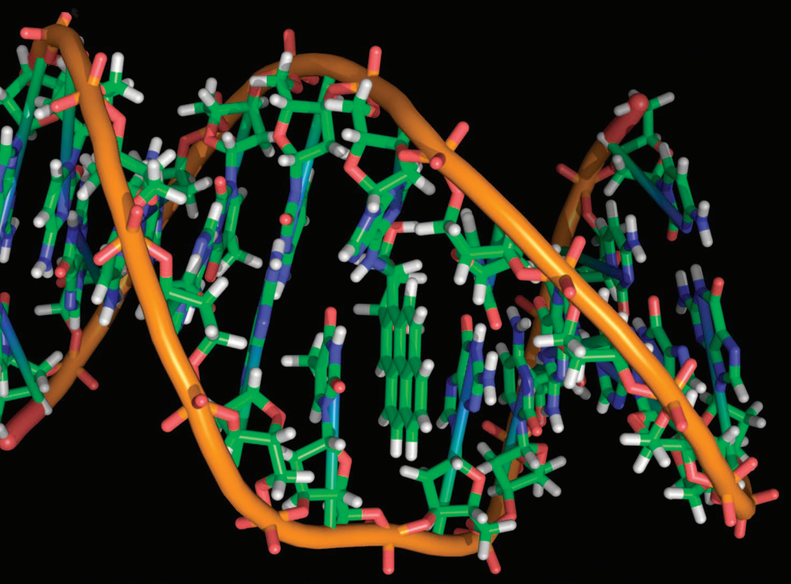
Figure 1 (from Girirajan &Eichler, in HMG, 2010, page R181)

model has been expanded to many other cases with unexplained severe phenotype, especially the ones with low de novo mutation rate (reviewed in Girirajan &Eichler, 2010). In other words, one hit is enough to reach a threshold just sufficient to induce select neuropsychiatric features but a second hit pushes one toward a more severe phenotype with intellectual disability and developmental delay (Figure 1).

Another concept, termed developmental brain dysfunction (DBD), has been used to integrate neurodevelopmental disorders caused by a variety of genetic variants of variable penetrance and expressivity. This conceptual framework has been initially used to describe abnormality of brain function and continuum of developmental disabilities (Capute & Palmer, 1980, Capute 1991). Genome-wide studies have shed some lights into this complexity, and, similar to variably expressed CNVs, have revealed that variants in the same gene might be associated with various neurodevelopmental phenotypes (http://www.nature.com/ng/journal/vaop/ncurrent/full/ng.2711.html). In other words, there is compelling evidence of variability in the clinical presentations of discrete genetic variants and sharing of genetic causes among clinically distinct brain disorders. These frequently co-occurring disorders have overlapping symptoms and often represent a significant challenge for clinicians and researchers (Girirajan et al., 2010; Banka et al., 2011). In light of these challenges and the technological progress, Ledbetter and colleagues have expanded the initial DBD concept to include the common denominator underlying neurodevelopmental, and some neurodegenerative, disorders (reviewed in De-Luca et al., 2013). Namely, the proposed DBD results in clinical manifestations that include the less severe disorders once encompassed by minimal brain dysfunction (Denckla M, 1978) or minimal cerebral dysfunction (i.e., learning disabilities, language disorders, developmental coordination disorder, and ADHD), the more severe classic neurodevelopmental disabilities (eg, ID, ASD), and also at least a subset of neuropsychiatric disorders (eg, schizophrenia and possibly major affective disorders). Both the 'second-hit' and the DBD models captures our hypothesis that the addition of a second genetic hit to the premutation is likely to increase the risk of autism or/and ID due to molecular changes leading to neuronal deficits (i.e., synaptic dysfunction).

Precise genetic origins such as certain CNVs and single-gene mutations (i.e., FMR1 gene) are shared among disorders that are held to be clinically distinct. For example, representing 'disorders of synapse,' an increasingly replicated link between FXS and at least a subset of ASD (Iossifov et al., 2012; Ascano et al., 2012) has been reported wherein FMRP is the critical unifying factor for FX-associated disorders (Sidorov et al., 2013) although its role in FX premutation is not yet delineated. This scientific breakthrough is important due to an enormous phenotypic heterogeneity in idiopathic ASD (Scherer &Dawson, 2011; Betancur, 2011). Nevertheless, the technological genomic advancement (Marshall et al., 2013) has led to recent several reproducible findings as for de novo mutations in idiopathic ASD (Jiang et al., 2013; Sanders et al., 2012; O'Roak et al., 2012; Neale et al., 2012; Iossifov et al., 2012; Michaelson et al., 2012; Kong et al., 2012), which begins to elucidate the genetic pattern of ASD. Specifically, an identifiable genetic etiology is attributable to 5-15% of individuals with ASD, which matches known CNVs changes or single gene disorders (i.e., FMR1 gene mutations). In addition, a recent focus in the field of ASD has yielded discovery of rare but apparently highly expressed de novo or inherited CNVs in upwards of 5-10% of cases with idiopathic ASD. These findings, coupled with genome sequencing data, suggest the existence of hundreds of ASD-risk genes, many of which are involved in synaptic function.

Furthermore, given the well-established role of genetic factors in idiopathic ASD (Zhao et al., 2007; Hallmayer et al., 2011), not only de novo variants but also rare autosomal and X-linked inherited variants in families were examined using whole-genome sequencing (Jiang et al., 2013). Among ASD probands, the authors identified pathogenic de novo mutations in 19% families and X-linked or autosomal inherited alterations in 31% families (some had combinations of mutations), including CAPRIN1 and AFF2 (both linked to FMR1). CAPRIN1, as an RNA-binding protein, may be involved in synaptic plasticity in neurons and cell proliferation and migration in multiple cell types. Moreover, Fatimy et al. (2012) recently showed that CAPRIN1 might modulate FMRP functions as they have in common at least two RNA targets (i.e., CaMKIIα and Map1b mRNAs). CAPRIN1 together with AFF2, a non-syndromal XLMR (FXMR2, Xq28), are also potential candidates for clinical drug trials, involving allosteric modulators of GABA receptors, which have ameliorated autism-like symptoms in mice (Henderson et al., 2012) and humans (Berry-Kravis et al., 2012). Additionally, rare variations in AFF2 could contribute to ASD susceptibility as it also may explain some of the male excess in ASD (Mondal et al., 2012).



The premutation FMR1 gene is typically unmethylated, and the phenotype is usually not associated with ID, but there are around 10% atypical cases. Progress in delineating X-Linked intellectual disability (XLID) has revealed over 150 syndromes, by far the most common of which is FXS, and XLID accounts for 5%–10% of ID in males. Mutations in 102 X-linked genes have been associated with 81 of these XLID syndromes and with 35 of the regionally mapped families with non-syndromal XLID (reviewed in Lubs et al., 2012). The association of ASD with mutations in at least eight of the 102 genes has been reported most frequently in FXS and Rett syndrome but also in disorders resulting from mutations in genes involved in synapse remodeling (NLGN4 and NLGN3) (Jamain et al., 2003; Yan et al., 2005; Darnell et al., in Cell 2011), RPL10, RAB39B, PTCHD1 (Papon et al., 2013), DLG3, the synapse scaffolding (CASK), and MED12. Importantly, NLGN3 (Darnell et al., 2011), DLG3 (http://goo.gl/eMJRBg) and CASK (http://goo.gl/yKYQ0) have several FMRP RNA-binding sites, and they are also linked to non-syndromal XLID. While all these genes affect a wide range of functions, and the cause of the clinical overlap is not clear, a synergistic synaptic pathology might impact the severity of the phenotype. In addition, it is compelling to speculate that even if the FMR1 mutation were not completely inactivating such as in premutation there still could be a synergistic pathology.

In conclusion, there are a variety of reasons for variable expression in premutation carriers and clearly potentially more than one mechanism involved. The level of FMRP may be affected and/or one or more FMRP targets may be also disrupted by a mutation, copy number, or epigenetic change, which could lead to a synergistic synaptic pathology. An additive or synergist effect could then impact the severity of the phenotype. Currently, a key goal in the fragile X field is to identify which proteins are regulated by FMRP and how increases or decreases in these proteins may account for phenotypes of the disorder (Sidorov et al., 2013). Since a comprehensive view of the effects of genetic and pathway aberrations is lacking, in silico tools that estimate the severity of the DNA base changes at the molecular level such as PolyPhen or SIFT, can be useful in stimulating new ideas, illuminating potential connections that are supported by existing evidence, and fostering clinical research. That said, it is also critical not to over interpret such predictive, and unvalidated, results. Future studies, including those that integrate a holistic molecular understanding of the interplay and consequences of FMR1 DNA, mRNA, and FMRP in context with detailed patient phenotypes, should help further advance understanding of likely complex mechanism(s) involved that underlies variable expression in premutation carriers.

Note: Relevant to this article, Dr Budimirovic is a co-author of a recent peer-reviewed article from a group of authors in the field titled 'Are genomic studies necessary in autism or neurological deficits of Fragile X premutation carriers?' (in-press). The group has presented at 1st International Conference on 'the FMR1 Premutation: Basic Mechanisms and Clinical Involvement' that was held in June of 2013, Perugia, Italy. Dr Budimirovic's oral presentation was titled 'High Resolution FMR1 Genetic and Epigenetic Molecular Assessments in a Well Characterized Cohort of Full Mutation and Premutation Fragile X Patients.'

Dejan B. Budimirovic, MD,
Medical Director, Fragile X Clinic, KKI, JHMI
Attending Child Neuropsychiatrist, KKI, JHMI
Co-Investigator physician, Clinical Trials Unit, KKI, JHMI
Assistant Professor of Psychiatry & Behavioral Sciences,
Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Fragile X: From Neurodevelopmental to Neurodegenerative Disorders

Chair: Randi J. Hagerman, MD, Medical Director of the M.I.N.D. Institute
Endowed Chair in Fragile X Research, UC Davis Health System, Sacramento, CA
Presenters: Randi Hagerman MD, Gary Latham PhD, Dejan Budimirovic MD, Craig Erickson, MD

Gary J. Latham, PhD
Vice President, Research
and Technology Development
Asuragen, Inc.

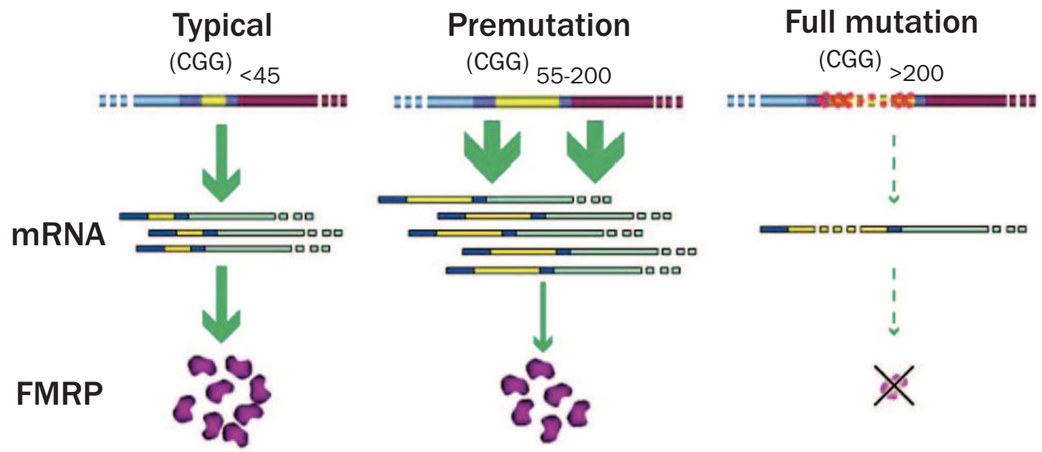
Craig Erickson, M.D.,
Assistant Professor of Psychiatry,
Cincinnati Children's Hospital
Medical Center,
Cincinnati, Ohio

Symposium titled 'Illuminating the fragile X spectrum of disease through molecular, clinical, and therapeutic advances' organized by Dr Budimirovic for American Academy of Child and Adolescent Psychiatry Annual 2014 Meeting.

Category: Genetics/Molecular Biology

The objectives of the symposium are to illuminate molecular, clinical and targeted treatment advances in fragile-X associated disorders (FXD) that are caused by premutation and full-mutation expansions in the fragile X mental retardation 1 (*FMR1*) gene. We seek to describe the following: (i) fragile X premutation-associated disorders in both development and aging (ii) recent advances in PCR that allow comprehensive molecular assessments of the FMR1 gene, and expand the types of specimens that can be analyzed, (iii) molecular-clinical profiles in a cohort of fragile X expansions patients of relevance to *FMR1* as a diagnostic and therapeutic marker, and (iv) the function of two potential mediators of dendritic deficits in the fragile X full-mutation.

FXD are caused by two types of CGG repeat expansions within the *FMR1* gene. First, in fragile X syndrome (FXS), the causal defect is epigenetic silencing of the *FMR1* gene and the consequent absence of FMRP (*FMR1* protein) due to large CGG expansions (>200 repeats; full-mutation). FMRP functions as a translational repressor ('brake') interacting with hundreds of mRNA transcripts, including ones for many genes implicated in autism spectrum disorder (ASD) (Ascano et al., 2012; Iossifov et al., 2012). As FMRP is important for proper synapse development and function, its absence causes intellectual disability and ASD. Second, the *FMR1* gene remains fully active in premutation expansions (55-200 repeats) but demonstrates increased *FMR1* mRNA production (Bailey et al., 2008; Chonchaiya et al., 2012; Hagerman and Hagerman, 2013).



From Hagerman et al. Pediatrics 123:378-90, 2009

It is noteworthy that the premutation-associated disorders arise through different pathogenic mechanisms than FXS. Namely, they are clinically apparent at opposite ends of the age range, and arise through different pathogenic mechanisms (i.e., gene silencing in FXS versus RNA toxicity in the premutation). The fragile X premutation is common in the general population (1 per 130-250 women and 1 per 250 - 810 men) (Tassone et al., 2012). After the discovery of *FMR*1 in 1991, premutation carriers were considered non-penetrant or unaffected. However, it was subsequently discovered that the premutation was a common cause of early ovarian failure (menopause before age 40), later named fragile X-associated primary ovarian insufficiency (FXPOI). In the 1990s, some children with the premutation were found to have developmental problems including ADHD, ASD and intellectual disability in addition to lowered levels of FMRP. In 2000 Tassone and colleagues reported elevated levels of *FMR1* mRNA ranging from 2 to 10 times normal depending on the number of CGG repeats. Subsequent to this discovery was the identification of older premutation carriers who suffered from an intention tremor, cerebellar ataxia, neuropathy and cognitive decline, sometimes leading to dementia. This was named the fragile X-associated tremor ataxia syndrome (FXTAS). While excessive RNA xpresion may well be the toxic element in FXTAS, Todd et al. (2013) recently showed that the accumulation of polyG in the ubitquitin-positive inclusions as the characteristic of FXTAS is linked to RNA toxicity as well. Clinically, FXTAS occur in approximately 40% of male carriers and 16% of female carriers with an average age of onset at 62 years (Jacquemont et al., 2004; Coffey et al., 2008). Dr Hagerman and her group leads the field and has published extensively as they, and others, have identified a variety of medical and psychiatric problems associated with the premutation either with or without FXTAS in controlled studies. To summarize, significant differences were found in prevalence for depression (in approximately 40% of carriers; 60% of those with FXTAS) and anxiety in 50 % of carriers with FXTAS (Bourgeois et al., 2009), migraines in 45% of females and 30% of male carriers, restless legs syndrome in 30% of carriers, hypertension in 50 to 60 % of carriers, fibromyalgia in 40% of women with FXTAS, hypothyroidism in 50% of women with FXTAS and sleep apnea is 35% of men with FXTAS. There is also a higher incidence of seizures in carriers, and for those with seizures the rate of intellectual disability and ASD is increased in carrier boys compared to their normal siblings. ADHD is also increased compared to controls in boys with the premutation compared to controls and also in adults who are carriers compared to controls. The premutation can also lead to neuroradiological chances including an increase in DTI FA changes versus controls in specific tracts and volumetric changes in carriers, particularly in the cerebellum and brainstem before the onset of FXTAS versus controls. It is important to note that involvement from the full mutation is related to deficits in FMRP and involvement in those with the premutation relates to the toxic effects of elevated *FMR1* mRNA and this is sometimes combined with a mild deficit of FMRP, particularly for those in the upper range of the premutation range (Hagerman and Hagerman 2013).

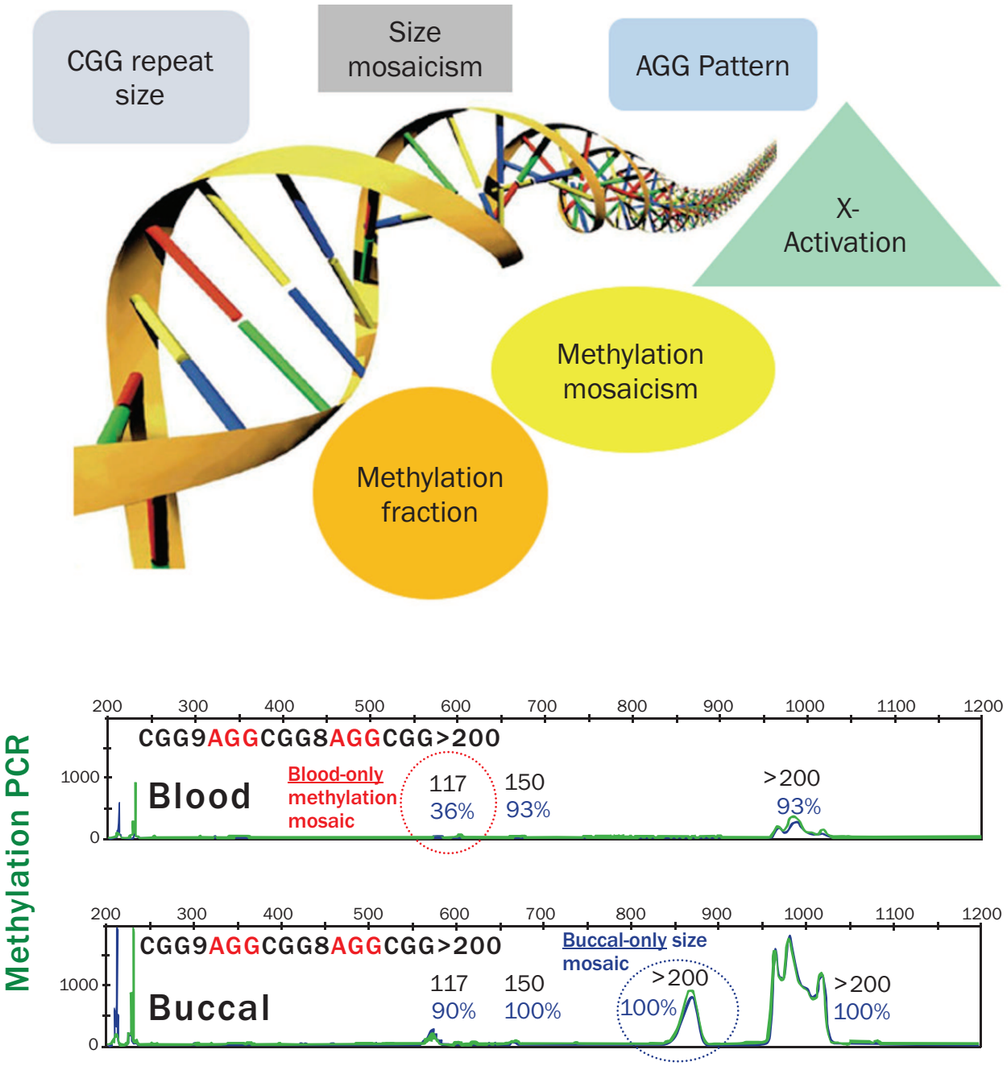
ASD is an enormous and growing public health (CDC, 2012) and economic (Levelle et al., 2014) challenge. This is a heterogeneous, behaviorally-defined group of disorders of mostly unknown etiology. Treatments targeting the core deficits in ASD are lacking. Thus, current research is focused on identifying shared pathways and common therapeutic targets among patients with causal genetic defects such as FXD, and, in particular FXS. It may be helpful to think of ASD as a "cloud" which represents a final common pathway of abnormal patterns of brain wiring. The cloud contains a common set of behavioral characteristics that are core features of ASD:

social-communication and interaction deficits and restricted and repetitive patterns of behaviors. Individuals with ASD and FXS represent a spot in the cloud where an individual meets criteria for ASD with higher social anxiety, hyperarousal, and other FXS-related differences. Owing to the prevalence of ASD in FXS and its shared neurophysiology with ASD, FXS has been extensively studied as a model for ASD (Budimirovic & Kaufmann, 2011), and offers a new hope to translate into rational therapy (Hagerman et al., 2009). The deficits in FMRP lead to upregulation of the mGluR5 and downregulation of the GABA systems, respectively, resulting in an excitatory-inhibitory imbalance. While the mediators of dendritic pathology in FXS include abnormalities in dendritic spins they remain inadequately understood. To date, rescue of the dendritic pathology of FXS has largely centered on use of mGluR5 antagonists (Gantois et al., 2013) and GABA-B agonists (Henderson et al. 2012). Other molecular systems in FXS that carry such potential as they also mediate FMRP expression are brain derived neurotrophic factor (BDNF) (Lauterborn et al., 2007) and secreted amyloid precursor protein alpha (sAPPα). In light of the progress with fragile X targeted therapeutics (Jacquemont et al., 2011; Berry-Kravis et al. 2012), further molecular-clinical studies are needed to understand the full value of *FMR1* as a diagnostic and therapeutic marker. This is possible only with innovative assays in the FMR1 gene diagnostics (Chen et al., 2010; 2011) as well as to further investigate other mediators of the dendritic dysregulation in FXS with profiling at baseline which persons with FXS may best respond to a particular treatment (Erickson and Wink, 2013); both aim to develop personalized medicine options for this disorder.

This symposium tackles recent molecular, clinical, and targeted therapeutics advances in FXD in humans, and particularly FXS as one of the 'autisms.' Presenters will review mostly the preliminary data on the molecular, diagnostics advanced methodology, genotype-phenotype profiles, and targeted treatments in the field. Dr Hagerman, the chair, will briefly introduce the topic, and then present molecular and clinical data on a rapidly emerging field of fragile X premutation-associated disorders in both development and aging. Multiple studies will be reviewed that are associated with a variety of psychiatric and neurological phenotypes, including ASD. Historically, molecular analysis of *FMR1* gene has been limited by difficult-to-amplify repeats sequences. Dr Latham will describe the emergence of rapid, information-rich fragile X PCR diagnostic methods and how they can enable improved patient-centered specimen collections across multiple *FMR1* markers. Using the same PCR methods, Dr Budimirovic will present preliminary molecular-clinical profiles on 19 subjects as for of relevance to understanding the full value of *FMR1* as a diagnostic and therapeutic marker. These preliminary data underscore the potential of highly sensitive and quantitative assays to inform genotype/phenotype linkages in patients with a range of genetic, cognitive, and behavioral characteristics. In particular, the observed differences in molecular profiles between mesodermal (venous blood) and ectodermal (buccal cell) lineages deserve further study. Below is an example of a full-mutation individual mosaic with fully a methylated full-mutation but differences in repeat length and methylation mosaicism between buccal and blood cell types. It will be important to understand significant which of these molecular profiles may be most clinically relevant.

In addition to a larger sample size, improved assays such as methyl-Seq and FMRP (LaFauci et al., 2013) that are being introduced in these studies are expected to provide a much more enlightened opportunity to find meaningful distinctions between ASD and non-ASD individuals of relevance for targeted therapeutics (Jacquemont et al., 2011). In the same line that the impact of deficient FMRP is likely wide and far reaching, Dr Erickson`s initial work with BDNF and sAPPα points to these two additional potential molecular systems that exhibit dysregulation in FXS. As a contributor to this pathophysiology, such preliminary data in 10 youths with FXS favors excessive sAPPα in the patients with FXS with autism compared to those with idiopathic autism versus potential overactivity of BDNF compared to matched neurotypical controls. Dr Erickson's group is currently working to confirm these finding in the *FMR1* KO mouse model. These initial findings and this line of research also hold promise (i) to guide therapeutics development as modulation of BDNF and/or APP derivatives maybe possible with targeted pharmacotherapy in FXS (Erickson, Wink et al. 2013), and (ii) that one or both of these markers may help to profile in the future which persons with FXS may best respond to a particular treatment.

In conclusion, these four studies demonstrate a significant progress in the fragile X translational model 'success story.' It begin to address the methodology, phenotyping gaps of relevance not only to fragile X CNS mechanisms targeted therapeutics but also to a potentially broader psychiatric spectrum of disorders, including ASD. Progress in basic neuroscience has led to discovery of molecular targets for treatment in FXS and other neurodevelopmental disorders; however, there is a gap in translation to targeted therapies in humans (Jacquemont et al., 2013; Berry-Kravis et al. 2013). It is important to underscore that the fragile X premutation and full-mutation are clinically apparent at opposite ends of the age range, and arise through different pathogenic mechanisms. Moreover, a rapid progress in understanding the *FMR1* premutation phenotype has left many clinicians unaware of the stark distinction between FXS (neurodevelopmental) and FXTAS (neurodegenerative) disorders, respectively (Hagerman and Hagerman 2013). The premutation can lead to medical and psychiatric problems throughout life related to RNA toxicity effects in the CNS, which are different pathogenic mechanisms than gene silencing in FXS. Radiological changes on DTI and volumetric studies can be seen in midlife well before the onset of FXTAS. Treatment recommendations can be made once a diagnosis of the premutation is made.



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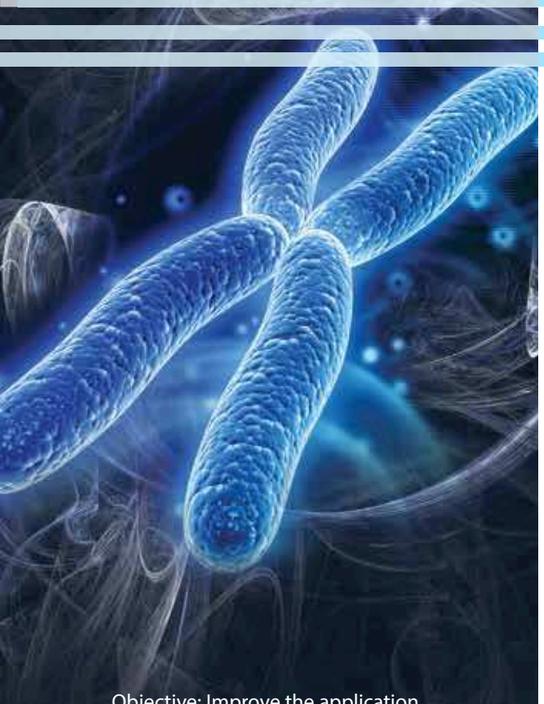
Dejan B. Budimirovic, MD,
Assistant Professor and Director
Co-Investigator Physician, Clinical Trials Unit
Kennedy Krieger Institute, The Johns Hopkins Medical Institutions
Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Stratification of fragile X syndrome and autism patients using tandem DNA and protein assays that can identify prognostic clinical features and help individualize therapy options

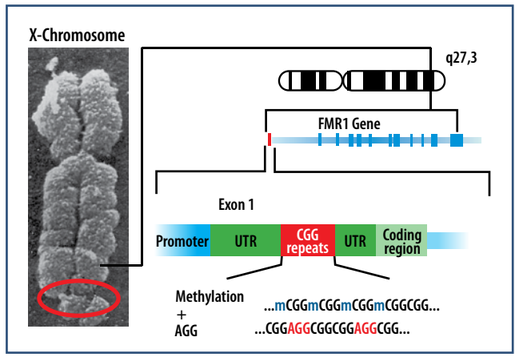


Objective: Improve the application of existing and emerging treatment options for fragile X syndrome (FXS) with and without autism patients using comprehensive molecular assays for the Fragile X Mental Retardation (*FMR1*) gene and its encoded fragile X mental retardation protein (FMRP).

Hypothesis: A novel, quantitative FMRP (qFMRP) assay in combination with next generation FMR1 DNA molecular assays will differentiate neurobehavioral profiles and clinical diagnoses/severity in patient friendly specimens from individuals with FXS.

Background

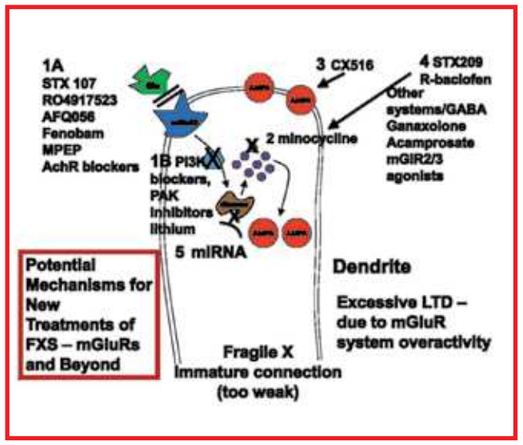
Clinical significance of FXS and related disorders: FXS is the best-understood single cause associated with autism spectrum disorder (ASD) that accounts for up to 5% of all cases of ASD [1]. FXS is caused by a full-mutation (FM, >200 CGG repeats) and epigenetic silencing of the *FMR1* gene, which results in a loss of its encoded protein (no FMRP) [2].



One gene, many markers. An expansion of the number of CGG nucleotide repeats in the FMR1 gene gives the appearance chromosome can easily break (red oval). This region is responsible for FXS and othe FXassociated disorders due to effects of the CGG repeats, AGG interruptions, and (hyper)methylation.

It is a genetic-medical diagnosis, unlike ASD which is a behaviorally-defined diagnosis. Clinically, individuals with FXS have a wide array of impairments in skills (i.e., intellectual disability-ID) [1] and behaviors that can include many features of syndromic ASD (refers to ASD in FXS), such as deficits in social interaction and communication (e.g., eye contact, peer relationships, social withdrawal-SW) [3], and restricted and repetitive behaviors [1, 3]. Moreover, two out of three boys with FXS meet criteria for a diagnosis of the causal ASD [1]. There are also evidence for existence of neurobehavioral subgroups in FXS based on whether individuals met criteria for ASD [4, 5], including subgroups based on severity of SW set of behaviors as a unifying factor of ASD and anxiety [6]. Indeed, the *FMR1* FM confers an especially high risk for anxiety disorders compared to general ID [7]. Yet, to date, there has been little work suggesting the existence of separable neurobiological phenotypes within FXS. Only two studies suggest that FXS may lack homogeneity at the neurobiological level despite arising from the single gene mutation [8, 9]. The work outlined here may identify endophenotypic subgroups within FXS through a model of molecular-behavioral stratification and may offer insights to understand the biological basis of neuropsychiatric pathology (e.g., the RDoC approach articulated by NIMH:http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml),including in individuals with ASD in FXS (https://www.grc.org/programs.aspx?year=2014&program=fragilex).

***FMR1* molecular diagnosis and new PCR technologies:** Advances in molecular methods have enabled the assessment of the degree of CGG expansion and promoter methylation as an aid in diagnosis of FXS. Asuragen offers a very successful suite of products under the AmplideX® and Xpansion Interpreter® *FMR1* methylation status (mPCR) brands. Nevertheless, the ability of these assays to predict the severity of clinical phenotypes is limited by the many variables that influence *FMR1* translational output: size and methylation mosaicism, X-chromosome inactivation, and other factors that regulate *FMR1*. Therefore, an important advance in the field is the development of an assay to reliably quantify FMRP [10]. By quantifying FMRP (qFMRP)—the functional endpoint of *FMR1* gene expression—a more comprehensive understanding of FXS biology may help improve the diagnosis, prognosis, and treatment options for affected individuals. Furthermore, the mPCR advanced method allows quantifying the spectrum of methylation characteristics in patients with *FMR1* expansions [11]. This is important as somatic mosaicism is well documented in FXS and this heterogeneity can confound molecular [11] and clinical interpretations [1].



FXS as a model for ASD, from biology to clinical management: Despite an early controversy, it is now clear that there is not only the clinical overlap between FXS and ASD [3] but also a substantial overlap in the molecular pathology of the two disorders [12]. FMRP is ubiquitously expressed in the brain and other tissues and interacts with about 4% of total mammalian brain mRNAs [13, 14], thus, regulates the levels of many important proteins involved with brain connections.

Since FMRP normally acts as a "brake" on protein synthesis in neural dendrites, the hallmark effects of the FMRP deficit are increased dendritic protein synthesis [12, 15] due to an overactive glutamatergic signaling mediated up-regulation in mGluR5 and down-regulation in GABA synaptic receptors [14, 16]. Many of the proteins involved in signaling pathways that regulate protein synthesis at brain synapses and interact with FMRP are associated with ASD in FXS [13, 14]. In addition, many of the proteins regulated by FMRP have been found to be associated with idiopathic ASD [17, 18]. Further, low FMRP levels have been documented in research samples of individuals with ASD in FXS [14, 19], and idiopathic

ASD [20]. Thus, deficits in FMRP seem to be the critical unifying factor linked, at a molecular and synaptic level [21], to dysfunction in brain pathways and links that lead to behavioral symptoms of ASD.

Emerging targeted therapies for FXS and ASD: A wide range of social deficits and maladaptive behaviors is common in individuals in FXS with and without ASD [1]. It causes enormous impairments in these individuals and their family's normal day-to-day function. FXS-nonspecific psychotropic drugs are often used to target different symptom clusters but only with partial benefits [1]. FXS-specific molecular modifying treatments present as a solution as they show potential to modify core social-communication and other behaviors in FXS with ASD [22]. Compelling evidence emerged that modification of the FMRP-deficit driven dysregulation of mGluR5 and GABA receptors in the *FMR1* knock-out animal model reversed the underlying pathophysiology of FXS. Specifically, several studies that used GABA-B receptor agonist STX209 [23] and mGluR5 antagonists [24, 25] rescued the FMRP-deficit caused protein excess in these mice.

Importantly, several phase 2 and 3 clinical trials showed benefits in social and other behaviors in individuals with ASD in FXS using a subtype-selective mGluR5 antagonist AFQ056 [9] and GABA-B agonist STX209 [26].

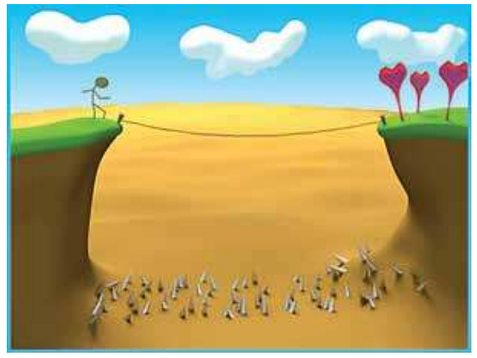
Furthermore, molecular characterization of epigenetic (full-methylation) patterns in ASD and FXS [9] has suggested that methylation status may constitute a treatment-sensitive biomarker for predicting response to a mGluR5 inhibitor [27, 28]. As noted earlier, this work is important to delineate neurobiological phenotypes within FXS. Yet, these clinical trials have also highlighted several challenges such as the populations heterogeneity, the lack of specific and sensitive outcome measures capturing the full range of improvements of patients with FXS, and a lack of reliable biomarkers that can track whether a specific mechanism is responsive to a new drug within relatively short period of time and whether the response correlates with clinical improvement [28].

Expected scientific impact of using the *FMR1* molecular diagnosis and new PCR technologies. The qFMRP assay [10] provides a reliable method for FMPR quantitation and is under development at Asuragen. A significant impact is expected as for subgrouping individuals with FXS through molecular-behavioral subphenotyping of relevance to clinical trials [27,28] as supported by the epigenetic pattern-biomarker to guide treatment in FXS with ASD [9]. FMRP data in males with FXS has significantly correlated with IQ, adaptive behavior, and possibly ASD [19, 29]. Moreover, a standardization of qFMRP application may further help patient stratification (endophenotyping) and advance clinical trials outcomes of multiple targeted therapies for syndromic ASD and possibly in idiopathic ASD. The latter is known to result from a variety of causes, and represents an enormous and growing public health issue. The aforementioned clinical trial studies have had promising results which offers new hope for rational therapy. This molecular-behavioral stratification approach may bode well with imaging-behavioral subtyping in which researchers found low and high functioning biologically and clinically separable neuro-phenotypes in FXS [8].

Next, buccal cells may be a better surrogate for brain cells than blood as they are closer in origin to ectodermal-derived neurological tissue than mesodermal blood [31]. This sampling approach also offers several patient-friendlier advantages over venipuncture, including convenience and decreased anxiety in patients. Moreover, such convenience may also enable better compliance and earlier diagnosis in FXS [33] and in idiopathic ASD [34]. An earlier age identification of the individuals with FXS with and without ASD and idiopathic ASD would typically lead to the initiation of early intervention services to treat these conditions. *Importantly, multiple candidates of targeted therapies for fragile X and idiopathic ASD that are in clinical trials may be more effective if administered as early as possible.* Together, this would lead to an improved outcome and the quality of life for these individuals and their families.

Lack of Models for Trials in FXS

- Before 2002 lack of significant moderate\large clinical trial experience in FXS with any "standard" drug
- No defined measure of behavioral improvement
- No "gold standard" outcome measure
- No template from any developmental disability about measuring cognitive outcomes when attempting to treat underlying disorder



Basic science has targets but mismatch in trial design/outcome measure development

In summary, testing FMRP levels in clinical specimens with the new qFMRP assay is one of cutting edge predictors of the molecular-behavioral stratification in FXS of relevance to FX-targeted clinical trials [27, 28]. This is important for individuals with FXS with and without ASD as well as non-FXS disorders because abnormally low FMRP levels have also been linked to non-FXS disorders in those with a normal *FMR1* genotype (i.e., major depression and bipolar disorder [34, 35], and schizophrenia [36]). These findings may support the use of the qFMRP test for much broader clinical applications other than just fragile X disorders.

Note: This text is an exert from Dr Dejan B. Budimirovic's recent application through the Johns Hopkins Medicine Brain Science Institute's Synergy grant mechanism. A collaboration of Drs. Marco Grados (The Johns Hopkins Hospital), Budimirovic (Kennedy Krieger Institute, the Johns Hopkins Medical Institutions) and Gary Latham (Asuragen, Inc.) proposed in this application is expected to grow into a larger group of studies (behavioral phenotype - biomarker studies) that will also include researchers in other genetic disorders.

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Abstracts:

Thursday, 19 June

Medical Genetics
Venue: Amphitheatre, University Children's Hospital, Tirsova 10

11.20 – 11.40

Title:

Newborn Screening – Current Status and Future Prospective

Presenter: Dimitar Gavrilov, M.D., Ph.D., Mayo Clinic, Rochester, MN, USA

Assistant Professor of Laboratory Medicine & Pathology - Mayo Clinic College of Medicine

Consultant (Joint Appointment) - Department of Medical Genetics, Mayo Clinic, Rochester, Minnesota

Consultant - Division of Laboratory Genetics, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Thursday, June 19, 2014

0800 - 1630

Pre-Conference Workshop
Venue 1: Main Conference Hall, Medical School, Belgrade
Includes Dean and ViceDean of MedSchool Belgrade address

CanMEDS & Residency Training: Impacting the Care of Patients (Royal College of Surgeons and Physicians of Canada)
Venue: Main Conference Hall, Medical School, Belgrade University, Dr Subotica 8

Facilitators: Dr. Goran Popovic Dr. Jerry Maniate

Learning Objectives:
At the end of this workshop, the participants will be able to:

① Define competency-based education
② Describe the CanMEDS Physician Competency Framework and outline its origins and rationale
③Understand the CanMEDS roles and how to relate it to daily practice
④Describe the competencies of the each Intrinsic CanMEDS Role
⑤Describe one teaching method for Intrinsic CanMEDS Role

Thursday, June 19, 2014

1100 – 1630

Medical Genetics
Venue: Amphitheatre, University Children's Hospital, Tirsova 10

Moderators: Mira Irons, Dusica Babovic, Goran Cuturilo

Learning Objectives:
At the end of this symposium, the participants will be able to:

① Understand the value of newborn screening programs and recognize acute presentation of metabolic disorders
② Know how to evaluate patients with suspected mitochondrial disease
③ Describe pediatric tumor-related syndromes
④ Understand advantages and limitations of new diagnostic methods in genetic testing (chromosome microarrays, next generation sequencing etc)
⑤ Familiarise with a novel high-resolution PCR technology (Amplidex) that allows understanding of genotype-genotype correlation in Fragile X-associated disorders

Newborn screening is a public health program aimed at conditions for which early intervention can prevent mortality, morbidity and disabilities. Over the past 50 years newborn screening evolved from "Guthrie test" for phenylketonuria to the current status when up to 31 core conditions and 25 secondary targets are included in the recommended panel of conditions to be screened in USA.

Several different methodological approaches are currently applied in newborn screening including enzymatic, MS/MS, immunologic and DNA based methods. To further improve the sensitivity and specificity and to reduce the number of false positive results, second tier testing for specific analytes and post-analytical tools have been developed and utilized.

Extensive work has been done to broaden the range of conditions to be screened and several new groups of diseases have been or soon will be added as targets for newborn screening.

Thursday, 19 June

Medical Genetics
Venue: Amphitheatre, University Children's Hospital, Tirsova 10

12.00 – 12.20

Title:

High Resolution FMR1 Molecular Assessments in a Cohort of Fragile X Full- and Premutation Patients.

Presenter: Dejan B. Budimirovic, MD, Medical Director, Fragile X Clinic, KKI, JHMI

Fragile X Clinical & Research Consortium

Attending Child Neuropsychiatrist, KKI, JHMI

Sub-Investigator Physician, Clinical Trials Unit, KKI, JHMI

Assistant Professor of Psychiatry & Behavioral Sciences,

Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Objective: To identify molecular-clinical profiles in a cohort of patients with fragile-X expansion mutations of relevance to autism spectrum disorder.

Background: Syndromic autism spectrum disorder (ASD) is common in males with an FMR1 full mutation (FM), but the premutation (PM) males may also represent a significant form of FMR1 in ASD. In light of the progress with fragile X targeted therapeutics and regarding FMR1's role as a diagnostic and therapeutic marker, we investigated a cohort of FM and PM patients for high resolution molecular testing using a panel of next generation PCR assays.

Methods: The clinical cohort (n=19, ongoing recruitment) was randomly selected from the Kennedy Krieger Institute's Fragile X Clinic. Clinical assessments and standardized measures of variety of skills (i.e., cognitive, adaptive, language) and autistic behavior (ADOS) were supplemented with parent rating scales. Genomic DNA was isolated from three collections per patient (EDTA blood, blood spotted on FTA cards, and buccal swabs). AmplideX(r) FMR1 PCR technologies (Asuragen) were utilized to report CGG repeat sizing, size mosaicism, X-activation, methylation status and AGG interruption patterns for each collection type.

Results: To date, 16 males, 3 females have been enrolled in the study. Out of ten (56%) individuals with ASD, 8 (44%) of them had DSM-IV diagnosis of autism, and three (12%) had PDD-NOS. All of the males and two females had an FMR1 FM, whereas one female had a PM. More than half (53%) of FM patients had at least one AGG interspersion, and >70% revealed some level of PM size mosaicism. Although AGG interruption patterns and methylation of the primary expanded allele was conserved across blood and buccal cell collections from the same patient, differences in repeat size and/or methylation mosaicism was evident in several cases.

Conclusions: We present pilot data of neurobehavioral assessments from a well characterized cohort representing clinically relevant FMR1 expansions. Our preliminary data underscore the meaningful potential of highly sensitive and quantitative assays to inform genotype/phenotype linkages in patients with a range of genetic, cognitive, and behavioral characteristics. In particular, the observed differences in molecular profiles between mesodermal (venous blood) and ectodermal (buccal cell) lineages deserve further study.

Thursday, 19 June

Medical Genetics
Venue: Amphitheatre, University Children's Hospital, Tirsova 10

14.20 – 14.40

Title:

Cancer Genetics in pediatric practice

Presenter: Janice Zunich, M.D.
Clinical Associate Professor, Department of Medical Genetics, Indiana University School of Medicine, Northwest Center for Medical Education, Gary, Indiana

Director, Genetics Center, Northwest Center for Medical Education, Gary, Indiana

Consulting Staff: Porter Memorial Hospital, Valparaiso, Indiana; St. Anthony Medical Center, Crown Point, Indiana; Community Hospital, Munster, Indiana; The Methodist Hospitals, Gary/Merrillville, Indiana; St. Margaret Hospital, Hammond, Indiana; LaPorte Hospital, LaPorte, Indiana; St. Mary Medical Center, Hobart, Indiana; Memorial Hospital, South Bend, IN, USA

Hereditary childhood tumors, such as retinoblastoma and Wilms tumor, are familiar to pediatricians. Less well known, however, may be hereditary cancer syndromes commonly considered adult conditions, such as Familial Adenomatous Polyposis, Li-Fraumeni syndrome and Familial Medullary Thyroid Carcinoma, which can have significant consequences for the pediatric population. Similarly, there are pediatric conditions, including Fanconi and Bannayan-Riley-Ruvalcaba syndrome, which can affect recommendations for cancer screening and surveillance in adult relatives.

These conditions and their implications for both the pediatric and adult population will be reviewed.

This text is an expanded version of an invited lecture by Dejan B. Budimirovic, M.D., to the Maryland Council of Child and Adolescent Psychiatry on November 12, 2004, in Baltimore, Maryland, USA. Dr Budimirovic is a member-at-large of the Council's Executive Committee.

Exclusive: USA´s science and medicine

Claudia Tan, an undergraduate premedical third year student studying Neuroscience and Psychology at the Homewood college campus of the Johns Hopkins University in Baltimore has edited and proofread this text. In fall of 2011, Dr Budimirovic has established the spring/fall semester Clinical practicum ("clinical shadowing") titled "Autism Spectrum Disorder: Basic Behavioral Phenotypes" for the Homewood students. To date, 12 students have completed the ongoing and well accepted practicum.

Fragile X-associated Disorders: New Insights into Targeted Therapeutics Clinical Trials in Fragile X and Autism Spectrum Disorder

Dejan Budimirovic, M.D.

Fragile X-associated Disorders are caused by a CGG repeat length expansion of the Fragile X Mental Retardation 1 (*FMR1*) gene on the X chromosome. The triplet repeat length (normal 30-45) ranges from premutation or 'carriers' (PM; 55-200) to full mutation (FM, >200). Fragile X syndrome (FXS) is the prototype of the disorders and affects 1:4,000 males and 1:6,000 females across all racial and ethnic groups (Sherman, 2002). Moreover, it is estimated that over one million individuals in the US and around 20 millions worldwide are fragile X carriers. There are two different, distinct pathophysiological mechanisms underlying PM and FM. In PM, an excess of mRNA accumulates and becomes toxic to the cell whereas in FM an epigenetic silencing of the gene causes lack of the gene's encoding protein, namely fragile X mental retardation protein (FMRP) (Hagerman et al., 2009). The most frequent clinically relevant situation involves a mother with PM and her son with FM (Hagerman et al., 2009). Premutation, which is about 10 times more common than FM, also puts some individuals at a higher risk for neurological disorders such as Fragile X-associated Tremor Ataxia Syndrome (FXTAS) (adult males) or gynecological Fragile X-associated Primary Ovarian Insufficiency (FXPOI) or 'early menopause' (adult females) (Hagerman and Hagerman, 2013). In FM, the *FMR1* gene is 'shut-down' (no FMRP) by an epigenetic (hypermethylation) mechanism, which leads to FXS at a very early age. That is to say that fragile X-associated disorder represents both neurodevelopmental (FXS) and neurodegenerative (FXTAS) disorders across the wide range lifespan. FMRP is found in nearly all cell types, particularly in dendritic synapses of the neurons, and regulates up to 4% of total RNA. Thus, FXS is associated with a wide array of physical and neurobehavioral problems (Kaufmann & Reiss, 1999; Hagerman, 2002; Hagerman et al., 2009) especially in males.

Neurobehavioral features of FXS consist of variable cognitive and language impairments as well as associated neurobehavioral problems (i.e., attentional difficulties, hyperactivity, anxiety, and autistic features). Social interaction disorders in FXS are autism spectrum disorder (ASD) and social anxiety. They are the most prevalent, severe, and highly debilitating phenotypes from both phenomenological (Brodkin, 2008) and therapeutic perspectives of FXS (Hagerman et al., 2009). Together, they constitute the major medical and educational concerns for patients with FXS (Kaufmann and Reiss, 1999; Hagerman RJ, 2002), especially in affected males. Typically, physicians may not consider FXS without a family history of intellectual disability or other dysmorphic features. However, these features are not present in approximately one third of individuals with FXS. Moreover, in contrast to some other genetic disorders (i.e., Down syndrome), FXS has no apparent physical features at birth. Therefore, it must be "detected" after atypical behaviors and delays in skill acquisition begin to emerge (Marschik et al., 2012; Hinton et al., 2013). Nevertheless, one survey revealed that almost 38% of parents of children eventually diagnosed with FXS underwent more than 10 symptom-related visits to their health care professional before the *FMR1* diagnostic test was ordered (Kemper and Bailey, 2009). The Fragile X Clinical & Research Consortium (FXCRC) has specific guidelines that begin with care by a physician-led team with expertise in fragile X-associated disorders. Guidance statements from professional organizations emphasize the need for fragile X testing in individuals with ASD. However, general clinical practice and available literature reveal that only one third (McLennan et al., 2008) of individuals with ASD are tested for *FMR1* mutations. The average age of diagnosis of FXS is 35 to 37 months (Bailey et al., 2009). Effectiveness of healthcare in this population has been hampered by the delay in diagnosis of idiopathic ASD (average age ~6) and delay in time from recognition to expert evaluation. The continued emphasis on early diagnosis and management, in conjunction with the identification of family members at risk for or affected by *FMR1* mutations, has led to intense discussion about the appropriate timing for early identification of *FMR1* mutations (Abrams et al., 2012). In addition, families are often confused by the relationship between FXS and ASD as it is not uncommon for a child to initially be diagnosed with ASD and later to receive an *additional* diagnosis of FXS. Regardless, these children also have ASD but its (genetic and epigenetic) cause is known.

Studies of FMRP continue to reveal relevant information as methodology improves. FMRP belongs to the family of RNA binding proteins (RBPs) and normally regulates translation (acts as a 'brake') in response to glutamate (i.e., mGluR5) signaling. FMRP and mGluR5 work in functional opposition (Bear et al., 2004; Bagni et al., 2012), and hallmark effects of *FMR1* silencing (no FMRP) are overactive glutamatergic signaling (the mGluR theory) (Bear et al., 2004). Indeed, while neurobiological studies in humans with FXS are scarce, available autopsy studies of FXS, and ASD, demonstrate increased amount of metabotropic glutamate receptors subtype 5 (mGluR5) in the frontal cortex, and a decrease in the inhibiting neurotransmitter GABA. Cytoplasmic FMRP Interacting Protein (CYFIP) family (Abekhoukh & Bardoni, 2014) and mTOR are up-regulated. Together, the lack of FMRP leads to increased dendritic protein synthesis and increased density of dendritic spines ("neuronal connections") (Kaufmann

Claudia Tan, JHU Class 2016

My name is Claudia Tan. I am currently an undergraduate premedical third year student studying Neuroscience and Psychology at the Homewood campus of the Johns Hopkins University in Baltimore, Maryland. I aspire to be a physician, and I have had the opportunity to take part in a Medical Tutorial this semester.

The Homewood Pre-professional Office together with the Johns Hopkins School of Medicine offer Medical Tutorials to select undergraduate students to take part in a one-semester program in which students work with a physician. They range anywhere from practicums, laboratory research, clinical research, to clinical shadowing and are a way for students to be introduced to medicine. The Medical Tutorial I am participating in allows me to shadow Dr. Dejan Budimirovic at the Kennedy Krieger Institute in Baltimore.

Being part of the tutorial has given me deeper insight to a day in the life of a physician. At the clinic I met a variety of patients, most of whom are affected with mild to severe Autism Spectrum Disorder (ASD) and/or Fragile X Syndrome (FXS), but also patients with conditions such as Depression, Attention Deficit and Hyperactivity Disorder (ADHD), Intellectual Disability (ID), and Oppositional Defiant Disorder (ODD). Each session, I listen to and observe interactions between the patient, family, and physician, giving me a glimpse of what it is like living with a psychiatric disorder and the impact it has on the daily life of both the patient and their family. I understand more about the care of a patient such as what medicine to treat certain symptoms/behaviors or programs to enroll in to help the patients and their families in coping with the psychiatric disorder and lead to an improved quality of life. I have seen the progress of some patients and the process of narrowing down causes of symptoms and behaviors of psychiatric conditions and figuring out what the next step for each patient should be. I have been able to compare and contrast patients with the same diagnosis and severity of the disorder, especially in patients with FXS, ASD, or both and the linkage between the two disorders. I learned a vast amount about and have become very interested in psychiatric conditions that I understood very little about before. This tutorial has greatly affected and further solidified my decision to become a physician, and I hope that in the future I will be able to apply the knowledge gained from such a unique and memorable experience and contribute to the field in practice.

Johns Hopkins University – Homewood Campus

& Moser, 2000; Darnell et al., 2005; Bassell & Warren, 2008). Consequently, the *FMR1* gene FM can alter the course of brain development, cognition, and behavior throughout life. Furthermore, FMRP interacts with half of the known genes associated with idiopathic ASD (Darnell et al., 2011; Iossifov et al, 2012), findings that were independently replicated by Ascano and colleagues (2012) and more recently in a large exome sequencing study by De Rubeis and colleagues (2014). The latter study strongly corroborates the findings that a set of synaptic genes were disrupted in ASD. Thus, both FXS and at least subsets of idiopathic ASD are considered the disorders of synapse; yet, idiopathic ASD is a behaviorally defined set of DSM-5 based symptoms. At this time, there is no medical test, such as a blood test or brain scan that can diagnose ASD whereas FXS is a genetic/medical diagnosis. Regardless, when diagnosing ASD the clinician should state whether it is associated with a known medical, genetic or environmental factor. They should also specify whether ASD is with or without accompanying intellectual impairment and with or without accompanying language impairment. Representing an enormous and growing public health challenge, idiopathic ASD is a heterogeneous group of disorders of mostly unknown etiology that afflict as many as 1 in 66 individuals in the general population. Of all genetic disorders associated with ASD, FXS is the best characterized and accounts for about 5% of all cases of ASD. As treatments for the core deficits in ASD are lacking, current research is focused on identifying shared pathways and common drug targets among patients with causal genetic defects such as FXS, the most common known genetic cause of ASD. Approximately two out of three boys with FXS has ASD (Bailey et al., 2008), which is characterized by social-communication and restricted and repetitive interests' impairments. The core deficits in FXS are intellectual disability, social anxiety, and hyperarousal. ASD in FXS is characterized by more social withdrawal and social avoidance/anxiety than in idiopathic ASD although social indifference ('aloofness') is also observed in FXS with severe forms of ASD. Moreover, both ASD in FXS disorders and without FXS include poor eye contact, social deficits, atypical language, and repetitive behaviors. While similar behavioral characteristics or symptoms may be present in individuals with ASD and individuals with FXS who meet criteria for ASD, clinicians working with individuals with FXS who meet diagnostic criteria for ASD observe distinct differences - e.g. lack of social initiative alone does not necessarily imply the absence of social awareness or social interest. Together, owing to the prevalence of ASD in FXS and its shared neurophysiology with ASD, FXS has been extensively studied as a model for ASD; and is deemed to be a "gold standard" to study neurodevelopmental disorders of relevance for targeted therapeutics for both FXS and ASD.

A significant progress in targeted treatments in FXS reflects the major preclinical breakthroughs (Bear et al., 2004) and shows promise in humans medical targeted therapeutics (Berry-Kravis et al., 2012; Jacquemont et al., 2011), namely agonists of GABA-B receptors and antagonists of mGluR5 receptors. Specifically, the FXS mouse model (the *FMR1* knockout) has shown that FXS can be "cured" (reversal of the excess protein synthesis) of the core phenotype after using agonists of GABA-B receptors (Henderson et al., 2012) or mGluR5 antagonists (Gantois et al., 2013). Yet clinical trials were not as effective in part due to high placebo effects rendering the drug in question not significantly effective. The varying responses to trials also highlight the challenges of conducting the studies ('building a bridge we walk on') and the need for new paradigms. A major problem is a lack of models for trials in FXS. For example, there were no significant moderate/large clinical trials in FXS with any "standard" drug before 2002, and there is no defined measure of behavioral improvement, "gold standard" outcome measure, or template from any developmental disability about measuring cognitive outcomes when attempting to treat the underlying disorder (Berry-Kravis et al., 2012). In order to improve the understanding of FXS and ASD, future clinical trials should take into account the study length, timing of intervention, appropriate clinical endpoints, use of a combination with psychopharmacological interventions, patient stratification into endophenotypic subgroups within FXS though different models (Jacquemont et al., 2013).

Published descriptive studies of severity of social withdrawal set of behaviors as a unifying factor of ASD (Budimirovic et al., 2006; Kaufmann et al., 2008) and/or anxiety (Cordeiro et al., 2011), emerging investigation of the biological basis of FXS through imaging-behavioral (Wolff et al., 2012), molecular-behavioral using next generation fragile X PCR that also allows fragile X testing to be simple and more efficient (Budimirovic et al., 2014) are examples of such helpful models. In parallel, an effort at identifying potential molecular mediators of dentritic overgrowth in FXS as new potential targets of treatment (Erickson et al., 2014), and integration of molecular and neurobiological data in FXS with ASD (i.e., FMRP, CYFIP1, mTOR) and it's still unknown and/or adequately understood targets (Sidorov et al., 2013) are needed. CYFIP type 1 'intermediate phenotype' link between ASD in FXS (Schenck et al., 2003; Abekhoukh & Bardoni, 2014) and subsets of idiopathic ASD such as 15q11-13 duplication (Nishimura et al., 2007; Oguro-Ando et al., 2014) emerges as a compelling example of the shared neurophysiology. Studies of educational, behavioral, and therapeutic interventions are also needed to generate evidence on which to base recommendations about supportive interventions and the similarities and differences between those recommendations for patients with FXS and ASD. As PM is also associated with ASD, further studies are clearly needed in this area, especially given much larger frequency of PM vs. FM. The integration of all these pieces of data is a major challenge and will be better addressed when additional data becomes available. Overall, future clinical trials implementing the aforementioned not only hold a hope but a meaningful clinical and functional progress in FXS and ASD, and improved quality of life for affected individuals and their families.

AACAP'S 61ST ANNUAL MEETING
OCTOBER 20-25, 2014 . SAN DIEGO, CA
MANCHESTER GRAND HYATT & MARRIOTT MARQUIS & MARINA
Please note that this schedule is subject to change.

Symposium 28 (open)

Fragile X-Associated Disorders: Illuminating the Spectrum of Disease Through Molecular, Clinical, and Therapeutic Advances

Friday, October 24, 2014; 8:30 AM-11:30 AM

Chair: Randi J. Hagerman, MD

28.1 Fragile X Premutation: From Neurodevelopmental to Neurodegenerative Disorders
Randi J. Hagerman, MD *University of California, Davis Health System Endowed Chair in Fragile X Research, Sacramento, CA*

28.2 Next-Generation Fragile X PCR; Breaking Barriers to Enable Comprehensive FMR1 Molecular Analyses From Patient-Friendly Specimens
Gary J. Latham, PhD *Asuragen Inc., Austin, TX*

28.3 Molecular-Clinical Profiles in Fragile X: Novel FMR1 Genetic and Epigenetic Molecular Assessments in a Cohort of Full-Mutation and Premutation Patients
Dejan B. Budimirovic, MD *Clinical Trials Center, Kennedy Krieger Institute The Johns Hopkins Medical Institutions, Baltimore, MD*

28.4 Identyfing New Targets of Treatment: Potential Molecular Mediators of Dendritic Overgrowth in Fragile X Syndrome
Craig A. Erickson, MD *Cincinnati Children's Hospital Medical Centre Cincinnati, OH*

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Acta SCIentiae

Dejan B. Budimirovic, M.D.,
Child Neuropsychiatry, JHMI

Exclusive: USA´s science and medicine

Exclusive from

The Solomon H. Snyder Department of Neuroscience
THE JOHNS HOPKINS UNIVERSITY

" Fragile X-*FMR1* gene research: story of a collaboration between a neuroscience PhD student and an MD clinician at Johns Hopkins"

My name is Megha Subramanian and I am a fourth-year Neuroscience PhD student at Johns Hopkins Medical School in Baltimore, Maryland. The vast frontier of neuroscience explores many fascinating questions regarding the fundamental biological principles governing brain function and complex human behavior. Throughout my undergraduate and graduate studies, I have been interested in the dynamic processes that drive brain development, and in particular, allow the developing nervous system to incorporate information from the external world in order to learn and adapt to changing environments. My current research interests focus on understanding the molecular basis of growth, signaling, and plasticity processes in the brain, and their dysregulation in the context of neurological and neurodevelopmental diseases.

Under the mentorship of Dr. Mollie Meffert, Associate Professor of Biological Chemistry and Neuroscience, I am studying the molecular mechanisms by which brain growth and sociocognitive development are disrupted in Autism Spectrum Disorders (ASD). ASD represents a range of neurodevelopmental conditions that are caused by changes in several genes known to affect brain development and function. The complex, heterogeneous nature of ASD has historically posed a challenge for elucidating the underlying etiologies of the disorder and for identifying targeted treatments. As a result, basic research has turned to rare monogenic syndromes wherein discrete known mutations in a single gene lead to increased risk for autism. One such disorder is Fragile X Syndrome, which is the most common single gene cause of autism and intellectual disability worldwide. In my research, I use a mouse model of Fragile X Syndrome, which recapitulates many of the neuronal and behavioral characteristics of ASD, in order to gain insights into the fundamental processes by which brain development differs in autism.

Megha Subramanian,
B.S. Neuroscience

Disruption in the number and function of brain synapses- the points of communication between neurons- is a characteristic feature in the development of autism and associated cognitive disabilities. It has been observed that children with ASD and autism mouse models display an early overproduction of synapses as well as a failure to remove inappropriate synapses in the brain. At the molecular level, these abnormalities could result from disruption of the cellular machinery that regulates the synthesis of key proteins involved in promoting neuronal and synaptic growth.

In the lab, I utilize molecular genetic tools in combination with behavioral assays in order to elucidate the role of an important class of molecules, known as microRNAs, in abnormal protein synthesis and synapse function in a mouse model of Fragile X Syndrome. MicroRNAs are short fragments of RNA that critically regulate protein production in cells. Since an individual microRNA species can target many genes, understanding microRNA regulation might elucidate how ensembles of neuronal proteins are dysregulated in ASD. I hope that this work will shed light on the underlying pathophysiology of ASD, and reveal novel biomarkers and therapies that effectively target the core causes of the disorder. In this regard, studying microRNAs is of particular significance, given that their stability makes them exceptional candidates for use as biomarkers and therapeutics. In fact, microRNA-based strategies are already under clinical trials and hold great promise for the early detection and treatment of cognitive and neurological diseases.

As I began to recognize the importance of the clinical aspects of my project, I realized that direct patient interaction was crucial to developing a comprehensive understanding of the nature of ASD and also for guiding my future research trajectory in the lab. Being at a premier research hospital like Johns Hopkins, I did not have to look very far to find clinical shadowing opportunities. Last year, I began shadowing Dr. Dejan Budimirovic, attending physician and medical director of the Fragile X Clinic at the Kennedy Krieger Institute. Dr. Budimirovic is not only a fantastic physician, but also a wonderful mentor, who takes great interest in sharing his work and passion for child psychiatry and autism research with students like myself.

At the clinic, I meet a variety of patients, most of whom are affected with mild to severe ASD and Fragile X Syndrome. In addition, I have also come across individuals with other conditions, such as Attention Deficit and Hyperactivity Disorder (ADHD), Depression, and Oppositional Defiant Disorder (ODD). Observing Dr. Budimirovic's interactions with patients has given me a better understanding of the day-to-day concerns and difficulties faced by families dealing with psychiatric disorders. In addition, it has also allowed me to recognize the striking clinical heterogeneity of ASD, wherein patients with similar diagnoses may display marked differences in symptom presentation and severity. The most memorable and important aspect of my experience so far has been the insightful discussions with Dr. Budimirovic about current progress in basic and clinical research in ASD. While I share promising new discoveries addressing the etiology and treatment of ASD, Dr. Budimirovic keeps me up-to-date on emerging drugs and ongoing clinical trials. I truly believe that fostering such a culture of collaboration between basic scientists and clinicians is crucial for translating basic mechanistic insights into useful therapeutic approaches.

During my time shadowing at the Fragile X Clinic, I have learned that impairments in the social, occupational, and cognitive realms continue to be major barriers faced by individuals with ASD and associated disabilities today. Most commonly prescribed medications do not reverse the core features of the disorder, but instead only treat secondary syndromes and problematic behaviors associated with ASD, such as anxiety and hyperactivity. This has given me a sense of the potential impact of my work and has truly cemented my dedication towards pursuing a career in autism research. I am excited to combine basic science with the clinical knowledge I gained from working with Dr. Budimirovic to inform my future research efforts in developing effective therapeutic interventions and biomarkers for ASD. I hope to continue to grow as an independent scientist and to make significant contributions to the field of autism and society at large in the years to come.

Adriana Bora, PhD

Exclusive: USA´s science and medicine

Dejan B. Budimirovic, M.D.,
Child Neuropsychiatry, JHMI

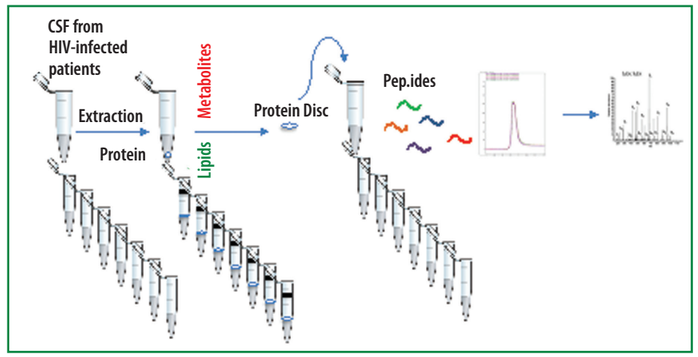
Medicine World Wide

My name is Adriana Bora and I am from Romania. I am a senior research fellow at the Johns Hopkins School of Medicine in the Pharmacology, Neurology and Molecular and Comparative Pathology Department where I pursue my research in the biomarker discovery of HIV-infected patients with neurological disorders. I became a neuroscientist in 2004 after I was awarded with my PhD in Neuroscience Program at University of Illinois at Urbana-Champaign with Professor Jonathan Sweedler, who not only inspired me but also he educated me to pursue any academic endeavour with confidence. At origins, I began my scientific journey as a physicist graduating with a Bachelor of Sciences from University of Bucharest, Physics Department.

As a neuroscientist, and early, in my career, I became aware of the multitude of applications of my field and the impact of my own research in studying the brain across clinical neurological disorders. Today, more than ever, brain research had gained high national and international consideration. The Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, the Human Connectome Project and the European Union Flagship Human Brain Project are major international, highly interdisciplinary, and collaborative endeavours that bring scientists and medical doctors together to help decipher how the brain works under normal and disease conditions. Being able to establish collaborations across many biomedical disciplines and learn new information on daily basis are motives that attracted me to pursue my postdoctoral fellowship in clinical research in neurological disorders at one the most prestigious institutions in the world, the Johns Hopkins School of Medicine, with world-renowned researchers and mentors such as Dr. Robert Cotter, Dr. Avindra Nath and Dr. Justin McArthur.

Integrative Omics, a new frontier of research in developmental disorders

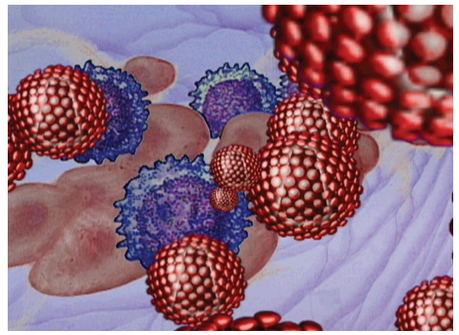
Proteomics ***Metabolomics*** ***Peptidomics*** ***Lipidomics***



My research interests have gravitated to studying neurological disorders utilizing state of-the art technologies and "omics" tools that comprise proteomics, metabolomics, lipidomics and functional neuropeptidomics. These scientific gears allow me to analyse, measure and characterize proteins, peptides, metabolites, and lipids, including their chemical modifications in human tissue, cell culture and biofluids (blood, cerebral spinal fluid, serum, saliva, urine, and others) as signatures of a disease or biomarkers.

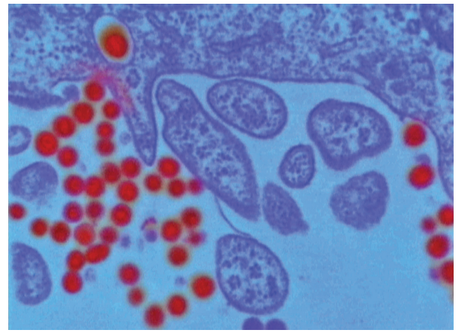
In practice, these biomarkers can help us understand the prediction, cause, diagnosis, and progression of a disease or the outcome of a treatment of a disease. Molecular biomarkers provide a dynamic and powerful approach to investigate and understand the spectrum of neurological diseases with applications in disease prevention, diagnosis and treatment.

Battle against AIDS



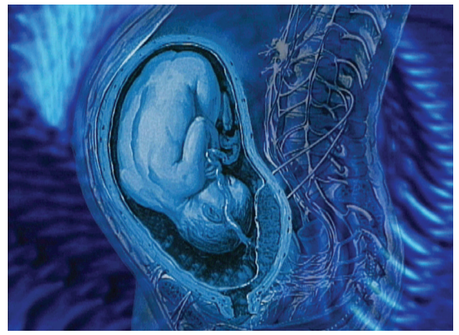
During HIV infection, millions of viral particles are present in the blood stream. They continuously infect cells of the immune system.

Action of NNRTI's



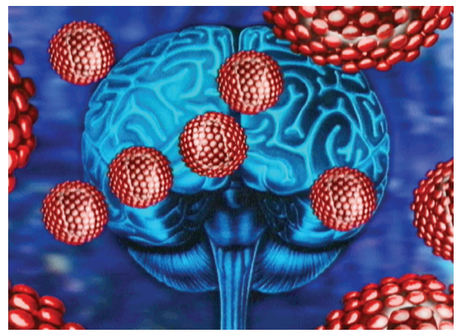
The battle between the viral infection and the cells of the immune system continues throughout the course of the infection.

HIV retrovirus



Perinatal transmission of HIV from mother to baby is the major cause of HIV infections in children worldwide.

HIV infection



HIV is able to infect the central nervous system (CNS), including the brain. The CNS has been shown to be a major reservoir of virus.

HIV is able to infect the central nervous system (CNS), which becomes a major reservoir of virus

My current research has led to the discovery of novel key proteins as putative biomarkers for early diagnosis of HIV-associated neurological disorders (HAND). Even under widely potent combination of antiretroviral therapy (cART), approximately fifty percent of HIV-infected individual will manifest some degree of neurological impairment that varies from the asymptomatic to severe forms, dementia. Soon after infection, the HIV virus enters the brain parenchyma via monocytes, perivascular macrophages, endogenous microglia cells and, perhaps to some degree, astrocytes. Although the utilization of cART has drastically reduced the number of patients with HIV-associated dementia, the increasing prevalence of patients with less severe forms of HAND remains a global concern. HAND progression is enhanced by additional factors such as aging, drugs of abuse, co-infections, oxidative stress, and other factors. Currently, there is no clinically approved biomarker for HAND; the diagnosis relies on patient performance of neuropsychological testing. Further validation of these key proteins in longitudinal studies of larger HAND cohorts will establish whether these proteins can be considered clinical biomarkers for early diagnosis of HAND. Another important aspect of my research is the investigation of molecular and cellular pathways that are affected by the HIV virus in the central nervous system using bioinformatics strategies such as correlation studies of these key proteins with metabolites and lipids extracted from the same cohort of patients.

Perhaps the most interesting aspect of neuroscience is that, indeed, it is the Holy Grail that gives us the tools to tackle a wide range of brain disorders. With a highly collaborative scientific and medical community, the Johns Hopkins provides the perfect environment to pursue research. Together with Dr. Dejan Budimirovic, an expert in Fragile X syndrome research and autism spectrum disorder, we discovered overlapping future clinical interests in the role of neuropeptides in the metabolism of autistic patients. Alteration in the function and number of brain synapses, the areas of communication between neurons, is a common characteristic in the development of autism and associated cognitive disabilities. Animal research has shown that these synaptosomes, the synaptic terminal of a neuron, contain neuropeptides that modulate behavior. Development and application of clinical assay that will establish a platform for detection and analysis of these signature brain peptides collected from human cerebral spinal fluid that modulate brain metabolism and human behaviour. In addition, by use of proteomic approach there is a high potential to discover a unique set of biomarkers not only for early detection of ASD but also the pathogenesis of this disorder. This knowledge can lead to an improved diagnostic accuracy and enable an early prevention of this disorder.

'Exclusive US science and medicine,' Editor
Dejan B. Budimirović, MD
Assistant Professor
Johns Hopkins University

Exclusive: USA´s science and medicine

Research essay submitted to the Society for Neuroscience for the Peter and Patricia Gruber International Research Award, supported by the Gruber Foundation

"Research essay submitted by Dr. Adriana Bora to the Society for Neuroscience for the Peter and Patricia Gruber International Research Award, supported by G.F. "
Author: Adriana Bora, PhD

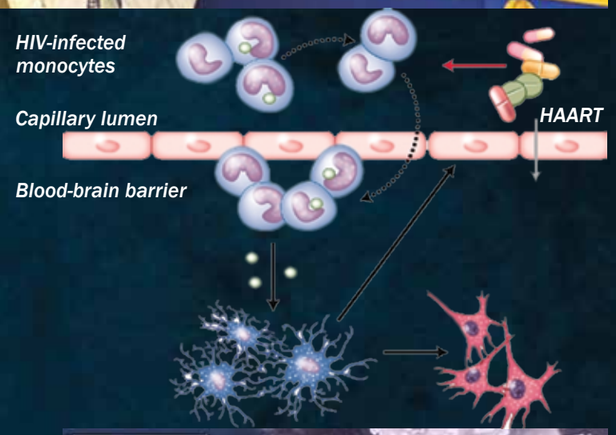
In the brain, HIV uses alternative cellular and molecular pathways that lead to neural and cognitive impairment. Even in the era of widely potent antiretroviral therapy, neurological disorders in HIV-infected patients remain a significant challenge for diagnosis and treatment. Approximately fifty percent of HIV-infected individuals will acquire a degree of neurocognitive impairment known as HIV-associated neurological disorders (HAND) that vary from asymptomatic forms of neuropsychological impairment to dementia. The neuropathogenesis of HAND is triggered by HIV systemic infection in the brain parenchyma via perivascular monocytes-derived macrophages, endogenous microglia and, to some degree, from infection of astrocytes [1-5]. Although the combination of antiretroviral therapy (cART) has dramatically decreased the severity of HAND, the overall prevalence of asymptomatic and mild forms remains high. Aging, drugs of abuse, neurotoxicity of long-term antiretroviral medications, co-infections, and oxidative stress are several factors that facilitate HAND progression. Neuropsychological performance testing remains the 'gold standard' method for diagnosis, although, for clinical diagnosis, additional biomarkers are needed for the early detection of HAND.

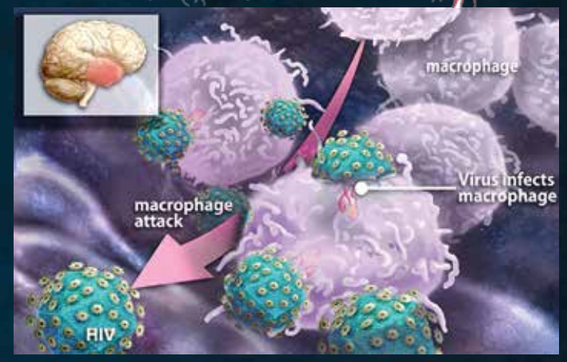
My post-doctoral research has led to a new understanding of key proteins as biomarkers for early diagnosis of HAND under cART therapy. With a team of international researchers we have developed a standardized protocol for collection of extracellular vesicles isolated from tissue, and biofluids of HIV-infected patients [6]. Specifically, I have developed highly efficient clinical assays to extract proteins, metabolites, and lipids from cerebral spinal fluid of a cohort of HIV-infected individuals with a spectrum of neurological disorders and analyzed them by mass spectrometry. These findings contributed to the discovery and characterization of an important class of glycoproteins and cell adhesion molecules that are involved in lipid metabolism, molecular transport, and small molecule biochemistry. Validation of these markers in larger patient cohorts for longitudinal studies can lead to early diagnosis markers of HAND.

As a neuroscientist, my future research is dedicated to find the alternative routes

Adriana Bora, PhD
Clinical Neurology
and Neuroimmunology, JHMI

that the virus uses to navigate in the brain that help differentiate between different stages of impairment. With an extensive expertise in *omics* sciences, I will develop novel clinical assays using functional peptidomics/metabolomics to monitor brain activity of individual HAND patients at different stages of impairment. By measuring changes in cerebral spinal fluid metabolites, including bioactive peptides and posttranslational modification of peptides, I can tackle the mechanism that HIV uses to alter the brain metabolism of HAND patients. Important emerging questions that my research will cover are: 1) whether these routes are completely new, overlap, or correlate with other cellular and molecular pathways differentially expressed across the broader spectrum of non-HIV neuroinflammatory disorders caused by aging, co-infections, or drug abuse; and, 2) whether these altered pathways are redundant within HAND phenotype(s) under specific type of cART or are completely novel with each regimen. With the multitude of factors that can contribute to the vulnerable brain infected by HIV, I will consider multidisciplinary collaborative strategies including computational medicine for central nervous system drug development. Eventually, HAND may require personalized patient-designed treatment aimed at suppressing systemic central nervous system inflammation based on age, lifestyle, type of cART, and concurrent co-infections.





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