

Biological Evidence of Significant Vaccine Related Side-effects Resulting in Neurodevelopmental Disorders.

**Presentation to the Vaccine Safety Committee of the Institute of Medicine,
The National Academies of Science, February 9, 2004.**
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Hypothesis

Evident for many individuals experienced with these issues, is the data supporting unprecedented levels of neurodevelopmental and immune disorders within the last two decades. The prevalence has risen to the point that many believe it has reached epidemic proportions. Our group of researchers and clinicians, have hypothesized that a subset of neurodevelopmental and medical disorders including: encephalopathy with autistic features, a unique inflammatory bowel disease, along with speech, learning and sensorimotor dysfunction, represent the manifestations of injuries related to vaccine components, especially mercury in the form of Thimerosal and measles virus from the MMR. Part of this hypothesis has been the existence of specific genetic vulnerability or environmental susceptibility cofactors. Our group represents researchers and clinicians with heterogeneous experience and expertise, that when taken together, provide the substance for a broad understanding of this phenomena.

Thesis

It must be recognized I am defining the data of a subgroup of children and in no way making estimates about autism or autism spectrum disorders in general. These terms will be discussed further in a moment. For what is often referred to as the broader autism phenotype, numerous candidate genes have been presented in the literature which I will not discuss here. To my knowledge, none of these genes would serve as susceptibility genes for measles viral persistence, mercury toxicity or autoimmunity of the type we will describe in this presentation, with the exception of the haplotype B44-SC30-DR4. Within this population, specific vulnerability factors, i.e. single nucleotide polymorphisms (SNP) within genes for enzymes regulating the methionine transsulfuration and glutathione systems, occur at statistically significant greater frequency than within the general or control populations. The biochemical defects predicted by these SNP are all present as well. These include: low methionine, thiol deficiencies, heavy metal (particularly mercury) accumulation, immunological disorders including autoimmunity and neurotransmitter malregulation, and the probability of viral persistence. Proteomic studies are presently underway and will help to further define and confirm these associations. Other environmental factors include oxidative stress, which has broad implications on chemistry, and *in utero* exposure to mercury (methylmercury from fish and Thimerosal in anti-Rho immunoglobulin preparations). In all endpoint issues, the findings have been reproduced by at least two independent laboratories, and as in the case of SNP identification and low thiols, by multiple laboratories using various methodologies. These data will be presented in summary form to the committee during the brief time allotted, but will be presented *in toto* in written form with supporting literature.

Vaccine Contribution to Public Health

The well-accepted benefits of vaccinations to prevention of many childhood and serious illnesses are without question. All the data presented herein are intended to improve vaccine safety and long-term public confidence in vaccines. When mass vaccination programs encompass billions of lives, even relatively rare toxicity events cannot be tolerated. While others may debate the frequency of specific adverse events and the appropriate detection and surveillance methodologies, medicine remains governed by the foundational tenet, *primum non nocere*. Where options exist, as is the case with Thimerosal, there is no acceptable rationale for continued use of a known, suspected or plausible neurotoxin in vaccines for developing children. Safe vaccine policy can now completely eliminate any concern of mercury for every age-group. This way, none of us need to fear even subtle potential effects of mercury.

The sixth month cut-off, which seems to be in practice (whereby children over 6 months are routinely advised to take vaccines with 25 mcg of ethylmercury, e.g. influenza vaccine) is equally untenable. As has been eloquently demonstrated by Landing et al, (full paper submitted: *Pediatric Pathology and Molecular Medicine* 21: 321-342, 2002) the organization of the six layers of the human neocortex undergoes repeated and dynamic changes during the exact time when a known neurotoxicant (ethylmercury) has been and is continuing to be administered. Strides to reduce Thimerosal in vaccines have been partially successful in the youngest children, but incompletely understood potential risks remain in older children who are still being exposed through Diphtheria Tetanus boosters, Influenza and other vaccines. Since no market-ready alternative for MMR exists in the US, this is a more challenging issue until new options emerge. I did receive notice from Secretary Tommy Thompson that a nasal measles vaccine was in development and expected within 2-3 years. No safety data is available, nor will be available for some time. Researchers at Johns Hopkins are working on a DNA MV vaccine. The concept is designed to further improve safety, but I have serious reservation regarding those within the population at risk for DNA hypomethylation, i.e. the same individuals with deficient methionine production secondary to folate deficiency, MTHFR defects or other factors. Methylation of viral DNA is a critical pathway to viral replication regulation/suppression. A discussion of this is beyond the scope of this paper.

Below are two graphs from the Landing paper, presented here to allow easier understanding of the timing issue on neocortical development.

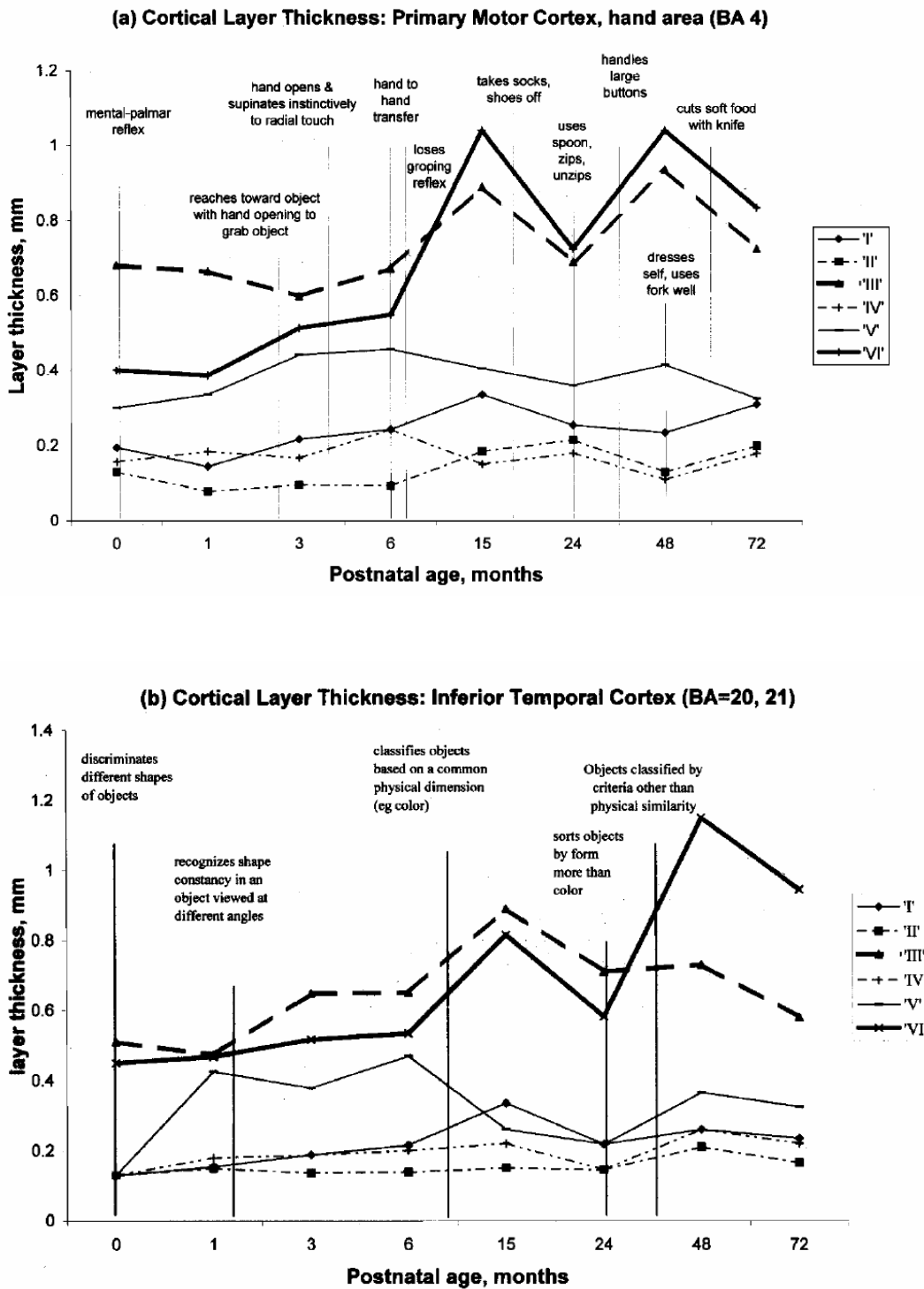


FIGURE 3(a,b). Development of cortical layer thickness for two cortical areas with well-defined cortical functions overlapped with the age at which there age-specific behaviors begin. Examples shown are for (a) the homuncular subdivision of the hand in the primary motor cortex (Brodmann area 4) subserving hand coordination; (b) the inferior temporal cortex (Brodmann areas 20, 21) subserving object recognition.

The sixth month timeframe is especially interesting to me since that is the historical time when children concluded the first cycle of Thimerosal exposure. Simultaneously, certain areas undergo a burst of proliferation, while layer “V” undergoes “pruning”. The precise significance of this timing is uncertain as it relates to vaccine Thimerosal, but the ability of ethylmercury to initiate apoptosis (Dr Baskin’s Presentation: July 2001 IOM) should be of great concern when considering Landing’s observations.

Causality Arguments

It is commonly held in medicine that when evaluating whether an association between exposure and outcome are causally linked, the criteria used to assess causality generally include:

1. Temporal relationship
2. Biological plausibility
3. Strength of association
4. Consistency of association
5. Reversibility
6. Dose response relationship/re-exposure effect

I would like to discuss these with regard to both Thimerosal and MMR in great detail but it would require more time than available prior to the scheduled date of this submission. Suffice it to say that the Committee will be dealing with these issues through its deliberations, so I will only highlight issues related to our research.

- 1) Temporal relationship.
 - a. Thimerosal: The inherent kinetics of ethylmercury’s effect on humans, particularly its rate of redistribution to the brain, are not well understood, so this is a nearly impossible point to evaluate with precision at this time. Comparisons to methylmercury are only partially applicable, and as is demonstrated in both the paper of Holmes et al scheduled to be presented by Dr Haley, and in our DMSA study, it appears the kinetics of mercury excretion are altered in at least some children presenting with the phenotypic symptoms of autism.
 - b. MMR: This has been presented since the original observations of Wakefield when he noted the parental observations of temporal relationship of MMR, bowel symptoms and autistic regression in the February 1998 Lancet publication, well known to the Committee. This has been generally dismissed by those opposed the hypothesis as a coincidence due to their belief that autistic regression routinely occurs or becomes evident during this timeframe. Nonetheless, the observation is one portion of the causality argument.
- 2) Biological Plausibility.
 - a. Thimerosal: The Committee accepted this for Thimerosal in its previous publication of findings on the subject.
 - b. MMR: The Committee rejected this on a population basis, but seemed to accept it for a potential small subgroup. Since the last round of

investigations on the subjects a significant number of technical molecular virology and immunological published studies appear to support the biological plausibility argument. This will be included in the later section and submitted. Additionally, new data on the persistence of MV genomes in multiple locations within affected children provides evidence of viral persistence. Permar *et al* have proposed that detection of MV in 2 or more body compartments is indicative of viral replication: “*Although we did not attempt to culture measles virus, we believe the presence of measles virus RNA represents continued measles virus replication, not simply the persistence of measles virus RNA after cessation of viral replication. This is supported by the detection of measles virus RNA from multiple clinical sites.*” (The Journal of Infectious Diseases 2001;183:532–8 - full article submitted). Our CSF study documenting the presence of MV genome in the CSF of children who additionally and simultaneously have MV genome in either or both, blood and ileum, satisfies this criterion. The first in a two part series of these observations has been submitted (full article submitted in pre-publication not final peer-reviewed form).

3) Strength of association

- a. Thimerosal: Several factors must be considered here. Typically the criteria applied involve epidemiological and/or case-controlled studies. Our study along with that of Holmes et al, are the only case-controlled or controlled studies to look at the specific question of mercury in the actual children affected. Other studies the committee will be dealing with applied retrospective chart analyses or database deconstructions to find possible trends. Since we are specifically looking for a different question to be answered, these database studies need to be taken with extreme caution when compared to case-controlled studies looking at specific biological markers. The hypothesis our group has been evaluating does not consider Thimerosal to be the exclusive cause of autistic features. I am aware that some have proposed this hypothesis. Rather more specifically, we believed the existence of specific vulnerability and environmental cofactors contributed to unique susceptibility of mercury toxicity and failed excretion. One obvious source of mercury was and is Thimerosal. Since the exposure to Thimerosal is generally voluntary or easily controlled through medical policy, it is an easier target for exclusion than background environmental sources of mercury. Clearly, based on our findings and those of Holmes et al, the autistic population deserves special evaluation for mercury exposure from all sources. Geier and Geier (part of our research team) have a study of the VSD (which they will present) in which they found a statistically significantly increased ($p < 0.0001$) relative risks of autism (relative risk = 27.6), mental retardation (relative risk = 5.7), and speech disorders (relative risk = 6.34) in a cohort receiving additional doses of mercury from thimerosal-containing DTaP vaccines in comparison to the cohort receiving thimerosal-free DTaP vaccines. Thus in both clinical case-controlled and epidemiological controlled studies

(which ask the question contained in our hypothesis) find strong associations between Thimerosal, mercury and the risk of autism.

- b. MMR: A complete discussion of the potential weakness and flaws of various epidemiological studies purporting to disclaim an association between MMR and autism is beyond the scope of my presentation. I address in more detail in that later section. The most recent study by DeStefano et al, which will be presented to the Committee, is a classic example of asking the wrong question. Neither Dr Wakefield nor the remainder of our group have ever proposed that the timing of MMR between 12 to 18 months versus 18 to 24 months, etc, was associated with bowel disorders or autistic regression. Inherent in their statistical assumptions is an unknown *presumption* that if MMR causes autism, the earlier the vaccine is given the more likely this is to occur. I am not aware of any data supporting this as a meaningful or scientifically supported observation. In fact, one could easily argue the reverse. Since maternal antibody persistence and breast milk augmentation of the immune system is variable and not controlled for in the study, great caution should be applied when interpreting the findings. (see below)

Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure.

J Pediatr. 1977 Nov;91(5):715-8.

Albrecht P, Ennis FA, Saltzman EJ, Krugman S.

A serologic study was made in 34 children immunized against measles at the age of 12 months. Using a sensitive virus neutralization test, it was found that many of the children had pre-existing maternal antibody to measles virus. Children with high pre-existing antibody titers failed to seroconvert. Children with lower pre-existing antibody titers seroconverted, but the resulting antibody titer was significantly lower than in children without pre-existing antibody titer. The results of this study demonstrate a probable mechanism for measles vaccine failure in 12-month-old children and support the recommendation of the Public Health Service Advisory Committee on Immunization Practices to postpone measles vaccination to 15 months of age.

Early loss of passive measles antibody in infants of mothers with vaccine-induced immunity.

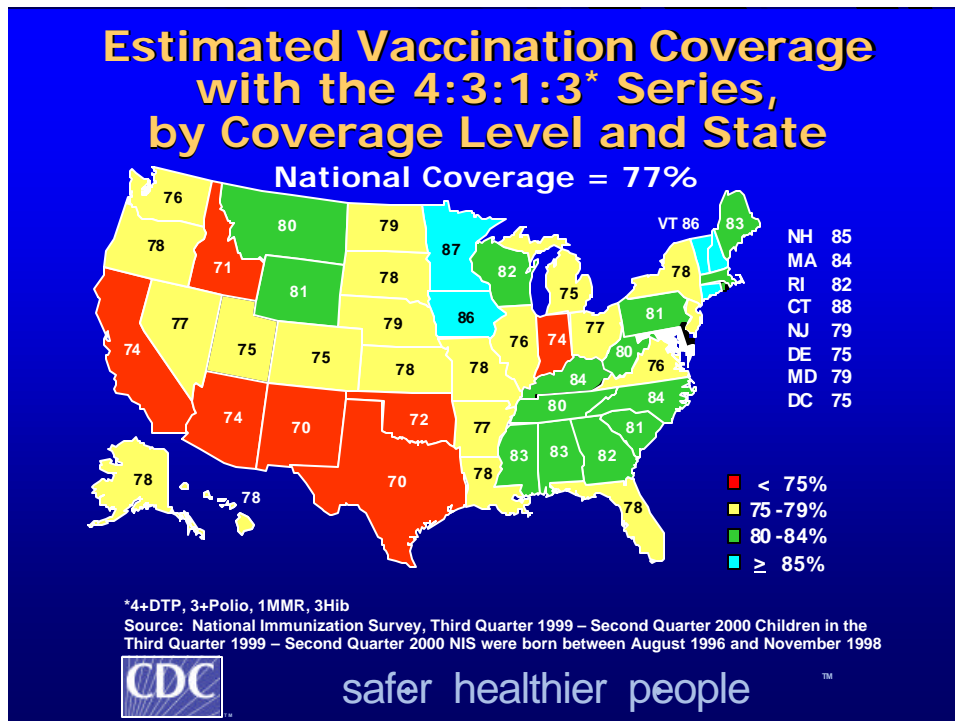
Pediatrics. 1995 Sep;96(3 Pt 1):447-50

Maldonado YA, Lawrence EC, DeHovitz R, Hartzell H, Albrecht P.

Department of Pediatrics, Stanford University School of Medicine, California 94305, USA.

BACKGROUND. Maternally derived passive measles antibody may interfere with vaccine-induced immunity in infants less than 12 months of age. However, early loss of passive measles antibody may occur in infants of women who received measles vaccine because measles vaccine induces lower antibody titers than does natural infection. **METHODS.** Persistence of passive neutralizing measles antibody was studied longitudinally in a group of normal infants as a function of maternal measles titer at birth and maternal date of birth. Maternal serum and cord blood specimens were tested from 162 women and their newborns, from 51 of these infants at 9 months of age and from 63 at 12 months of age. **RESULTS.** Seventy-one percent of sera from 9-month-old infants (36 of 51, 95% confidence interval 68% to 84%) and 95% of samples from 12-month-old infants (60 of 63, 95% confidence interval 89% to 101%) had no detectable neutralizing measles antibody. Measles geometric mean titers were significantly higher at delivery in mothers whose infants were seropositive at 9 and 12 months compared with mothers whose infants were seronegative at 9 and 12 months. All infants with detectable measles antibody at 9 or 12 months had mothers born before 1963, before the vaccine era, and both maternal and cord blood measles geometric mean titers decreased significantly with decreasing maternal age. **CONCLUSIONS.** Persistence of passive measles antibody is uncommon by 12 months of age; earlier antibody loss is related to lower maternal age and maternal measles titer. *(However 5% did have persistence at 12 months and it is obvious the potential MMR – Autism population is a much smaller group than this. How persistence of low levels of maternal antibodies in this subset might affect MV persistence is not known. Numerous patients in the ICDRC database are of mothers born prior to 1960. This affect has not been adequately studied to allow the Atlanta study findings to be based on this comparison).*

Of greater concern in the Atlanta study is the appearance that they had access to a large group of children (N~120) who were not vaccinated until after the onset of typical autism symptoms, and yet they claimed to be unable to discern onset of symptoms to be able to use this data to evaluate if autism symptoms occurred prior to MMR, or were only after MMR, or exacerbated by MMR. Since they also claimed to have access to extensive medical records and highly trained autism experts this would have seemed straight forward. Also, given the late onset of vaccination in some of the individuals a better case-controlled study whereby vaccinated versus non-vaccinated could have been compared. The study identified 624 autistics in Atlanta and based on accepted prevalence data from the CDC of 1 child with autism in 165 to 200 total children, the total population represented in the study was ~ 120,000. Given an ~ 20% non-uptake rate in the Georgia population (see below) they would have seemed to have the potential for a much more meaningful study.



The epidemiological findings are but one component of the strength of association argument. The data we are presenting herein are (to my thinking) considerably more specific and compelling. They represent the detection of MV genome in the inflamed GI biopsies, the circulating lymphocytes and the CSF in a significant number of children whose parents reported autistic regression following MMR. MV is rarely detected in controls and the only positive control we have obtained for our CSF study has both lymphoma and leukemia. All other leukemia patients did not have MV in the CSF (cases v. controls RR = 25.90; CI 3.96-181.58, p<0.00001). Where strain specificity can be determined it is nearly 100% of vaccine origin.

4) Consistency of the Findings.

- a. Thimerosal: The in vivo and vitro data are very consistent for the subgroup of children who seem to be retaining or accumulating mercury to a greater degree. The findings are consistent with animal models of Thimerosal effects and not dissimilar from other mercury forms. The association of cysteine deficiency and apparent defects in the methionine transsulfuration pathway, further combined with the MTHFR genomic and other gene studies of James provide further evidence of a consistent affect.
- b. MMR: The post-infectious autoimmune encephalopathy of wild-type MC is known to have autoimmune features. These are described by Singh and others in the ASD populations. ASD as has been shown MV wild infections are associated increased risk of autism (43). MV is in both apparent target locations: bowel and CSF. Other viruses are not consistently present. The animal Borna viral model is also supportive in a non-specific fashion. The

defect in MTHFR and/or methionine transsulfuration would be expected to increase the risk of autoimmunity and allergy and MMR has been reported by Imani and Kehoe to do the same thing (full paper submitted: Clin Immunol. 2001 Sep;100(3):355-61.). Antibodies to myelin basic protein, while nonspecific to MV are also consistent with past or ongoing MV infections. The increased frequency of seizures well described in ASD is further consistent with an ongoing CNS process and low-grade encephalitis. This is discussed further in the submitted papers.

5) Reversibility.

- a. Thimerosal: Heavy metal intoxication, even subtly during critical phases of cortical development has little assurance of reversibility. This has certainly been demonstrated over the long history of lead intoxication and has a similar inconsistent recovery history in mercury intoxication as well. Despite the poor prognosis, children with ASD are being shown to recover after various methodologies used for mercury chelation. Caution must be used in interpreting these results since all chelators have additional properties including those of antioxidants and thiols. Nonetheless, Lonsdale et al (44) did demonstrate efficacy in ASD, and Dr Rimland has posted parental surveys on the Autism Research Institute website and presented his data at scientific assemblies documenting DMSA chelation as the single most effective parental rated intervention for autism (www.autism.com/ari).
- b. MMR: No MV specific antiviral exists. Treatment regimens for wild-type MV persistence, as in subacute sclerosing panencephalitis, have met with inconsistent and inadequate results. The nature of vaccine strain persistence and the means the virus uses to persist seems to be different than its wild-type progenitor. As such it is unlikely that this criterion is applicable directly to viral persistence. However, since the presumed mechanism of pathogenesis in MV diseases similar to this is autoimmune, those studies documenting responses to human immunoglobulin and other immune modifying approaches are fair proxies for antiviral therapies. I will submit our own findings on IVIG published in the Congressional Record and those of Gupta and others.

6) Dose response relationship/re-exposure effect

- a. Thimerosal: The Geiers' studies on Thimerosal do show a significant dose response effect as did the original studies from the CDC as presented at the Simpsonwood Meeting. Subsequent reworking of the data has not shown this according to some looking at the VSD dataset, but looking directly at similar vaccines with and without Thimerosal, as mentioned above, shows a very significant risk.
- b. MMR: The IOM was presented preliminary findings of the so-called "double-hit" effect with MMR and intestinal pathology and intestinal symptoms. The one child in our CSF PCR study showed subacute neurological symptoms, seizures and diarrhea after a second dose of MMR. Here I will present on the behalf of Dr Wakefield et al, the findings of that more detailed and submitted for publication study.

Terminology Regarding Autism, ADHD and Related Symptoms: Intrinsic to this thesis is the appropriate use of terms related to both subtle and dramatic neurodevelopmental injuries. Psychological and psychiatric disorders represent symptom clusters, are often nonspecific and overlapping, and fail to distinguish underlying biochemical and medical disorders. As such we see *Autism*, *Autism Spectrum Disorders* and *the Autism Broader Phenotype* all being used in published works regarding potential toxicological and immunovirological injuries precipitating neurodevelopmental disorders more properly termed – encephalopathies, or even on some occasions, encephalitides. Since autism and all similar spectrum related terms represent behavioral disorder terminology dating back to observations of Dr Leo Kanner in the late 1930's and Dr Hans Asperger in 1944, it is scientifically untenable to continue to use these archaic terms to define the specific genomic and biological subgroups of neurodevelopmental disorders related to vaccine effects. Medicine has abandoned other archaic terms throughout its history, and it would be equally inappropriate to continue using *dropsy* or *consumption* to define low cardiac output or tuberculosis pneumonia.

The history of the present-day term *Attention Deficit Hyperactivity Disorder* is equally colorful and equally archaic when considered in light of definable subgroups and distinguishing neurochemistry. In 1902, Dr. Still, a British doctor, documented cases involving impulsiveness. He called it "Defect of Moral Control." He did believe, however, that this was a medical diagnosis, rather than a spiritual one. And by 1922, the collection of symptoms now associated with ADHD, were defined as *Post-Encephalitic Behavior Disorder*. This later term, in retrospect, is actually more specific with regard to etiopathology than our DSM-IV.

Inherent in all of these symptom generated behavioral diagnoses is the tendency to confuse the science more than to define it. Since we have no idea how many different substances, toxins, viruses or genomic - related biochemical disorders interfere with brain function to the point of precipitating some constellation of these symptoms, we must look at the epidemiological data with extreme caution to prevent us from missing the truth presented by the biomedical data gathered about the individual child suffering with neurodevelopmental dysfunction. Regrettably, we cannot escape using these terms when trying to compare our works to previous publications.

MMR Epidemiology – Briefly Summarized

I am not an epidemiologist, but as a clinician, I see serious problems with the epidemiology which purports to refute an association with Thimerosal or MMR and autism, and therefore presumes either to be safe in the subgroup we have defined.

Epidemiological studies that have examined the possible MMR-autism association have concluded that the data provide no evidence in support of this hypothesis ⁽¹⁻⁵⁾. These studies have been challenged on a number of counts including inappropriate methodology (6), lack of statistical power and lack of a control group (7,8), indiscriminate diagnostic

groupings (9) and non-disclosure of relevant data (10). Re-analysis of the data of Dales et al (4) has, in fact, identified a positive association for some children (11).

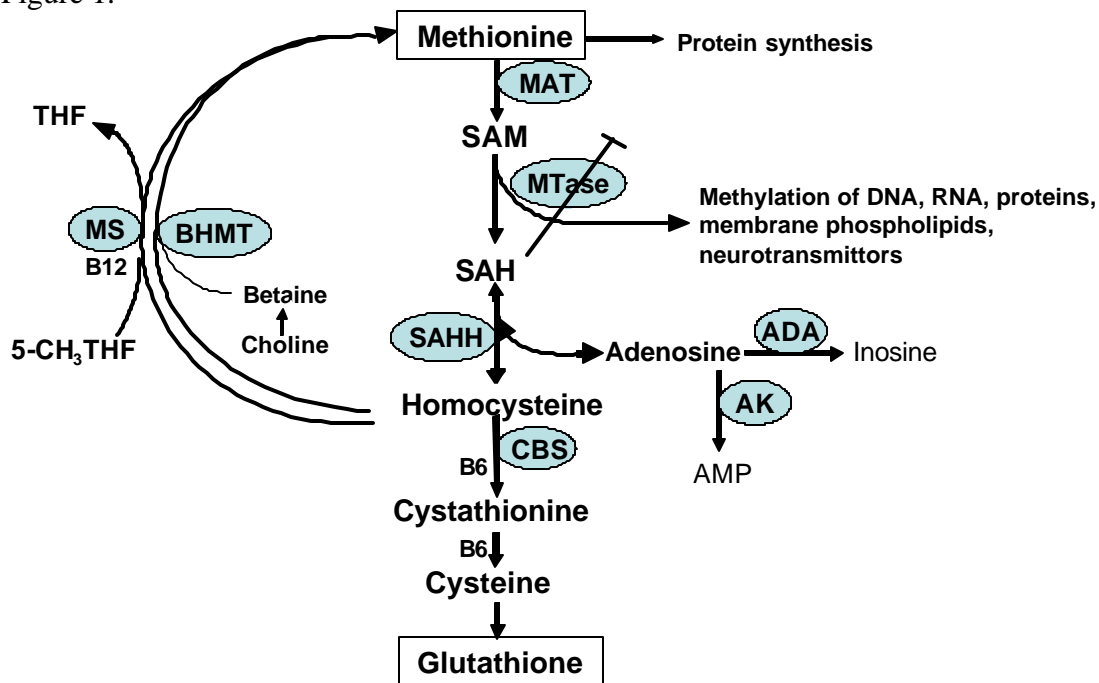
Clearly, meaningful epidemiological studies should test *a priori* hypotheses that derive from all clues evident in the clinical histories of affected children. Thus far, this has not happened. A crucial question relates to what makes a child susceptible to a possible adverse event to MMR vaccine. Possible risk factors are beginning to emerge in the histories of affected children including: familial autoimmunity, pre-existing dietary allergy/intolerance, vaccination with MMR while unwell, including current or recent antibiotic administration, and receipt of multiple simultaneous vaccine antigens, with the associated potential for immunological interference (12-14), particularly for mumps upon measles virus (13). The growing burden of infant vaccines may increasingly skew the immune response away from optimal anti-viral immunity towards a dominant T-helper cell type 2 repertoire (15). The rapid increase in numbers of children with dietary allergy, itself associated with reduced CD8 cell numbers, prolonged viral infections and familial autoimmunity (16) and increasing antibiotic use in infants over the last 10-15 years, suggests that the number of children who may be at risk of aberrant responses to atypical infectious challenges will have risen in the last decades. A likely autoimmune component to the pathogenesis of regressive autism suggests that any causal association with MMR vaccine would lead to a continuing upward trend in incidence after vaccine introduction - in developed-world but not developing-world populations, in parallel with other autoimmune lesions.

Based on our current scientific capabilities, children now can and should be defined by individual genomics, resulting biochemistry, and then evaluated medically for likely pathologies as determined by the history, physical examination and genomic profile.

Thimerosal Background Studies

Further, compounding the ability to find the truth is the misuse of science, especially toxicology. One illustration of this is demonstrated by confusion over blood half-life and body half-life of mercury as in the study by Pichichero, et al, (17). Serum is known to be an insensitive indicator of mercury deposition in brain, and does not reflect body burden. Half-life of mercury in the blood is highly individualistic and influenced by selenium, copper, zinc, oxidative stress and thiols – particularly cysteine, glutathione and metallothionein. At the genomic level, common single nucleotide polymorphisms of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene result in significant reduction in the production of methionine and therefore cysteine, glutathione and metallothionein through decreased kinetics within the methionine transsulfuration pathway. See figure 1.

Figure 1.



Abbreviations: THF: tetrahydrofolate; MS: methionine synthase; BHMT: betaine-homocysteine methyltransferase; MAT: methionine adenosyltransferase; SAM: S-adenosylmethionine; SAH S-adenosylhomocysteine; SAHH: SAH hydrolase; ADA: adenosine deaminase; AK: adenosine kinase; CBS: cystathionine beta synthase (From Jill James PhD, full paper submitted).

The rate limiting step in the production of both glutathione (the main thiol antioxidant in the brain) and metallothionein is the availability of cysteine.

Other polymorphisms, as in methionine synthase, would be equally important to the understanding of Thimerosal kinetics and toxicology on an individual basis. Within the context of this discussion, I have obtained permission from several colleagues to incorporate their data regarding both genomic susceptibility and biochemical evidence of methionine transsulfuration defects in the population of possibly vaccine - injured children. When combined with our data and that published or presented by others in different forums, the evidence is substantial that this type of genetically predisposed individual would be expected to encounter difficulty with vaccine constituents, particularly mercury and aluminum. I understand, the need for gene product analysis and the proteomic studies intrinsic in these pathways will be fully evaluated in the near future.

The Human Genome Project has reinforced our understanding that while humans may all be 97 to 99.9+% the same genetically, single polymorphisms are capable of generating significant differences in individual disease or susceptibility factors to environmental toxins(18). These must now be considered in light of the biological findings we present here.

Equally important to evaluating Thimerosal toxicity issues and susceptibility risks are nutritional factors, e.g. micronutrients, protein quality, vitamins, essential fatty acids and antioxidants (19-24). Neither the previous IOM proceedings, nor epidemiology available regarding vaccine safety take these factors into adequate consideration.

The present body of medical literature supporting the influence of both genomics and nutrition on endpoint disease is so large as to defy cataloguing without the aid of a vast team.

Therefore, as the IOM undertakes its deliberations on the subject of vaccine safety, it must take into account the new individual genomic profiles assembled and presented herein, as well as the unique biochemical, nutritional, immunological and molecular virological data which has been published, presented or is in the process of being published (but included herein).

Genomic, Toxicological, Immunological and Virological Data Regarding Vaccine Safety Concerns

The data come from a consortium of clinicians and research scientists working together and numerous institutions all sharing their ideas and efforts through regular “think tanks” held at least twice annually. When considered with data collected from other independent scientists, the previously murky picture of vaccine related neurodevelopmental injury is now clear and compelling. In presenting this data, I do not speak for any of the authors directly. They may have individual opinions regarding causality or the implications of the findings. In general, their papers speak to the issues directly.

THE THIMEROSAL ISSUE

Mercury Body Burden: The IOM has specifically asked me to address the case-control study on mercury in children with autism spectrum disorders (25). The study was simple and designed to retrospectively review our database to determine if heavy metals, and of particular interest - mercury, were present at increased levels in children with this disorder. We had clinically been asking the question on an individual basis for a few years and had a large collection of data on post-oral chelation urine levels of various toxic metals. The study is a refinement of the data we presented to the IOM in pre-published form during the 2001 Thimerosal session. The study we conducted is a proxy marker for relative mercury load, since children were compared under matched dosing schemes for DMSA. As such it can be used to estimate the *relative* burden of mercury between the two populations. It is not a precise measurement of *total* body mercury burden (which may from the title, and in retrospect, be somewhat misleading to traditional toxicologists, but which is clearly defined in the study).

While we were aware of the methods of determining true body burden (rather than relative body burden) we had historically deemed it to be very challenging logistically in the ASD population. This would require collection of all DMSA-chelated mercury until it

falls to zero – or below detection. Since we had no way of known total reserves, the rate of DMSA elimination in ASD versus neuro-typical children, nor did we have a rapid detection method to know we had reached endpoint, and since the studies were not externally funded, we elected the relative body burden assessment. Further, this was a retrospective look at our data. A true prospective study of actual body burden would be ideal, but by the time funding might have been feasible, the vaccine schedule had changed to the point of making it moot from that perspective.

This is a body of data which to my knowledge cannot be reproduced in the US population due to changes in the vaccine schedule. Since many other nations still use full dose Thimerosal containing vaccines a prospective total body burden study could be undertaken in one of these nations. I have concerns about matching the datasets however, since ideally the data should now be considered in light of the genomic profile of the two populations. MTHFR and methionine synthase (MS) SNP occur at differing rates in various ethnic gene pools.

It had always seemed reasonable to screen children with neurodevelopmental disorders for heavy metals, given the obvious neurotoxicities of lead, cadmium and mercury, and the overlapping symptoms of chronic heavy metal exposure with developmental delays. Given the growing concerns about mercury and Thimerosal, we collated our data retrospectively. It was becoming clear the mercury exposure during the course of childhood vaccinations could present a significant source of mercury, in addition to the obvious concerns about environmental methylmercury.

This becomes of even greater importance to the Thimerosal issue when put into the context of the report in MMWR. A comment from the abstract is included here:
*“Methylmercury exposures to women of childbearing age are of great concern because a fetus is highly susceptible to adverse effects. This report presents preliminary estimates of blood and hair Hg levels from the 1999 National Health and Nutrition Examination Survey (NHANES 1999) and compares them with a recent toxicologic review by the National Research Council (NRC). The findings suggest that Hg levels in young children and women of childbearing age generally are below those considered hazardous. **These preliminary estimates show that approximately 10% of women have Hg levels within one tenth of potentially hazardous levels indicating a narrow margin of safety for some women and supporting efforts to reduce methylmercury exposure**”.* (Blood and hair mercury levels in young children and women of childbearing age--United States, 1999. MMWR Morb Mortal Wkly Rep. 2001 Mar 2;50(8):140-3.)

This indicated to us that a significant portion of children were already at risk for increased mercury levels at the time of vaccine exposure to additional mercury. The exact percentage of children at risk would be nearly impossible to determine due to the large number of factors already discussed which regulate mercury susceptibility. Three years ago we hypothesized that this group of children were the likely subgroup reacting adversely to vaccine mercury. This was reinforced by Professor Adams from Arizona State University when he informed me of the results of his survey which demonstrated

significant association of maternal fish consumption and autism in children of those high-fish consuming mothers.

Since we had a small group of neurologically normal children of different vaccination status whose parents had requested screening for heavy metal toxicity, we felt a comparison of the ASD population and the neurologically normal group could be helpful.

There were no differences in the neurologically normal group based on their vaccine status. Granted the number of children is small, but it agrees with other environmental studies that find low body burden of mercury in neurologically normal children (26). Trepka et al, had shown dental amalgams were the strongest predictors of urinary mercury following DMSA chelation challenges (27). We controlled for this by excluding children with amalgams from both the subjects and controls.

The results of the study can be summarized by this quote from the paper: *“This study compares mercury excretion after a three-day treatment with an oral chelating agent, meso-2,3- dimercaptosuccinic acid (DMSA), in children with autistic spectrum disorders and a matched control population. Overall, urinary mercury concentrations were significantly higher in 221 children with autistic spectrum disorders than in 18 normal controls (Relative Increase (RI)=3.15; P < 0.0002). Additionally, vaccinated cases showed a significantly higher urinary mercury concentration than did vaccinated controls (RI=5.94; P < 0.005). Similar urinary mercury concentrations were observed among matched vaccinated and unvaccinated controls, and no association was found between urinary cadmium or lead concentrations and autistic spectrum disorders.”*

Placing this study into context with other mercury data.

Dr Haley will be presenting the ASD hair study, but this was further discussed in the case-control study above. Since the ASD population appears to be uniquely less able to excrete mercury in hair, at the very least, hair cannot be used as a predictor of body burden in this population. But it likely implies disordered sulfation. This has now been documented as will be discussed in depth in the paper co-submitted by Dr Jill James.

I will not go into detail regarding the wide-ranging effects of decreased glutathione or cysteine since Dr James treats this subject so well. I refer the IOM to her paper directly. In context, this excerpt is nearly identical to my own findings, so I will include the table from her paper here.

**Impaired transsulfuration and oxidative stress in autistic children:
Improvement with targeted nutritional intervention**

Abnormal methionine transsulfuration metabolites in autistic children:

Baseline levels of methionine transsulfuration metabolites were measured in plasma from twenty autistic children using HPLC with electrochemical detections^{20,21}. Relative to levels measured in age-matched control children, the plasma thiol profile of autistic children was severely abnormal.

Table 1	Control Children n=33	Autistic Children n=20	p value
Methionine (μmol/L)	30.6 ± 6.5	19.3 ± 9.7	0.001
SAM (nmol/L)	90.0 ± 16.2	75.8 ± 16.2	0.01
SAH (nmol/L)	20.1 ± 4.3	26.1 ± 5.4	0.001
Homocysteine (μmol/L)	6.3 ± 1.2	5.4 ± 0.9	0.01
Adenosine (μmol/L)	0.28 ± 0.16	0.39 ± 0.19	0.05
Cysteine (μmol/L)	210 ± 18.5	163 ± 14.6	0.001
Total glutathione (μmol/L)	7.9 ± 1.8	4.1 ± 0.5	0.001
Oxidized Glutathione (nmol/L)	0.3 ± 0.1	0.55 ± 0.2	0.001
GSH/GSSG Ratio	25.5 ± 8.9	8.6 ± 3.5	0.001

Because methionine is the precursor for cysteine, the rate-limiting amino acid for glutathione synthesis, it is not surprising that low methionine levels are associated with the low cysteine and glutathione levels in these children. The significant increase in adenosine is consistent with the increase in SAH since adenosine binds to the active site of SAH hydrolase and inhibits its activity. The increase in SAH is of concern in autistic children because of its ability to inhibit SAM-dependent methyltransferase activity and cellular methylation reactions. The reduction in total glutathione (GSH) and increase in oxidized glutathione resulted in a 3-fold reduction in the ratio of reduced (active) GSH to oxidized glutathione (GSSG). This is of major concern because it reflects a significant decrease in antioxidant capacity associated with an increase in oxidative stress in the autistic children.

We are preparing for publication a retrospective review of the ICDRC database regarding cysteine and sulfate as a means of validating her observations. Cysteine/cystine deficiency in ASD was originally described by Dr Jon Pangborn in 1984 (28). As such we have three separate observations of this vital and from the brain's perspective, essential amino acid. The brain lacks the cystathionine beta-synthase enzyme to convert homocysteine ultimately to cysteine and glutathione. This makes the brain dependant on either dietary cysteine or liver export of glutathione which is converted to cysteine/cystine. Cystine is then transported to astrocytes and glial cells and eventually finds its way in to the neuron. And excellent review of this chemistry has been published by Ralf Dringen and will be submitted *in toto* (Dringen, et al. MINIREVIEW.

Glutathione metabolism in brain. Metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. *Eur. J. Biochem.* 267, 4912±4916. 2000).

Understanding glutathione metabolism's importance to the brain is central to understanding the toxicology of Thimerosal and other mercury species.

As is apparent in Table 2, we are observing, despite using a different lab and completely distinct populations, the same sulfation issues as James.

Table 2. Plasma cysteine and plasma sulfate concentrations among children with autism spectrum disorders in comparison to normal controls

Type of Measurement	Controls Mean Concentration (mg/dL) [population size]	Cases Mean Concentration (mg/dL) [population size]	Percent Difference	p-value
Plasma Cysteine	3.50 ∓ 0.20 [N=41]	2.76 ∓ 0.45 [N=27]	21%	P < 0.0001
Plasma Sulfate	5.05 ∓ 0.125 [N=52]	4.50 ∓ 0.55 [N =19]	11%	P < 0.0006

James observed 22% lower cysteine from normal and we observed a 21% difference. This demonstrates remarkable consistency within two distinct data sets. As James describes in her paper, Since cysteine is a critical rate-limiting step in the production of both glutathione and metallothionein, decreased defense against heavy metals would be an expected consequence of this finding. So our observations regarding heavy metal burden are completely consistent with these data.

Walsh reported abnormal copper-zinc ratios in children with ASD and felt it represented a metallothionein deficiency (29). An excerpt from the presentation states this: *“In a study of 503 autism-spectrum patients, we found abnormal levels of copper and zinc in blood of 499 of 503 patients (p<0.0001) indicating defective functioning of metallothionein (MT) proteins. In humans, MT proteins regulate blood levels of these metals, detoxify mercury and other heavy metals, and assist in neuronal development. The expected consequences of defective MT during gestation or early infancy are consistent with several classic symptoms of autism. It appears that defective functioning of MT proteins may represent a primary cause of autism.”*

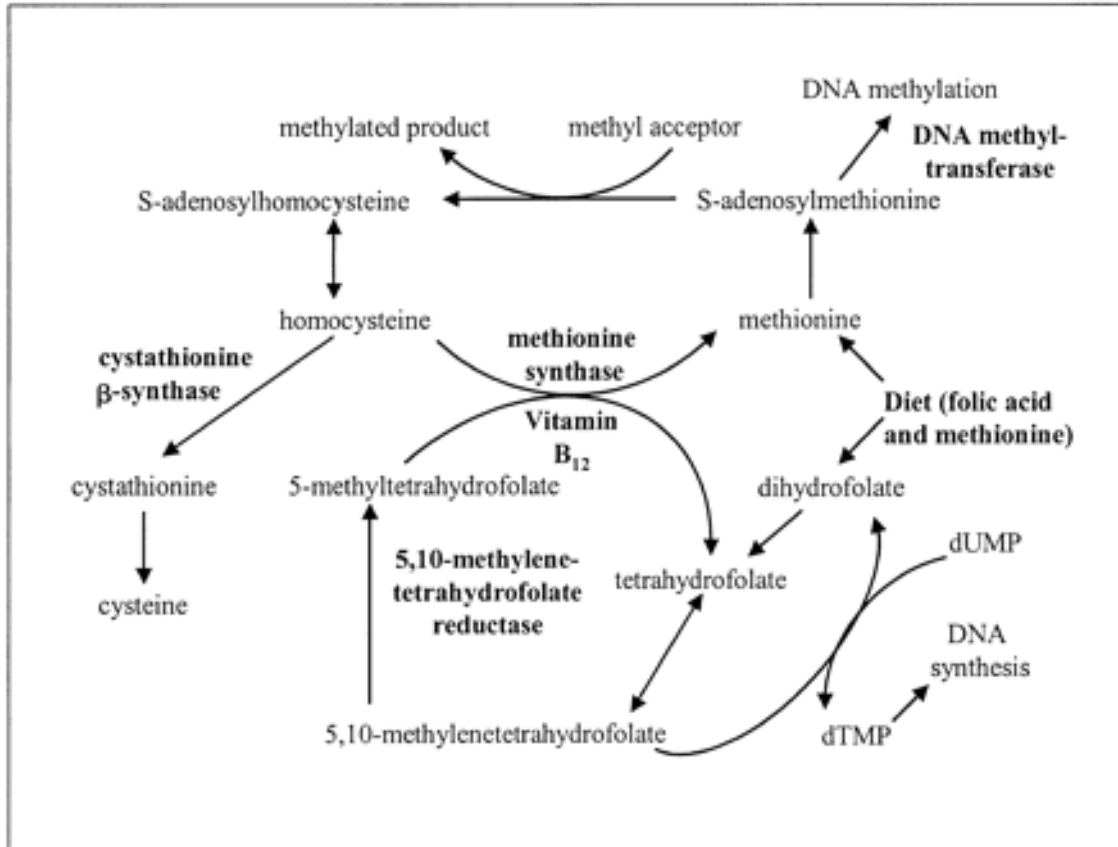
Our data and those of James support Walsh's observation, however, as demonstrated by James, the defect is not limited to metallothionein. The more complete picture is that of decreased methionine transsulfuration which would simultaneously adversely effect methylation and sulfation, with resultant disruption in numerous critical biochemical pathways.

With these data, it is obvious the population of children so affected would be uniquely vulnerable to Thimerosal. While we cannot, as yet, determine the incidence of these disorders in all groups potentially influenced by Thimerosal, it appears a significant subset of children labeled with autism, represent a group which is simultaneously accumulating mercury from all sources, and therefore likely adversely influenced by

Genomic Profiling

Based on the biochemical aberrations presented above, the likely candidate genes may lie in the folate – methionine cycle. Fortunately, these genes are well characterized secondary to their importance in numerous other major disorders including cancer, neurological disorders and vascular disease. I will submit selected papers on the genomic issues as background for the committee.

Presently, three groups (ours included) have extensive data on various SNP within the folate – methionine cycle. I have obtained permission from Boris and Goldblatt to submit their paper and summarized larger dataset on the occurrences of SNP in the MTHFR genome. That paper which has been submitted for publication is included as: **Association of 5,10-Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphisms with Autistic Spectrum Disorders**. The data they have continued to collect on siblings, parents and children with ASD and ADHD indicate remarkable consistence and support the hypothesis that ASD and ADHD share certain vulnerabilities to mercury toxicity and methionine transulfuration deficits. Their larger dataset represents over 400 individuals and the frequency mutation in the MTHFR genome is much higher than the reported or control group prevalence of the SNP. In the larger series of Boris and Goldblatt, 369 of 413(89.3%) were positive for at least one SNP in the MTHFR. As will be shown, this data completely agrees with our findings in the population of cysteine deficient children. While our numbers available at this writing are relatively small, they are significant, 14 of 15 (93%) of cysteine deficient children with encephalopathy presenting with features of ASD, have at least one SNP in the MTHFR gene. We have already collected specimens for genomic profiling on an additional 20 children and have expanded our survey genes to include methionine synthase (a B12 dependent enzyme) and other genes in the pathway. See the diagram below:



The abstract for Boris et al in their first data set is included here.

Abstract: 168 children referred to our facility with a confirmed diagnosis of autism or Pervasive Developmental Disabilities had blood specimens evaluated through PCR DNA techniques to determine the frequency of the C677T and A1298C allelic variants in the MTHFR gene. The homozygous mutant C677T allele (TT) frequency was 23% in the autistic children compared to 11% in the control population ($p < 0.0001$). The heterozygous C677T allele (CT) was present in 56% of the autistic children compared to 41% in the control population ($p < 0.0001$). The compound C677T and A1298C heterozygotes were more prevalent in the autistic population, 25%, than controls, 15% ($p = 0.01$). The normal A1298C allele was significantly higher in the autistic group, 55%, compared to the controls, 44% ($p < 0.05$). The data supports the hypothesis that autistic spectrum disorders are associated with genetic mutations in the MTHFR gene. Although the C677T variant allele is significantly higher and the A1298C variant allele is significantly lower, these associations alone can provide only a partial organic explanation of autistic spectrum disorders. A search for additional genetic and environmental risk factors should be undertaken. Nonetheless this study suggests that abnormalities in the folate-homocysteine metabolic pathway are associated with autistic spectrum disorders.

While our three groups find differences in the distributions in the MTHFR mutations, the pattern is consistent with the biochemical abnormalities observed by James and ICDRC. In her paper James found the following genomic profile:

Preliminary Genotyping Results:	
1. Methylenetetrahydrofolate Reductase (MTHFR) 677 C? T polymorphism	
Frequency in Control individuals:	11%
Frequency in Autistic Individuals:	16.6%
2. MTHFR 677 C? T /1298 A? C polymorphism	
Frequency in Control individuals:	15%
Frequency in Autistic Individuals:	25%
3. Glutathione-S-Transferase M1 null	
Frequency in Control individuals:	53%
Frequency in Autistic Individuals:	50%
4. Glutathione-S-Transferase T1 null	
Frequency in Control individuals:	14%
Frequency in Autistic Individuals:	29%
5. GST M1/GST T1 null	
Frequency in Control individuals:	8%
Frequency in Autistic Individuals:	16.6%
These preliminary results are consistent with a genetic weakness in the ability to detoxify environmental oxidative stressors and are consistent with the metabolic profile in Table 1 (above).	

Of additional concern, oxidative stress further inhibits the functioning of the methionine transsulfuration system (discussed in the paper by James). Oxidative stress has been consistently reported in autistic individuals (30,31). Thus, again we see a consistent pattern of mercury and neuronal vulnerability in this population.

Mercury Toxicology

A complete discussion of this is beyond the scope of this paper, but several critical issues need discussion. As previously reviewed by the IOM, Grandjean noted subtle cognitive changes in children exposed to “safe” levels of mercury (32). So by adding the now known issues in methionine transsulfuration and low plasma cysteine, we should expect at least subtle defects in motor coordination, and visual processing in the ASD population if mercury were causal in at least part of their symptoms.

These are all consistent findings in this population. Face processing, eye movement, crawling and rollover motion, attention, motor control, gait disturbances and perception are all effects of low dose mercury exposure and reported in ASD as well (33-38).

Clearly, three papers all published in January of 2004 add an extra measure of support for Thimerosal’s toxic role in neurodevelopment and autoimmunity. Waly and colleagues have defined a mechanism whereby extremely low levels of Thimerosal inhibit critical

pathways in membrane phospholipid methylation (39). This is highly significant given the already established defects in the body's main methylation pathway. A few quotes from this vital paper deserve highlighting here:

“Our studies also provide evidence that ethanol, heavy metals and the vaccine preservative Thimerosal potentially interfere with MS (methionine synthase) activation and impair folate-dependent methylation. Since each of these agents has been linked to developmental disorders, our findings suggest that impaired methylation, particularly impaired DNA methylation in response to growth factors, may be an important molecular mechanism leading to developmental disorders.”

“A single thimerosal-containing vaccination produces acute ethylmercury blood levels of 10– 30 nM,⁶⁴ and blood samples in 2-month-old infants, obtained 3–20 days after vaccination, contain 3.8–20.6nM ethylmercury.⁶⁵ Our studies therefore indicate the potential for thimerosal to cause adverse effects on MS activity at concentrations well below the levels produced by individual thimerosal-containing vaccines.”

Now we see that Toshiko et al, have demonstrated Thimerosal has nearly identical effects to methylmercury on cerebellar neurons and adjusting for actual mercury content in Thimerosal (apparently overlooked by the authors) would seem to make Thimerosal even more toxic (40). Again, it seems appropriate to quote the authors:

“The potency of thimerosal was similar to that of methylmercury in the presence of l-cysteine. Both agents at 1 microM or more similarly decreased the cellular content of glutathione in a concentration-dependent manner, suggesting an increase in oxidative stress. Results indicate that thimerosal exerts some cytotoxic actions on cerebellar granule neurons dissociated from 2-week-old rats and its potency is almost similar to that of methylmercury.”

Based on his published data, I assume Dr Singh will be discussing his observations regarding autoimmunity to various brain proteins and the relationship he finds to measles virus immune responsiveness. I will review MMR issues later, but new data on the effect of Thimerosal on autoimmunity deserves special attention here (41). The authors report the following:

“Thimerosal induces in genetically susceptible mice a systemic autoimmune syndrome very similar to that seen after treatment with inorganic mercury, although a higher absorbed dose of Hg is needed using thimerosal. The autoimmune syndrome induced by thimerosal is different from the weaker and more restricted autoimmune reaction observed after treatment with an equipotent dose of methylmercury...There exist no specific data for ethylmercury in humans, but it seems unlikely that males should absorb more mercury or be at higher risk for autoimmune manifestations than females. However, recent discussions regarding the autoimmune effect of mercury are not only, or even mainly, concerned with the risk of inducing de novo autoimmune condition, but further the possibility that mercury might accelerate or aggravate spontaneously occurring systemic autoimmune conditions”.

As the Geier's have published with the VAERS and will present with the VSD databases, when appropriate grouping of the Thimerosal related vaccines are examined, strong associations with Thimerosal and Autism and other neurodevelopmental disorders are quite apparent.

THE MMR ISSUE

Unfortunately and inappropriately, the burden of presenting the molecular virology on this subject falls to me alone. To adequately understand this subject and its criticality to a subgroup of children, the IOM **must** directly evaluate the published works of the Dr O'Leary's group in Dublin and the totality of the gastrointestinal findings as presented by the ICDRC and Royal Free groups.

With regard to the molecular virology of MV vaccine strain persistence in autism, Dr Kawashima has replicated this work for circulating lymphocytes and found MV vaccine strain in children with ASD. As part of the UK class action lawsuit, another university laboratory has been able to reproduce the findings of Drs Sheils and O'Leary in both CSF and Bowel. Unfortunately, and even though I have seen the data and the filed reports, the legal system in the UK has restricted discussion of the specifics outside of the proceedings. That is regrettable. I am aware of several replication studies underway in the US at this time. No one has published any data which refutes these findings and Dr Singh's data supports it.

There is also support from another source, the Ring and colleagues found a statistically significant occurrence of autism measles outbreaks. It is generally held that the attenuated virus can mimic wild-type diseases. This is generally observed in immune deficiency states.

As the IOM is aware, immune disorders are commonly described by numerous authors in the ASD population. Since the Ring paper is not on Medline I am inserting the abstract here and including the entire paper with my report.

Evidence for an infectious etiology in autism

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Abstract

Objective: Cyclical trends in birth rates that vary from general population patterns have been reported for several neuropsychiatric disorders. These data are used to test etiological hypotheses. Monthly and seasonal variations have been demonstrated for Autism possibly implicating an environmental pathogenic factor. We analyse data from Israel, focusing on viral epidemics. **Method:** Data covering a 30 year period (1960–1989), including general population live births, autistic births, and incidence of viral encephalitis, viral meningitis, rubella, measles, and poliomyelitis were collected from Israel's Ministry of Health, Central Bureau of Statistics and National League for Autism. Periodicity and correlations between variables were analyzed. **Results:** Over the period studied, 290 autistic births were evaluated. Spectral analysis using the cosine function demonstrated a significant periodicity (tau) of 17.6 years ($P = 0.011$) and near-significant periodicities of 3.2 years ($P = 0.07$) and 4.1 years ($P = 0.27$). Superposition of these three periods showed a highly significant fit to the annual autistic birth pattern ($r = 0.749$, $P = 0.042$). Multiple regression analysis of variables (encephalitis, meningitis, rubella, measles and poliomyelitis), yielded two significant findings: (a) during the period 1968–1989 the incidence of measles correlated with the rhythmicity of Autistic births ($r = 0.49$, $P = 0.036$); (b) during the entire study period the incidence of viral meningitis correlated with this rhythmicity ($r = 0.37$, $P = 0.042$). It is note worthy that the only risk factor demonstrating a cyclical trend was viral meningitis (tau = 3.0 years, $P = 0.032$). **Conclusions:** Cyclical trends are significant in Autistic births in Israel. Autistic birth patterns are partially explained by the rates of measles and viral meningitis in the general population. There is a statistically significant environmental association between autism and both viral meningitis and measles that should be further investigated. © 1997 Elsevier Science B.V.

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In a similar light, DeLong writes (42):

"In seeking the neurologic substrate of the autistic syndrome of childhood, previous studies have implicated the medial temporal lobe or the ring of mesolimbic cortex located in the mesial frontal and temporal lobes. During an acute encephalopathic illness, a clinical picture developed in three children that was consistent with infantile autism. This development was reversible. It was differentiated from acquired epileptic aphasia, and the language disorder was differentiated aphasia."

These data provide significant support for the MMR connection when combined with the new data presented below.

THE DOUBLE-HIT PHENOMENA- New Evidence of Causality

When last the IOM met on the MMR issue they reported that evidence of exposure/re-exposure worsening of symptoms would be strong evidence of a causal association. That evidence exists and has been submitted for publication. I will insert part of that paper here. Also, it should be noted that child 2 in the first CSF paper (below) was such a case. This ties together re-exposure and CSF MV persistence.

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Patients & Methods

Children with normal early development and autistic regression who had received more than one dose (“re-exposed”) of a polyvalent measles vaccine (MCV) (n=23) - were compared with once-exposed children. The latter were children with normal early development and autistic regression who had received only one MCV (n=23) – matched for sex, age and time-elapsd from first exposure to endoscopy. Comparisons included: secondary physical symptoms and observer-blinded scores of endoscopic and histological disease.

Results

Re-exposed scored significantly higher than once-exposed for: 2 physical symptoms (p<0.0001), including incontinence (p=0.009); presence of severe ileal lymphoid hyperplasia (p=0.002); acute inflammation including number of children affected (p<0.05), proportion of biopsies affected (p<0.001), greater severity (p<0.05); and epithelial damage (p<0.05).

Conclusion

The data identify a re-challenge effect on symptoms and a biological gradient effect on intestinal pathology, linking MCV exposure to regressive autism and enterocolitis.

MV Persistence in Children Presenting with ASD Symptoms

The first paper on this has been submitted and I will include a copy of it in the pre-publication format here, subject to the understanding that it may undergo subtle changes in the peer-review process. It was our belief that once O’Leary’s group documented viral persistence in one location, that we were obligated to look at CSF where possible in children who experienced regression following MMR. The analytical methods had evolved to a point by mid-2001 to make this possible.

This data has been presented to the US Congress and to Claude Allen, the Deputy Secretary of HHS with representatives of the CDC and Congressman Weldon present. Given the potential public health policy issues we have delayed publication to allow officials an opportunity to respond. Since it doesn’t appear a reaction from CDC is forthcoming despite nearly two years of waiting, we will proceed. The abstract is presented below:

Detection of Measles Virus Genomic RNA in Cerebrospinal Fluid in Children with Regressive Autism by TaqMan RT-PCR: A Report of Three Cases.

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SUBMITTED: JAN 28, 2003.

Abstract

In light of encephalopathy, presenting as autistic regression (autistic encephalopathy; AE) closely following MMR vaccination, three children underwent cerebrospinal fluid (CSF) assessments including studies for measles virus (MV). All three children had concomitant onset of gastrointestinal symptoms and had already had MV genomic RNA detected in biopsies of ileal lymphoid nodular hyperplasia (LNH). Presence of MV Fusion (F) gene was examined by TaqMan RT-PCR in cases and control CSF samples, obtained from three non-autistic MMR-vaccinated children with indwelling shunts for hydrocephalus. None of the cases or controls had a history of measles exposure, other than MMR vaccination. Serum and CSF samples were also evaluated for antibodies to MV and myelin basic protein (MBP). MV F-gene was present in CSF from all three cases, but not in controls; genome copy number ranged from 3.7×10^4 – 2.42×10^7 . Serum anti-MBP autoantibodies were detected in all children with AE. Anti-MBP and MV antibodies were detected in the CSF of two cases, while the third child had neither anti-MBP nor MV antibodies detected in his CSF. The findings are consistent with a MV etiology for the AE, and indicate the possibility of a virally driven cerebral immunopathology in some cases of regressive autism.

The abstract for our next paper (nearly submitted) is similar, but in this context we have expanded the observations to a much larger group and with significantly more controls. This paper has not yet been submitted, but it is not expected to undergo significant redrafting from the abstract perspective.

Abstract (for the larger series)

In light of encephalopathy, presenting in children as autistic regression closely following MMR vaccination and in view of our previous findings of MV genome in the CSF of this population, affected (ASD) children (n = 28) underwent lumbar puncture and examination of cerebrospinal fluid (CSF) for measles virus (MV) genomic RNA. Presence of MV Fusion (F) gene was examined by TaqMan RT-PCR. Control CSF

samples (n = 37) were obtained from children in remission from leukaemia (n = 20), children undergoing shunt insertion for hydrocephalus (n = 3) and young adults with either multiple sclerosis (n = 7) or encephalitis other than MV related (n = 7). All ASD cases and pediatric controls had received MMR vaccine. None had a history of wild-type measles infection. MV haemagglutinin (H) gene allelic discrimination (AD) assay was performed on cases where adequate MV amplicon was obtained. MV F-gene was present in CSF from 19 of 28 (68%) cases and in one of 37 (3%) controls (RR = 25.90; CI 3.96-181.58, p<0.00001). Where data were available on CSF (5 cases), allelic discrimination assay confirmed that the MV H-gene product was consistent with vaccine strain. The findings confirm a highly significant statistical association between the presence of MV RNA in CSF and autistic regression following MMR vaccination.

These papers stand atop the base of understanding built by O'Leary, Wakefield, Singh, and others. Since the epidemiology has tested the wrong hypotheses and since we have formidable evidence for an association, which is most likely causal in nature, there appears to be a subgroup of children experiencing significant disorders as a result of MMR. I trust Dr Singh to submit all of his papers, and I will contribute all of the pertinent Wakefield and O'Leary team publications.

IN SUMMARY

The criteria of causality for both Thimerosal and MMR in neurodevelopmental disorders appear to be adequately demonstrated. Further, although somewhat preliminary in nature, our database appears to contain sufficient evidence of a large overlap between the MTHFR-SNP, low cysteine population, elevated body burden of mercury, and measles virus persistence populations. It is further likely that the defects in the methionine transsulfuration pathway provide a possible link between Thimerosal and MMR observations. Additionally, with likely contribution from oxidative stress, nutritional deficiencies and environmental exposure to toxins like methylmercury, this may provide a central genomic/biochemical mechanism of certain neurodevelopmental disorders, while explaining the nature of vaccine susceptibility and injury in some cases.

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