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The biological basis of autism spectrum disorders: Understanding causation and treatment by clinical geneticists

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Autism spectrum disorders (ASDs), also known as pervasive developmental disorders (PDD), are a behaviorally defined group of neurodevelopmental disorders that are usually diagnosed in early childhood. ASDs disproportionately affect male children. Mercury (Hg), a heavy metal, is widespread and persistent in the environment. Mercury is a ubiquitous source of danger in fish, drugs, fungicides/herbicides, dental fillings, thermometers, and many other products. Elevated Hg concentrations may remain in the brain from several years to decades following exposure. This is important because investigators have long recognized that Hg is a neurodevelopmental poison; it can cause problems in neuronal cell migration and division, and can ultimately cause cell degeneration and death. Case-reports of patients have described developmental regressions with ASD symptoms following fetal and/or early childhood Hg exposure, and epidemiological studies have linked exposure to Hg with an elevated risk of a patient being diagnosed with an ASD. Immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs were reported following Hg intoxication with similarities extending to neuroanatomy, neurotransmitters, and biochemistry. The sexual dimorphism of ASDs may result from synergistic neurotoxicity caused by the interaction of testosterone and Hg; in contrast, estrogen is protective, mitigating the toxicity of Hg. Mercury exposure may significantly increase androgen levels, and as a result, patients diagnosed with an ASD may significantly benefit from anti-androgen therapy. Finally, the clinical geneticist has a wealth of biomarkers to evaluate and treat patients diagnosed with an ASD.

Key words: autistic, estradiol, ethylmercury, merthiolate, methylmercury, Thimerosal

INTRODUCTION

Autism spectrum disorders (ASDs), also known as pervasive developmental disorders (PDD), are a behaviorally defined group of neurodevelopmental disorders that are usually diagnosed in early childhood. ASDs disproportionately affect male children (roughly, 5 males per 1 female) (Austin 2008). ASDs are characterized by early onset of impairments in social interaction and communication, and the development of unusual stereotyped behaviors. Unable to learn from the natural environment as most children, the child diagnosed with an ASD generally shows little interest in the world or people around him/her. Although a few children with an ASD develop normal and even advanced skills in particular areas, most exhibit a wide range of profound behavioral problems and delayed or undeveloped skills. Further, a child diagnosed with an ASD may display a range of problem behaviors such as hyperactivity, poor attention, impulsivity, aggression, self injury and tantrums. In addition, many frequently display unusual responses to sensory stimuli such as hypersensitivities to light or certain sounds, colors, smells, or touch and have a high threshold of pain (Austin 2008). Further, common co-morbidity conditions often associated with an ASD diagnosis include gastrointestinal disease and dysbiosis (White 2003), autoimmune disease (Sweeten et al. 2003), and mental retardation (Bolte and Poustka 2002). Therefore, in the absence of treatment, an ASD is, in general, a lifelong developmental disability that profoundly affects the way a person comprehends, communicates and relates to others.

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Historically, autism is a new disorder only described by Dr. Leo Kanner from Johns Hopkins University among a cohort of children born in the early 1930s. Subsequently, autism remained a very rare disorder affecting less than 1 in 2 500 children. Starting in the late 1980s/early 1990s, significant increases in the prevalence rate of diagnosed ASDs were first observed in the United States (US). Researchers have been unable to fully explain the increasing rates of ASD diagnosis in the US by changing diagnostic criteria or improved diagnostic systems (Blaxill 2004, Hertz-Picciotto and Delwiche 2009). In January 2004, an Autism A.L.A.R.M. was issued by the American Academy of Pediatrics (AAP) and the US Centers for Disease Control and Prevention (CDC), stating that 1 in 166 children in the US suffers from an ASD, and far worse, that 1 in 6 children suffers from a developmental and/or behavioral disorder. The most recent survey data estimates that, for 8 year-old children born in the early 1990s, more than 1 in 150 children in the US may have an ASD diagnosis and that more than 1 percent of children may have an ASD diagnosis in certain regions of the US (Centers for Disease Control and Prevention 2007). These epidemic rates for ASD diagnoses in the US have apparently coincided with a sharp rise in fetal and infant exposures to mercury (Hg) (Austin 2008).

MERCURY EXPOSURE: IN REVIEW

Mercury, a heavy metal, is widespread and persistent in the environment. Exposure to hazardous Hg levels can cause permanent neurologic and renal impairment. Elemental Hg or inorganic Hg, is released into the air or water from sources such as coal-burning power plants or manufacturing facilities and it becomes methylated in the environment where it accumulates in animal tissues and increases in concentration through the food chain. The US population is primarily exposed to methyl-Hg by eating fish. The exposure to methyl-Hg is of greatest concern because fetuses are highly susceptible to Hg's adverse effects. In addition, numerous prescription and over-the-counter drugs have contained or continue to contain inorganic or organic forms of Hg. As a result, Hg is a ubiquitous source of danger in drugs for the eye, ear, nose, throat, and skin; in bleaching creams; as a preservative in cosmetics, tooth paste, lens solutions, vaccines, allergy test and immunotherapy solutions; in antiseptics, disinfectants, and contraceptives; in fungicides and herbicides; in dental fillings and thermometers; and many other products (Geier et al. 2008b).

The most recent data by Lederman and coworkers (2008) from the US CDC evaluated a cohort of several hundred individuals in New York. It was observed that about 6% of women of child bearing age had blood Hg levels above the safety limit (≥5.8 μg/L) established by the US Environmental Protection Agency (EPA) (Centers for Disease Control and Prevention 2004). Far worse, when evaluating the blood Hg levels present in new born babies, about 1 in 3 infants tested had blood Hg levels above the US EPA safety limit, and a number of infants had blood Hg levels above the level defined by the US CDC as the threshold level for Hg poisoning (≥10 μg/L) (Belson et al. 2005).

Furthermore, most infants in the US have and continue to receive additional doses of Hg following birth as part of the routine childhood vaccination schedule. The Hg compound added to vaccines to help prevent bacterial and fungal contamination in multi-dose vials is called Thimerosal. Thimerosal, an antiquated drug component which has never been adequately tested for its safety in humans, contains organic Hg (ethyl-Hg) and is about half Hg by weight (Geier et al. 2007).

During the mid-1980s, infants received a cumulative dose of 100 μg during the first 18 months from the 25 μg Hg in each DTP vaccine routinely administered at 2, 4, 6, and 18 months of age. Additionally, during this time, infants may have incurred additional Hg exposure through breast milk if they were born to mothers with Hg amalgam fillings and/or Rh-negative mothers, since many Rho(D)-immune globulin formulations, used to prevent isoimmunization in the Rho(D) negative individual exposed to Rho(D) positive fetal blood, contained Thimerosal (10.5 to >50 μg Hg/dose). Rho(D)-immune globulins were routinely recommended for administration to these mothers within 72 hours of birth (Geier et al. 2008b).

Starting in the late-1980s/early-1990s, the cumulative dose of Hg children received from Thimerosal-containing childhood vaccines/biologics almost tripled. Specifically, a Thimerosal-containing *Haemophilus influenza* type b (Hib) vaccine (25 μg Hg/dose) was recommended for routine administration at 2, 4, 6 and 18 months of age. Furthermore, a Thimerosal-containing hepatitis B vaccine (12.5 μg Hg/dose) was recommended for routine administration at birth, 2 and 6 months. As a result, an infant could have potentially received a cumulative dose of 237.5 μg Hg during the first 18 months of life. Furthermore, since many formulations of Rho(D)-immune globulins were Thimerosal-containing

(10.5 to >50 μg Hg/dose) and were recommended for routine administration to all Rh-negative pregnant women at 28 weeks of gestation starting in the late-1980s/early-1990s (in addition to the recommendation for its routine administration within 72 hours of birth), the cumulative dose of Hg received from Thimerosal-containing vaccines/biologics was certainly even higher for many US infants (Geier et al. 2008b).

By the summer of 1999, the realization that the Hg exposure American infants were incurring through the immunization schedule exceeded some, if not all, safety limits, caused alarm among both private health organizations and public agencies. On July 7, 1999, the US Public Health Service (USPHS) and the AAP issued a joint statement that urged "all government agencies to work rapidly toward reducing children's exposure to Hg from all sources". The statement recommended that Thimerosal be removed from vaccines as soon as possible as part of this overall process. Between 1999 and 2001, many of the Thimerosal-preserved vaccines recommended for children less than 6 years of age began to be made available in reduced-Thimerosal ("preservative free") formulations in the US (Geier et al. 2008b).

Despite the call, issued by the USPHS and the AAP, to reduce children's exposure to Hg from all sources, Thimerosal was re-introduced into the US routinely recommended childhood vaccine schedule in 2002 with recommendations to administer two doses of influenza vaccine in the first year of life (starting at 6 and 7 months of age) and to vaccinate all children who were 6 months to 23 months of age (Geier et al. 2008b). In addition, recommendations were made to vaccinate all pregnant women who would be in their 2nd or 3rd trimester of pregnancy during the US-"flu" season (December to March) as well as those who have medical conditions that might increase their risk for complications from influenza, regardless of the stage of pregnancy. It is important to note that the significant majority of influenza vaccines have contained and continue to contain Thimerosal (25 μg Hg/dose). Moreover, the 2002 recommendation has been continually expanded to the point that, in 2008, the US CDC recommended that all pregnant women should receive an influenza vaccine (without regard to the trimester of pregnancy) and that all infants should receive two doses of influenza vaccine in the first year of life, with one influenza vaccine administered on a yearly basis thereafter until a patient is 18 years-old (Geier et al. 2008b).

The administration of Thimerosal-containing vaccines to infants in the US was found to result in increased blood Hg levels (Stajich et al. 2000, Pichichero et al. 2008) with some infants having blood Hg levels in excess of the blood safety limit from the US EPA, as well as the blood Hg level defined by the US CDC as the threshold level for Hg poisoning. In addition, administration of Thimerosal-containing vaccines to infants in the US was found to induce hair Hg levels in excess of the hair Hg safety limit established by the US EPA for significant periods of time during the first several years of life (Redwood et al. 2001, Marques et al. 2007).

All told, researchers have reported that Hg exposures in early childhood from both potential environmental and vaccine sources resulted in some infants receiving in excess of 350 μg Hg during the first 6 months of life. It was estimated that about 50% of the total Hg doses to which some infants were exposed came from routinely recommended Thimerosal-containing childhood vaccines. The cumulative exposure resulted in infants receiving doses of Hg in excess of Hg exposure limits established by the US EPA, US CDC, US Food and Drug Administration (FDA), and Health Canada during key developmental periods during the first year of life (Bigham and Copes 2005).

MERCURY DISTRIBUTION AND PERSISTENCE FOLLOWING EXPOSURE

Furthermore, research studies in monkeys reported that significant, persistent Hg concentrations were present in the brain following administration of ethyl-Hg (Takahashi et al. 1971) and injection of Thimerosal-containing childhood vaccines comparable to the dosing schedule (weight- and age-adjusted) that US children received (Burbacher et al. 2005). In addition, it was observed that a significant fraction of Hg observed in the infant monkey's brain following administration of Thimerosal-containing childhood vaccines was found to not significantly decrease in concentration more than 120 days following the last dose administered to the infant monkeys. In addition, Olczak and coworkers (2009) observed persistent significant Hg levels in infant rat brains following administration of Thimerosal mimicking the dosing that US children received. Also, similar results were observed in infant monkey's brain following oral administration of low doses of methyl-Hg (Burbacher et al. 2005). Some

researchers have described that Hg may have the potential to remain in the brain from several years to decades following exposure (Sugita 1978).

BIOLOGICAL PLAUSIBILITY OF MERCURY INDUCED ASDS

The importance of persistent increased brain Hg levels stems from the fact that researchers have long recognized Hg is a neurodevelopmental poison (Clarkson et al. 1985). This means that Hg exposure can severely disrupt the normal neurodevelopmental processes in the human brain. As a result, Hg may cause problems in normal neuronal cell migration and division, as well as inducing neuronal cell degeneration, and ultimately cell death. Based upon this knowledge, for example, Nelson (1991) from the National Institute for Occupational Safety and Health (NIOSH) of the US CDC reported that organic Hg was among the compounds known to induce behavior disorders such as autism. Subsequently, other researchers reported the specific biological effects of Hg exposure on neuronal development to be compatible with brain pathology observed in autism (Faustman et al. 2000). In addition, published case-reports of patients have described developmental regressions with ASD symptoms following fetal and/or early childhood Hg exposure (Chrysochoou et al. 2003, Geier and Geier 2007b, Corbett and Poon 2008). Researchers reported that exposure to Hg can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs, and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry (Austin 2008, Geier et al 2008b). Finally, a scientific consensus statement developed by the Collaborative on Health and the Environment's Learning Developmental Disabilities Initiative (2008) on environmental agents associated with neurodevelopmental disorders declared there was no doubt Hg exposure causes learning and developmental disorders including conditions such as ASDs.

EPIDEMIOLOGICAL EVIDENCE OF A LINK BETWEEN MERCURY EXPOSURE AND ASDS

While epidemiology is not intended to provide an absolute demonstration of drug safety or harm to an individual, use of this academic discipline as a surveillance tool is appropriate in determining whether low levels of Hg exposure may have contributed to an increased risk for a child developing an ASD.

In order to investigate the relationship between levels of Hg exposure from Thimerosal-containing vaccines and ASDs, a meta-analysis epidemiological study was performed on data from the Vaccine Adverse Event Reporting System (VAERS) database (Geier and Geier 2006b). The VAERS is an epidemiological database that has been maintained by the US CDC since 1990 as a surveillance tool to evaluate vaccine safety. Using techniques developed and published by the US CDC, unique VAERS reports stating that a DTP or Thimerosal-containing DTaP was administered were assigned to the Hg exposed group, and the unique VAERS reports stating that DTPH or Thimerosal-free DTaP was given were assigned to the Hg unexposed group. The Biological Surveillance Summary reports from the US CDC, sorted by vaccine manufacturers, indicated that there were a total of 57 151 417 vaccine doses administered to children in the exposed group, those receiving additional doses of Hg from Thimerosal-containing vaccines (i.e. the DTP or Thimerosal-containing DTaP vaccines), and 47 985 230 vaccine doses administered to children in the unexposed group, those receiving lower doses of Hg from vaccines (i.e. the DTPH or Thimerosal-free DTaP vaccines). The following numbers of study-associated neurodevelopmental disorder adverse events were identified in VAERS: autism (133 reports), speech disorders (115 reports), mental retardation (143 reports), personality disorders (124 reports), thinking abnormalities (41 reports), ataxia (41 reports), and neurodevelopmental disorders in general (374 reports). It was observed that there were significantly increased adjusted risk ratios for neurodevelopmental disorder adverse events reported to VAERS in the exposed group, when compared to the unexposed group, for the outcomes of autism, speech disorders, mental retardation, personality disorders, thinking abnormalities, ataxia, and neurodevelopmental disorders in general. By contrast, none of the control adverse events (note: these were selected on an a priori as not biologically plausibly linked to an increased risk following additional doses of Hg from Thimerosal-containing vaccines) of conjunctivitis, febrile seizures or lymphadenopathy reported to VAERS had a significantly increased risk ratio in the exposed group when compared to the unexposed group.

Young and coauthors (2008) examined possible associations between neurodevelopmental disorders and exposure to Hg from Thimerosal-containing vac-

cines by evaluating automated medical records for patients in the US CDC's Vaccine Safety Datalink (VSD) database. A total of 278 624 subjects were identified in birth cohorts from 1990-1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of Disease, 9th revision (ICD-9) specific neurodevelopmental disorders (selected a priori as having a biologically plausible association with Hg exposure) and control outcomes (selected a priori as not having biologically plausible association with Hg exposure) were calculated. Exposure to Hg from Thimerosal-containing childhood vaccines was calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from Thimerosal-containing childhood vaccines. Consistent significantly increased rate ratios were observed for autism, ASDs, tic disorders, developmental disorder/learning disorder, attention deficit disorder, and emotional disorders with Hg exposure from Thimerosal-containing childhood vaccines. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from Thimerosal-containing childhood vaccines.

Gallagher and Goodman (2008) investigated the relationship between Hg exposure from Thimerosal-containing hepatitis B vaccination in 1,824 children age 1-9 years and developmental disability based upon examination of data from the US CDC's 1999-2000 National Health and Nutrition Survey. These researchers observed that the odds of developmental disabilities were about nine times greater for boys receiving Thimerosal-containing hepatitis B vaccinations than those who were unvaccinated. These researchers concluded that their study provided significant evidence for an association between Thimerosal-containing hepatitis B vaccination to US infants and an increased risk of developmental disabilities.

Marques and coworkers (2008) evaluated pre- and postnatal variables associated with neurodevelopment at 180 days in a cohort of 82 exclusively breastfed infants using principal component analysis. This multivariate method was applied to identify hierarchy and sets of interrelated variables. The principal component analysis yielded a significant inverse relationship between exposure to Hg from Thimerosal-containing childhood vaccines and neurodevelopment (measured by Gesell scores) in the areas of motor development, language development, adaptive development, and general development.

A recent study evaluated the potential adverse effects of prenatal Hg exposure from Thimerosal-containing Rho(D) immune globulins (TCRs) routinely administered to Rh-negative mothers in the US prior to 2002 (Geier et al. 2008c). It was hypothesized: (1) if prenatal Rho(D)-immune globulin preparation exposure was a risk factor for neurodevelopmental disorders then more children with neurodevelopmental disorders would have Rh-negative mothers compared to controls; and (2) if Thimerosal in the Rho(D)-immune globulin preparations was the ingredient associated with neurodevelopmental disorders, following the removal of Thimerosal from all manufactured Rho(D)-immune globulin preparations in 2002, the US frequency of maternal Rh-negativity among children with neurodevelopmental disorders should be similar to control populations.

Maternal Rh-negativity was assessed at two sites (Clinic A-Lynchburg, VA; Clinic B-Rockville and Baltimore, MD) among 298 Caucasian children with neurodevelopmental disorders and known Rh-status. As controls, maternal Rh-negativity frequency was determined from 124 Caucasian children (born 1987-2001) without neurodevelopmental disorders at Clinic A, and the Rh-negativity frequency was determined from 1 021 Caucasian pregnant mothers that presented for prenatal genetic care at Clinic B (1980–1989). Additionally, 22 Caucasian patients with neurodevelopmental disorders born from 2002 onwards (Clinics A and B) were assessed for maternal Rh-negativity. There were significant and comparable increases in maternal Rh-negativity among children with neurodevelopmental disorders (Clinic: A=24.2%), ASDs (Clinic: A=28.3%, B=25.3%), and attention-deficit-disorder/attention-deficit hyperactivity-disorder (Clinic: A=26.3%) observed at both clinics in comparison to both control groups (Clinic: A=12.1%, B=13.9%) employed. Children with neurodevelopmental disorders born post-2001 had a maternal Rh-negativity frequency (13.6%) similar to controls.

In contrast, some studies have failed to find a consistent significant association between Hg exposure from Thimerosal-containing childhood vaccines and neurodevelopmental disorders (Verstraeten et al. 2003, Thompson et al. 2007, Tozzi et al. 2009). For example, Thompson and colleagues (2007) reported among the 42 neuropsychological outcomes evaluated, they were

able to detect only a few significant associations between dose-dependent exposure to Hg from Thimerosal and deficits in the areas attention, executive functioning, and speech articulation, but concluded that their study does not support a causal association between early exposure to Hg from Thimerosal-containing vaccines/immune globulins and deficits in neuropsychological functioning at the age of 7 to 10 years because the associations observed were small and almost equally divided between positive and negative effects. As another example, Verstraeten and coworkers (2003) described significantly increased relative risks for tics and language delay with dose-dependent increasing cumulative exposure to Hg from Thimerosal-containing childhood vaccines, but the investigators concluded that no consistent significant associations were found between Thimerosal-containing childhood vaccines and neurodevelopmental disorders. Tozzi and others (2009) reported among 24 neuropsychological outcomes evaluated, increasing Hg exposure from Thimerosal-containing childhood vaccines was associated with significantly worse mean finger-tapping with the dominant hand test scores and Boston Naming test scores, but the investigators concluded the associations found, although statistically significant, might be attributable to chance, and their clinical relevance remains to be determined.

In yet another study, a prospective, blinded assessment was undertaken to examine the hypothesis that increased Hg exposure from maternal dental amalgams during pregnancy may significantly impact the severity of ASD diagnoses (Geier et al. 2009e). A total of 100 qualifying participants born from 1990-1999 and diagnosed with DSM-IV autism (severe) or ASD (mild) were prospectively recruited from patients presenting for outpatient genetic consultations at the Genetic Centers of America. Logistic regression analysis (age, gender, race, and region of residency adjusted) by quintile of maternal dental amalgams during pregnancy revealed the ratio of autism: ASD (severe: mild) was about 1 (no effect) for ≤5 amalgams and increased for ≥6 amalgams. Fitting the model with a binary maternal dental amalgam level during pregnancy revealed that subjects whose mothers had ≥6 amalgams during pregnancy were 3.2-fold significantly more likely to be diagnosed with autism (severe), in comparison to ASD (mild), than subjects whose mothers had ≤5 amalgams during pregnancy. This study concluded that elevated Hg exposure from maternal dental amalgams during pregnancy is associated with an elevated risk of increasing autism severity.

Finally, a series of epidemiological studies conducted in the US (in California, Louisiana, and Texas) and in South America have all also found significant associations between environmental sources of Hg exposure and ASDs (Counter et al. 2002, Palmer et al. 2006, 2009, Rury 2006, Windham et al. 2006). In the study published from California (supported by the US CDC), 283 children with ASDs and 657 controls, born in 1994 in the San Francisco Bay area, were examined (Windham et al. 2006). These researchers assigned exposure level by census tract of birth residence for 19 chemicals. Among these 19 chemicals to which children were exposed, Hg was found to be the single largest risk factor associated with ASDs. When comparing high Hg exposure relative to low Hg exposure, there was a significant increase of the risk, which was about double, for being diagnosed with an ASD. Furthermore, Palmer and coauthors (2009) demonstrated that distance from point sources of Hg exposure were significantly related to the risk of an individual being diagnosed with an ASD, even after adjustment of potential covariates. Furthermore, Schweikert and others (2009) undertook an evaluation in the US, on a state by state basis, of ASD prevalence among 3 to 5 year-old children from 2000 to 2006 and environmental Hg exposure levels from 1996 to 2006. These investigators observed that Hg concentration in the environment among children 1 year-old or younger had a significant association with ASD prevalence three years later.

ANIMAL MODELS OF MERCURY EXPOSURE INDUCED ASDS

Several researchers examined the potential adverse effects of administration of Thimerosal-containing childhood vaccines mimicking the US vaccination schedule (weight- and age-adjusted) to mouse (Hornig et al. 2004, Minami et al. 2010), rat (Olczak et al. 2009), and hamster (Laurente et al. 2007) animal systems. It was observed that pathological and clinical symptoms similar to those seen in patients diagnosed with an ASD were observed following the dosing regimen. For example, Hornig and coworkers (2004) from Columbia University observed that a genetically sensitive mouse strain exhibited many of the symptoms and altered brain structure observed in children diagnosed with ASDs, including: growth delay, reduced locomo-

tion, exaggerated response to novelty, decreased numbers of Purkinje cells, increased brain size, densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Laurente and colleagues (2007) found significantly decreased bodyweight and brain weight, and smaller stature in hamsters. They also observed damage to the hippocampus, cerebral cortex, and cerebellum (Purkinje cells and granulose cells); with decrease in neuronal density, neuronal necrosis, axonal demyelinization, and gliosis. In addition, research in rats showed impaired pain reactions (Olczak et al. 2009). Similar results were observed following low-dose methyl-Hg exposure during perinatal periods in several different animal model systems (Burbacher et al. 1990, Burke et al. 2006, Falluel-Morel et al. 2007, Bourdineaud et al. 2008, Fischer et al. 2008, Montgomery et al. 2008, Yochum and Wagner 2009).

CLINICAL EVIDENCE OF SUSCEPTIBILITY AND TOXICITY FROM MERCURY IN ASDS

With animal models demonstrating the toxic effect of low-dose Hg exposure upon the developing brain, and epidemiological studies suggesting a correlation between Hg exposure and the rate of ASDs, one must assess whether this association is theoretical or actual. This requires an assessment of Hg body-burden and/or detoxification between children diagnosed with an ASD and neurotypical controls. Among individuals diagnosed with an ASD relative to controls, data have demonstrated: increased brain Hg levels (Sajdel-Sulkowska et al. 2008); increased blood Hg levels (DeSoto and Hitlan 2007, Geier et al. in press); increased Hg levels in baby teeth (Adams et al. 2007); increased Hg levels in hair samples (Fido and Al-Saad 2005); increased urinary porphyrins-associated with Hg intoxication (Austin and Shandley 2008, Geier and Geier 2006c, 2007c, Nataf et al. 2006, 2008) increased Hg in urine/fecal samples (Bradstreet et al. 2003, Geier and Geier 2007b); and decreased of Hg through first baby haircuts (Holmes et al. 2003, Adams et al. 2008).

Furthermore, it was observed in blinded studies of children diagnosed with an ASD that the greater the Hg body burden (as measured by Hg-associated porphyrins), the more severely affected the child diagnosed with an ASD, as measured by a professional evaluation based upon the Childhood Autism Rating Scale (CARS) (Geier et al. 2009b, 2009b,c). It was also observed from regression analyses that the body burden of toxic metals, particularly Hg, as assessed by urinary excretion before and after detoxification therapy, was significantly related autism severity, as measured by a professional evaluation based on the Autism Diagnostic Observation Schedule (ADOS), among children diagnosed with an ASD (Adams et al. 2009).

In heavy metal toxicity, Hg binds to cysteine thiol (-SH) groups on intracellular proteins and inactivates their function. The cysteine-SH group of glutathione binds Hg and protects essential proteins from functional inactivation. The synthesis of glutathione has been directly linked to the rate of Hg excretion and cellular protection from Hg-induced damage. Individuals with genetic deficiencies in glutathione synthesis will be less able to excrete Hg and will be more sensitive to its adverse effects. Several studies in patients diagnosed with an ASD relative to controls demonstrate transsulfuration pathway abnormalities, including significant reductions in plasma cysteine, plasma sulfate, plasma reduced glutathione (GSH), and total glutathione levels. A series of studies showed that there were significant correlations between genetic changes associated with reduced functioning in Hg detoxification pathways (i.e. gene deletions/polymorphisms) in patients diagnosed with ASDs in comparison to controls (Geier et al. 2009a, 2009d). A significant increasing correlation was also found between increased body-burden of Hg as measured by urinary porphyrins and glutathione unavailability as measured by plasma oxidized glutathione (GSSG) (Geier et al. 2009b).

James and others(2009) utilized lymphoblastoid cells (LCLs) derived from children diagnosed with autism and from unaffected controls. These cells were used to assess relative concentrations of GSH and GSSG in cell extracts and isolated mitochondria as a measure of intracellular redox capacity. The results indicated that the GSH/GSSG redox ratio was decreased and percentage oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. Exposure to oxidative stress via Thimerosal exposure resulted in a greater decrease in the GSH/GSSG ratio and increase in free radical generation in autism compared to control cells. Acute exposure to physiological levels of nitric oxide decreased mitochondrial membrane potential to a greater extent in the autism LCLs, although GSH/GSSG and ATP concentrations were similarly decreased in both cell lines. These investigators concluded that it is plausible to hypothesize that exposures to prooxidant

environmental toxins, including Thimerosal, would have the greatest effect on individuals with a preexisting fragile redox homeostasis or depleted glutathione reserves due to concurrent infection, or who are simultaneously exposed to other prooxidant contaminants that in combination can reach a toxic threshold. These potentially vulnerable sub-populations need to be identified and evaluated independently because large population epidemiologic studies do not have the sensitivity to detect minor high-risk subpopulations.

CELLULAR MECHANISMS OF MERCURY-INDUCED ASDS

In considering the potential for an exposure to induce a disorder, it is important to demonstrate that the exposure can induce the pathological findings characterizing the disorder in an in vitro model system. Furthermore, estimating the amount of exposure necessary to induce the pathology observed in the disorder and evaluating this in the context of other exposures that may produce and/or contribute to the disorder is essential.

Recent studies have demonstrated that Hg, and in particular Thimerosal, was able to induce significant mitochondrial dysfunction, reduced cellular oxidative–reduction activity, cell death, and cellular degeneration in a concentration- and time-dependent fashion (Geier et al. 2009f). The LC50 for mitochondrial dysfunction following 24 h incubation with Thimerosal ranged between 9.7 and 337 nM. Additionally, following 48 hours of incubation with Thimerosal containing media, the LC50 for cell oxidative–reduction activity in the neuroblastoma cells studied was 7.6 nM Thimerosal. Furthermore, different cell types showed different levels of susceptibility to Thimerosal-induced cellular toxicity. The observed order of the sensitivity of the cell types studied to Thimerosal-induced cellular toxicity was: human fetal cells >human neuroblastoma cells >human astrocytoma cells (Geier et al. 2009f). Similarly, Parran and coworkers (2005) described the LC50 for cell death in SH-SY-5Y human neuroblastoma following incubation with Thimerosal (without serum) at 38.7 and 4.35 nM, at 24 and 48 hours, respectively. Yel and coworkers (2005) demonstrated that Thimerosal, in a concentration- and time-dependent manner, even in nanomolar concentrations as low as 25 nM (these researchers did not examine any concentrations of Thimerosal lower than 25 nM), significantly increased cell death in neuroblastoma cells. These researchers demonstrated that the cell death induced by Thimerosal was visually characterized by visual phenomena, including: nuclear morphology of apoptosis, vacuolization, and chromatin condensation and shrinking. Further, Humphrey and coauthors (2005) reported on mitochondrial mediated Thimerosal-induced apoptosis in SKN-SH human neuroblastoma cells. These researchers visually observed that low-concentration Thimerosal exposure rapidly induced neuronal cell degeneration characterized by alterations in membranes, characteristic of cellular blebbing seen in apoptosis. In addition, cell shrinkage, and detachment were also observed. Finally, researchers demonstrated that low-dose Thimerosal exposure rapidly inhibited important neurodevelopmental pathways in neurons (Waly et al. 2004, James et al. 2005, Ariano et al. 2006, Herdman et al. 2006, Lawton et al. 2007, Deth et al. 2008)

The concentrations of Thimerosal used in many of the aforementioned studies to induce significant neuronal cell toxicity are comparable with physiological levels known to be induced by fetal and early infant exposure to Hg from Thimerosal-containing biologics and vaccines (Burbacher et al. 2005). Further, the observed effects induced by Thimerosal are consistent with recently emerging evidence documenting the brain pathophysiology present in patients diagnosed with an ASD. For example, it was shown by Geier and coworkers (2009f) and James and others (2005) that human neuroblastoma cells were significantly more susceptible to Thimerosal-induced damage than human astrocytoma cells. Further, Toimela and Tahti (2004) evaluated co-cultures of neuroblastoma and astrocytoma cells following organic and inorganic Hg exposures. In co-cultures of neuroblastoma and astrocytoma cells, researchers visually observed that neuroblastoma cells were significantly damaged at Hg concentrations which had little or no adverse effects on the astrocytoma cells. Consistent with these observations, Lopez-Hurtado and Prieto (2008) found striking differences in the density of glial cells, the density of neurons and the number of lipofuscin-containing neurons in brain regions associated with the production and processing of speech when patients diagnosed with an ASD were compared to controls. Specifically, it was observed that the brains in the patients diagnosed with an ASD had significantly greater mean densities of glial cells in comparison to controls. By contrast, the density of neurons was significantly

decreased in the brain samples of patients diagnosed with autistic disorders in comparison with controls. It is important to note that visual images obtained from the brain samples of patients diagnosed with an ASD were virtually identical in morphology with those observed in co-cultures of neuroblastoma and astrocytoma cells exposure to Hg by Toimela and Tahti (2004). These researchers observed that patients diagnosed with an ASD had significantly increased numbers of lipofuscin-containing neurons in comparison to controls. The presence of lipofuscin in neurons is significant with regard to Hg toxicity, because lipofuscin is a depot for heavy metals such as Hg (Opitz et al. 1996). Finally, it was observed in several studies that significant mitochondrial dysfunction and impaired oxidative–reduction are significant mechanisms underlying neuronal cell damage induced by Thimerosal (Humphrey et al. 2005, James et al. 2005, Yel et al. 2005, Herdman et al. 2006, Geier et al. 2009f). Consistent with these observations, studies identified evidence for mitochondrial dysfunction and impaired oxidative–reduction in patients diagnosed with an ASD (Chauhaun and Chauhaun 2006).

AN EXPLANATION OF THE MALE/FEMALE RATIO IN ASDS

As was stated previously, ASDs disproportionately affect male children (roughly, 5 males per 1 female), and hence, any causal factors for ASDs must be able to explain this phenomena. In animal models and in human poisonings, males were found to be significantly more susceptible to Hg toxicity (including following exposure to Thimerosal) than females (Clarkson et al. 1985, Grandjean et al. 1998, Vahter et al. 2007, Branch, 2009). Also, in a series of tissue culture experiments, testosterone was able to potentiate the neuronal toxicity of Hg (including Thimerosal), whereas estrogen lessened the toxicity (Haley 2005). Further, increased testosterone and associated androgen metabolites were observed to occur in tissue culture, in animals, and in humans following low-dose Hg exposure (Freeman and Sangalang 1977, Veltman and Maines 1986, Barregard et al. 1994). Mercury was even shown to significantly reduce the functional activity of several enzymes, including: 21-hydroxylase (21-OH), hydroxysteroid sulfotransferase (HST), and aromatase that would be expected to raise testosterone and other androgen levels (Ryan and Carroll 1976, Veltman and Maines 1986, Gerstenberger et al. 2000, Xu et al. 2002). In addition, the enzyme HST is dependent upon sulfation, glutathione, and is inhibited by inflammation (Ryan and Carroll 1976, Kim et al. 2004). Finally, it was previously observed in pink disease, known to be primarily caused by the use of mercuric chloride teething powers in infants, that altered states of adrenocorticol secretion, excessive production of androgen hormones, and pseudohermaphroditism in infancy and childhood were common occurrences (Cheek et al. 1951) and Geier and coworkers (2010) observed significantly increased rate ratios for medically diagnosed ICD-9 premature puberty following additional doses of mercury exposure from Thimerosal-containing childhood vaccines in the VSD.

A series of clinical studies have examined androgen metabolites in patients diagnosed with an ASD. These studies have revealed hormonal patterns consistent with significantly elevated androgen levels in patients diagnosed with an ASD relative to controls. For example, Tordjman and coauthors (1997) examined a case series of patients diagnosed with an ASD relative to controls, and found that 1 in 3 pre-pubertal age children diagnosed with an ASD had significantly increased plasma testosterone levels relative to age- and sex-matched controls. Similarly, in a case series of patients diagnosed with an ASD, Geier and Geier (2006a) found that patients had significantly increased dehydroepiandrosterone (DHEA) and serum testosterone levels relative to age- and sex-specific normal laboratory reference ranges from the Laboratory Corporation of America (LabCorp). Subsequently, Geier and Geier (2007c) evaluated a moderate size cohort of patients diagnosed with an ASD (n=70) for androgen metabolites relative to age- and sex-specific laboratory reference ranges from LabCorp.

The results of the study showed significantly increased relative mean levels for serum testosterone (158%), serum free testosterone (214%), percent free testosterone (121%), DHEA (192%), and androstenedione (173%) among patients diagnosed with an ASD in comparison with controls. In addition, on an individual test basis, greater than 20% of ASD patients tested had levels greater than the laboratory age- and sex-specific reference range upper limit values for each androgen attribute examined. On the whole, it was observed that 81.4% (57 of 70) patients diagnosed with an ASD had at least one androgen that was greater than the pertinent upper limit for their laboratory

age- and sex-specific reference range. Additionally, among those with an ASD diagnosis, the female patients studied had a significantly increased overall relative percentage of mean levels for both serum testosterone and serum free testosterone, in comparison to male patients examined. In addition, others have observed that patients diagnosed with an ASD also have lower-than-expected 2nd to 4th digit (2D: 4D) ratios (Manning et al. 2001, de Bruin et al. 2006), which is correlated with higher ratios of fetal testosterone (FT) to fetal estradiol (Lutchmaya et al. 2004).

The hypothesis that biochemical blocks in the hormonal synthesis pathway of patients diagnosed with an ASD may result in increased testosterone and other androgens is supported by multiple different clinical observations. Testosterone production is significantly regulated by the conversion of DHEA to the storage metabolite of dehydroepiandrosterone-sulfate (DHEA-S) by the enzyme HST. Any significant decrease in the ability to make this conversion would be expected to result in elevated DHEA levels that could proceed through the androgen synthesis pathway to produce testosterone. Based upon the known increase in Hg body-burden, as well as dysfunction within the transsulfuration pathway (i.e. low sulfate and low glutathione) in patients diagnosed with an ASD, it is expected that DHEA-S levels should be significantly decreased. Strous and collaegues (2005) confirmed that patients diagnosed with an ASD had significantly lowered plasma DHEA-S levels in comparison to controls. This may have a significant impact on testosterone because most DHEA is supposed to be converted into DHEA-S. As a result, even slight perturbations in the conversion of DHEA to DHEA-S by HST may have a significant impact on increasing testosterone levels.

Also, consistent with the notion that elevated testosterone is the result of biochemical blockage within the androgen synthesis pathway, Geier and Geier (2007c) evaluated follicle-stimulating hormone (FSH) levels in patients diagnosed with an ASD relative to pertinent age- and sex-specific reference ranges, and found that the relative mean level of FSH (51%) was significantly decreased. This was observed despite the fact that these same patients had significantly elevated androgen levels as was described previously. Further, examination of the patients by head MRI (for pituitary tumors) and abdominal ultrasound (for adrenal tumors) was unable to account for the significant elevations observed in androgen levels. As a result, it was postulated that the pituitary in patients diagnosed with an ASD was attempting to down regulate androgen synthesis, but was unsuccessful because of significant biochemical blockage within the androgen synthesis pathway.

The importance of biochemical blockage within the androgen synthesis pathway and ASDs is further supported by genetic studies examining the clinical symptoms of patients with steroid sulfatase deficiency. Kent and others (2008) reported that X-linked ichthyosis (XLI) (steroid sulfatase deficiency) is caused by deletions or point mutations of the steroid sulfatase (STS) gene on chromosome Xp22.32. Deletions of this region can be associated with cognitive behavioral difficulties including autism. Animal work suggests the STS gene may be involved in attentional processes. Cases of XLI were recruited from families originally discovered fetuses with STS deficiency through a routine maternal screening program. Boys with XLI were assessed for attention deficit-hyperactivity disorder (ADHD) and autism using standardized questionnaires and interviews. Deletions of the STS gene were identified and characterized by analysis of genomic DNA and/or fluorescent in situ hybridization. The study examined 25 boys with XLI who were assessed for autism and ADHD. Forty percent fulfilled DSM-IV criteria for a diagnosis of ADHD, 80% of which were inattentive subtype. ADHD diagnoses were present in those with both deletions and presumed point mutations of STS. Additionally, five boys, from three unrelated families, fulfilled criteria for an autistic spectrum disorder or related language/communication difficulty. These investigators concluded that STS deficiency may be a risk factor for ADHD with predominantly inattentive symptoms and those with XLI deletions are also at increased risk of developing autism and related disorders.

The elevations in testosterone and other androgens with decreased levels of estradiol and other estrogens may also have other important biochemical functional consequences in patients diagnosed with an ASD. Elevated levels of testosterone, and possibly other androgen metabolites, may have a negative impact on the transsulfuration pathway. A series of studies demonstrated that testosterone administration at least partially blocks the conversation of homocysteine to cystathionine, whereas estrogen administration had the opposite effect. Additionally, researchers have shown significant positive correlations between homocysteine

and androstenedione levels and glutathione and DHEA-S levels in humans. Thus, high levels of androgens are expected to block the transsulfuration pathway. The apparent result, as demonstrated in those with an ASD diagnosis, is significantly increased homocysteine, *S*-adenosylhomocysteine (SAH), or adenosine levels, in comparison to controls (Geier and Geier 2007c, Geier et al. 2008b).

Prudova and coworkers (2007) reported that clearance of homocysteine via the transsulfuration pathway provides an endogenous route for cysteine synthesis and represents a quantitatively significant source of this amino acid needed for glutathione synthesis. Men have higher plasma levels of total homocysteine than do women, but the mechanism of this sex-dependent difference is not known. In this study, the researchers investigated regulation by testosterone of cystathionine β-synthase (CBS), which catalyzes the committing step in the transsulfuration pathway. These investigators reported that testosterone downregulates CBS expression via a posttranscriptional mechanism in an androgen-responsive human cell line. This diminution in CBS levels is accompanied by a decrease in flux through the transsulfuration pathway and by a lower intracellular glutathione concentration. The lower antioxidant capacity in testosterone-treated human cells increases their susceptibility to oxidative stress conditions. These results demonstrate regulation of the homocysteine-clearing enzyme, CBS, by testosterone and suggest the potential utility of targeting this enzymatic block to help control glutathione levels.

In putting these pieces together, environmental exposures (particularly Hg exposure) that adversely effect HST and the transsulfuration pathway can cause a cyclical biochemical interaction pattern to develop between the transsulfuration and androgen pathways that directly correlates with the biochemistry observed in those with an ASD diagnosis. As expected, this interaction pattern and androgen elevations are consistent with the behavioral/physical traits associated with or defining those who have an ASD diagnosis.

UNDERSTANDING THE TREATMENT OF HORMONAL DISTURBANCES IN ASDS

Elevated androgens in patients diagnosed with an ASD may have a very significant impact on the clinical symptoms observed. Investigators reported that individuals with an ASD tend to display a hypermasculine profile on many cognitive tasks. In addition, patients diagnosed with an ASD tend to show lower verbal and higher numerical intelligence. Some neuroanatomical studies comparing the brains of individuals with and without an ASD reveal structural differences associated with high levels of FT, including hemispheric asymmetries. Girls with abnormally high FT levels as a result of congenital adrenal hyperplasia (CAH) have a higher number of autistic traits than their unaffected sisters. Clinical examination of patients with an ASD has revealed that on average, girls with an ASD show a significant delay in the onset of menarche (excess androgens have been linked to menstrual problems) and are more likely to display elevated rates of testosterone-related disorders than neurotypical controls (Geier and Geier 2007c, Geier et al. 2008b). Dorn and others (1999) even observed a significant increase in ASD symptoms (including psychosocial problems such as increased social problems, increased social withdrawal, increased behavior problems, increased depression, increased internalizing/externalizing behaviors, reduced vocabulary, reduced verbal scale IQ, and reduced information/processing) among previously neurotypical children with premature adrenarche (with increased blood levels of DHEA and androstenedione) in comparison to matched control children with on-time adrenarche.

In clinically considering the apparent biochemical abnormalities in the androgen pathway among those having an ASD diagnosis, and the significant importance of abnormalities that the androgen pathway may have on the clinical symptoms observed in patients diagnosed with an ASD, some have suggested that therapies which address the steroid hormone pathways in ASD cases may help to improve clinical outcomes (Geier and Geier 2005). Recently, several studies with drugs having known anti-androgen effects including: leuprolide acetate, cyproterone acetate, spironolactone, risperidone, haloperidol, and pioglitazone were reported to have beneficial effects in ASDs. These studies noted that the therapies utilizing these drugs resulted in significant clinical ameliorations in hyperactivity/impulsivity, stereotypy, aggression, self injury, abnormal sexual behaviors, and/or irritability behaviors that frequently occur in those with an ASD diagnosis (Geier and Geier 2007c, Geier et al. 2008b).

Among the aforementioned drugs, leuprolide acetate appears to hold an especially interesting potential to significantly help patients diagnosed with an ASD

because of its previously reported ability to help significantly ameliorate many of the clinical symptoms present in those diagnosed with an ASD.

For example, Umathe and colleagues (2008b) described a correlation between the neuronal mechanism of anxiety and the neuroanatomic expression/neuromodulatory role of gonadotropin-releasing hormone (GnRH). These researchers investigated the influence of GnRH agonists and antagonist on the anxiety-like behavior of rats in the elevated plus-maze and social interaction tests. Leuprolide acetate administration significantly increased percentage of open arms entries, time spent in open arms, and time spent in social interaction in rats, and the observed anxiolytic effect of leuprolide acetate administration was comparable with diazepam (an anxiolytic medication). Further, in other animal studies it was observed that leuprolide acetate administration significantly improved hyperactivity (Umathe et al. 2008b) and depressive (Umathe et al. 2008c) conditions. In addition, Bryan and coauthors (2010) demonstrated that leuprolide acetate administration improved cognitive function in the Morris water maze and Y-maze tests. Importantly, these investigators observed pathways associated with improved cognition such as CaMKII and GluR1-Ser831 were up-regulated by leuprolide treatment.

Loosen and coworkers (1994) evaluated the effects of acute gonadal suppression on sexual function and behavior in a clinical trial conducted on eight normal men. Administration of a potent GnRH antagonist reduced levels of serum testosterone, luteinizing hormone, and follicle-stimulating hormone. These effects coincided with significant reductions in outward-directed aggression, sexual function, sexual desire, anger, and anxiety. Further, Giammanco and coauthors (2005) described an important role for elevated testosterone levels in aggressive behavior, and that administration of an antagonist of hypophysial GnRH reduced testosterone levels and aggressiveness in man.

Umathe and others (2008d) reported that GnRH and sex steroids are known to modulate the immune system. These investigators examined mice treated with leuprolide acetate to determine whether it could be useful to prevent stress-induced immunosuppression. Leuprolide prevented stress-induced decrease in relative weights of thymus, total leukocyte count, and sheep red blood cells challenged humoral and cell-mediated immune reaction, and thus, the investigators concluded that leuprolide acetate significant prevented stress-induced immunosuppression.

Mathias and his lab team (1998) conducted a double-blind, placebo controlled trial using leuprolide acetate (Lupron Depot), in the treatment of moderate to severe symptoms (especially abdominal pain and nausea) in patients with functional bowel disease (FBD). Pain is the hallmark of patients with FBD. Patients in the Lupron Depot-treated group showed consistent improvement in symptoms, including for abdominal pain and nausea compared to placebo. Patient quality of life assessments and global evaluations completed by both patient and investigators were highly significant compared to placebo. All reproductive hormone levels significantly decreased for both Lupron Depot-treated groups by week 4 and were significantly different compared to placebo at week 16. These investigators concluded that their study shows that leuprolide acetate is effective in controlling the debilitating symptoms of abdominal pain and nausea in patients with FBD.

In evaluating the effects of leuprolide acetate administration to patients diagnosed with an ASD, investigators have described their clinical experience following its administration to nearly 200 patients with an ASD diagnosis. Leuprolide acetate administration significantly lowered androgen levels and resulted in very significant overall clinical improvements in socialization, sensory/cognitive awareness, and health/physical/behavior skills, with few non-responders and minimal adverse clinical effects to the therapy. It was also observed that leuprolide acetate administration resulted in significant clinical ameliorations in hyperactivity/impulsivity, stereotypy, aggression, self injury, abnormal sexual behaviors, and/or irritability behaviors (Geier and Geier 2007c, Geier et al. 2008a).

CONCLUSIONS

The dramatic increase in the prevalence of diagnosed ASDs in recent years suggests external or environmental factors have increased. Examination of childhood vaccine schedules reveals a dramatic increase in Hg exposure beginning for the most part in the 1990s. In addition to vaccines, other sources, such as drugs, fish and other foods, dental amalgams, and air Hg sources (e.g. coal burning power) can add to the exposure. Mercury is known to accumulate in the brain, persist, and be lethal to neurons. Several studies

have shown that children with an ASD diagnosis have higher levels of Hg burden relative to neurotypical controls. Moreover, the neurological damage caused by Hg is consistent with the abnormalities found in the brains of children diagnosed with an ASD. Unfortunately, Hg not only directly kills brain cells but also disrupts the critical mechanisms and pathways needed to eliminate it. Mercury creates a pathophysiological environment that potentiates its toxicity by: (1) increasing the presence of androgens; (2) inhibiting

Table I

A summary of clinically available lab testing and clinically available drugs to treat such conditions among biomarkers associated with ASDs

Autism Biomarker	Clinical Laboratory Testing [LabCorp Test#][1]	Clinical Treatment Options
Porphyrins	Random Fractionated Urinary Porphyrins [120980]	Detoxification Therapy (DMSA, DMPS)
Transsulfuration	Homocysteine [706994], Cystathionine [911032], GSH [853002], Taurine [910844]	Methylcobalamin (vitamin B12), Folinic Acid, Pyroxidine (Vitamin B6)
Oxidative Stress/ Inflammation	Oxidative Stress Panel (Catecholamine, GSH, Lipid Peroxides, GSH-Px, SOD) [853047], Neopterin [140335]	ALDACTONE® (Spironolactone)
Hormones	Testicular Function Profile II (FSH, LH, Testosterone, Free Testosterone) [035113], DHEA [004101], DHEA-S [004697], Androstenedione [004705], Androstane Diol Glucuronide [140442], Dihydrotestosterone [500142], Estradiol [140244], Estrone [004564], Total Estrogens [004549]	LUPRON® (Leuprolide Acetate), ANDROCUR® (Cypertyrone Acetate), ALDACTONE® (Spironolactone)
Mitochondria	Carnitine [706500], Pyruvic Acid [004788], Lactic Acid [004770], Ammonia [007054]	CARNITOR® (L-Carnitine)
Genetic	Blood Chromosomes [052019], Chromosome Microarray [510002], DNA Rett Syndrome [511180], Angelman/Prader Willi Syndrome Methylation Assay [511210], Fragile X Syndrome [510065], MTHFR [511238], APOE [822098]	Genetic Counseling (Prenatal, Pediatric, Predictive)

(APOE) Apolipoprotein E; (DHEA) Dehydroepiandrosterone; (DHEA-S) Dehydroepiandrosterone-Sulfate; (DMP) 2,3-Dimercapto-1-propanesulfonic acid; (DMSA) Meso 2,3-dimercaptosuccinic acid; (FSH) Follicle-stimulating hormone; (GSH) Glutathione; (GSHPx) Glutathione Peroxidase; (LH) Luteinizing hormone; (MTHFR) Methylenetetrahydrofolate reductase; (SOD) Superoxide Dismutase

[1] Laboratory testing described is available from the Laboratory Corporation of America (LabCorp), and is covered by most major insurance companies.

the transsulfuration pathway; and (3) decreasing the production of glutathione which is essential for the elimination of Hg (all of these characteristics are interrelated through biochemical pathway interactions). By addressing the abnormal levels of androgens and using safe and effective medications, clinicians can significantly improve the androgen and transsulfuration pathways. As a result, the negative cycle of interaction between the androgen and transsulfuration pathways that is found to occur in patients diagnosed with an ASD can be significantly reversed. Further, such treatment may significantly help to improve the clinical symptoms of patients diagnosed with an ASD.

Overall, it is apparent that many patients diagnosed with an ASD have significant medical conditions that require evaluation and potential treatment. Table 1 summarizes the specific types of biomarkers that a clinical geneticist may employ to help evaluate and treatment patients diagnosed with an ASD (Geier and Geier 2008a). It is clear that as additional research is done, careful attention will be needed by the clinician to incorporate new testing and treatment options for the benefit of their patients on the autism spectrum.

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REFERENCES

Adams JB, Baral M, Geis E, Mitchell J, Ingram J, Hensley A, Zappia I, Newmark S, Gehn E, Rubin RA, Mitchell K, Bradstreet J, El-Dahr JM (2009) The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels. J Toxicol 2009: 532640.

Adams JB, Romdalvik J, Ramanujam VM, Legator MS (2007) Mercury, lead, and zinc in baby teeth of children with autism versus controls. J Toxicol Environ Health A 70: 1046–1051.

Adams JB, Romdalvik J, Levine KE, Hu LW (2008) Mercury in first-cut baby hair of children with autism versus typically-developing children. Toxicol Environ Chem 90: 739–753.

Ariano P, Erriquez J, Gilardino A, Ferraro M, Lovisolo D, Distasi C (2006) Calcium signals and the in vitro migration of chick ciliary ganglion cells. Cell Calcium 40: 63–71.

Austin D (2008) An epidemiological analysis of the 'autism as mercury poisoning' hypothesis. Int J Risk Saf Med 20: 135–142.

Austin DW, Shandley K (2008) An investigation of porphyrinuia in Australian children with autism. J Toxicol Environ Health A 71: 1349–1351.

Barregard L, Lindstedt G, Schutz A, Sallsten G (1994) Endocrine function in mercury exposed chloralkali workers. Occup Environ Med 51: 536–540.

Belson MG, Schier JG, Patel MM (2005) Case definitions for chemical poisoning. MMWR Recomm Rep 54: 1–24.

Bigham M, Copes R (2005) Thimerosal in vaccines: balancing the risk of adverse effects with the risk of vaccine-preventable disease. Drug Saf 28: 89–101.

Blaxill MF (2004) What's going on? The question of time trends in autism. Public Health Rep 119: 536–551.

Bolte S, Poustka F (2002) The relation between general cognitive level and adaptive behavior domains in individuals with autism with and without co-morbid mental retardation. Child Psychiatry Hum Dev 33: 165–172.

Bourdineaud JP, Bellance N, Benard G, Brethes D, Fujimura M, Gonzalez P, Marighetto A, Maury-Brachet R, Mormede C, Pedron V, Philippin JN, Rossignol R, Rostene W, Sawada M, Laclau M (2008) Feeding mice with diets containing mercury-contaminated fish flesh from French Guiana: a model for the mercurial intoxication of the Wayana Amerindians. Environ Health 7: 53.

Bradstreet J, Geier DA, Kartzinel JJ, Adams JB, Geier MR (2003) A case-control study of mercury burden in children with autistic spectrum disorders. J Am Phys Surg 8: 76–79.

Branch DR (2009) Gender-selective toxicity of Thimerosal. Exp Toxicol Pathol 61: 133–136.

Bryan KJ, Mudd JC, Richardson SL, Chang J, Lee HG, Zhu X, Smith MA, Casadesus G (2010) Down-regulation of serum gonadotropins is as effective as estrogen replacement at improving menopause-associated cognitive deficits. J Neurochem 112: 870–881.

Burbacher TM, Sackett GP, Mottet NK (1990) Methylmercury effects on the social behavior of Macaca fascicularis infants. Neurotoxicol Teratol 12: 65–71.

Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T (2005) Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing Thimerosal. Environ Health Perspect 113: 1015–1021.

Burke K, Cheng Y, Li B, Petrov A, Joshi P, Berman RF, Reuhl KR, DiCicco-Bloom E (2006) Methylmercury elicits rapid inhibition of cell proliferation in the developing brain and decreases cell cycle regulator, cyclin E. Neurotoxicology 27: 970–981.

Centers for Disease Control and Prevention (2004) Blood mercury levels in young children and childbearing-aged women-United States, 1999-2002. MMWR Morb Mortal Wkly Rep 53: 1018–1020.

Centers for Disease Control and Prevention (2007) Prevalence of autism spectrum disorders-autism and developmental disabilities monitoring network, 13 sites, United States, 2002. MMWR Surveill Summ 56: 12–28.

Chauhan A, Chauhan V (2006) Oxidative stress in autism. Pathophysiology 13: 171–181.

Chrysochoou C, Rutishauser C, Rauber-Luthy C, Neuhaus T, Boltshauser E, Superti-Furga A (2003) An 11-month-old boy with psychomotor regression and auto-aggressive behavior. Eur J Pediatr 162: 559–561.

Cheek DB, Hetzel BS, Hine DC (1951) Evidence of adrenal cortical function in pink disease. Med J Aust 2: 6–8.

Clarkson TW, Nordberg GF, Sager PR (1985) Reproductive and developmental toxicity of metals. Scand J Work Environ Health 11: 145–154.

Collaborative on Health and the Environment's Learning and Developmental Disabilities Initiative (2008) LDDI Scientific consensus statement on environmental agents associated with neurodevelopmental disorders. Seattle, WA

Corbett SJ, Poon CSC (2008) Toxic levels of mercury in Chinese infants eating fish congee. Med J Aust 188: 59–60.

Counter SA, Buchanan LH, Ortega F, Laurell G (2002) Elevated blood mercury and neuro-otological observations in children of the Ecuadorian gold mines. J Toxicol Environ Health A 65: 149–163.

de Bruin EI, Verheij F, Wiegman T, Ferdinand RF (2006) Differences in finger length ratio between males with autism, pervasive developmental disorder-not otherwise specified, ADHD, and anxiety disorders. Dev Med Child Neurol 48: 962–965.

DeSoto MC, Hitlan RT (2007) Blood levels of mercury are related to diagnosis of autism: a reanalysis of an important data set. J Child Neurol 22: 1308–1311.

Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M (2008) How environmental and genetic factors combine to cause autism: A redox/mythylation hypothesis. Neurotoxicology 29: 190–201.

Dorn LD, Hitt SF, Rotenstein D (1999) Biopsychological and cognitive differences in children with premature vs. on-time adrenarche. Arch Pediatr Adolesc Med 153: 137–146.

Falluel-Morel A, Sokolowski K, Sisti HM, Zhou X, Shors TJ, Dicicco-Bloom E (2007) Developmental mercury exposure elicits acute hippocampal cell death, reductions in neurogenesis, and severe learning deficits during puberty. J Neurochem 103: 1968–1981.

Faustman EM, Silbernagel SM, Fenske RA, Burbacher TM, Ponce RA (2000) Mechanisms underlying children's susceptibility to environmental toxicants. Environ Health Perspect 108(Suppl 1): 13–21.

Fido A, Al-Saad S (2005) Toxic trace elements in the hair of children with autism. Autism 9: 290–298.

Fischer C, Fredriksson A, Eriksson P (2008) Neonatal coexposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244: 157–165.

Freeman HC, Sangalang GB (1977) A study of the effects of methyl mercury, cadmium, arsenic, selenium, and a PCB, (Aroclor 1254) on adrenal and testicular steroidogeneses in vitro, by the gray seal Halichoerus grypus. Arch Environ Contam Toxicol 5: 369–383.

Gallagher C, Goodman M (2008) Hepatitis B triple series vaccine and developmental disability in US children aged 1-9 years. Toxicol Environ Chem 90: 997–1008.

Geier DA, Geier MR (2006a) A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. Horm Res 66: 182–188.

Geier DA, Geier MR (2006b) A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States. Neuro Endocrinol Lett 27: 401–413.

Geier DA, Geier MR (2006c) A prospective assessment of porphyrins in autistic disorders: a potential marker for heavy metal exposure. Neurotox Res 10: 57–64.

Geier DA, Sykes LK, Geier MR (2007a) A review of Thimerosal (Merthiolate) and its ethylmercury breakdown product: specific historical considerations regarding safety and effectiveness. J Toxicol Environ Health B Crit Rev 10: 575–596.

Geier DA, Geier MR (2007b) A case series of children with apparent mercury toxic encephalopathies manifest-

ing with clinical symptoms of regressive autistic disorders. J Toxicol Environ Health A 70: 837–851.

Geier DA, Geier MR (2007c) A prospective assessment of androgen levels in patients with autistic spectrum disorders: biochemical underpinnings and suggested therapies. Neuro Endocrinol Lett 28: 565–573.

Geier DA, Geier MR (2007d) A prospective study of mercury toxicity biomarkers in autistic spectrum disorders. J Toxicol Environ Health A 70: 1723–1730.

Geier DA, Geier MR (2008a) Autism spectrum disorder-associated biomarkers for case evaluation and management by clinical genetics. Expert Rev Mol Diagn 8: 671–674.

Geier DA, King PG, Sykes LK, Geier MR (2008b) A comprehensive review of mercury provoked autism. Indian J Med Res 128: 383–411.

Geier DA, Mumper E, Gladfelter B, Coleman L, Geier MR (2008c) Neurodevelopmental disorders, maternal Rh-negativity, and Rho(D) immune globulins: a multicenter assessment. Neuro Endocrinol Lett 29: 272–280.

Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Geier MR (2009a) A prospective study of transsulfuration biomarkers in autistic disorders. Neurochem Res 34: 386–393.

Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Nataf R, Geier MR (2009b) Biomarkers of environmental toxicity and susceptibility in autism. J Neurol Sci 280: 101–108.

Geier DA, Kern JK, Geier MR (2009c) A prospective blinded evaluation of urinary porphyrins verses the clinical severity of autism spectrum disorders J Toxicol Environ Health A 72: 1585–1591.

Geier DA, Kern JK, Geier MR (2009d) A prospective study of oxidative stress biomarkers in autistic disorders. Electron J Appl Psychol 5: 2–10.

Geier DA, Kern JK, Geier MR (2009e) A prospective study of prenatal mercury exposure from maternal dental amalgams and autism severity. Acta Neurobiol Exp (Wars) 69: 189–197.

Geier DA, King PG, Geier MR (2009f) Mitochondrial dysfunction, impaired oxidative-reduction activity, degeneration, and death in human neuronal and fetal cells induced by low-level exposure to Thimerosal and other metal compounds. Toxicol Environ Chem 91: 735–749.

Geier DA, Kern JK Geier MR (in press) Blood mercury levels in autism spectrum disorder: is there a threshold level? Acta Neurobiol Exp (Wars).

Geier DA, Young HA, Geier MR (2010) Thimerosal exposure and increasing trends of premature puberty in the Vaccine Safety Datalink. Indian J Med Res 131: 500–507.

Geier MR, Geier DA (2005) The potential importance of steroids in the treatment of autistic spectrum disorders and other disorders involving mercury toxicity. Med Hypotheses 64: 946–954.

Gerstenberger SL, Heimler I, Smies R, Hutz RJ, Dasmahapatra AK, Tripoli V, Dellinger JA (2000) Minimal endocrine alterations in rodents after consumption of lake trout (*Salvelinus namaycush*). Arch Environ Contam Toxicol 38: 371–376.

Giammanco M, Tabacchi G, Giammanco S, Di Majo D, La Guardia M (2005) Testosterone and aggressiveness. Med Sci Monit 11: RA136–RA145.

Grandjean P, Weihe P, White RF, Debesa F (1998) Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. Environ Res 77: 165–172.

Haley BE (2005) Mercury toxicity: genetic susceptibility and synergistic effects. Med Ver 2: 535–542.

Herdman ML, Marcelo A, Huang Y, Niles RM, Dhar S, Kiningham KK (2006) Thimerosal induces apoptosis in a neuroblastoma model via the cJun N-terminal kinase pathway. Toxicol Sci 92: 246–253.

Hertz-Picciotto I, Delwiche L (2009) The rise in autism and the role of age at diagnosis. Epidemiology 20: 84–90.

Holmes AS, Blaxill MF, Haley BE (2003) Reduced levels of mercury in first baby haircuts of autistic children. Int J Toxicol 22: 277–285.

Hornig M, Chian D, Lipkin WI (2004) Neurotoxic effects of postnatal thimerosal are mouse strain dependent. Mol Psychiatry 9: 833–845.

Humphrey ML, Cole MP, Pendergrass JC, Kiningham KK (2005) Mitochondrial mediated Thimerosal-induced apoptosis in a human neuroblastoma cell line (SK-N-SH). Neurotoxicology 26: 407–416.

James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW (2009) Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. FASEB J 23: 2374–2783.

James SJ, Slikker W 3rd, Melnyk S, New E, Pogribna M, Jernigan S (2005) Thimerosal neurotoxicity is associated with glutathione depletion: protection with glutathione precursors. Neurotoxicology 26: 1–8.

Kent L, Emerton J, Bhadravathi V, Weisblatt E, Pasco G, Willatt LR, McMahon R, Yates JR (2008) X-linked ichthyosis (steroid sulfatase deficiency) is associated with increased risk of attention deficit hyperactivity disorder, autism, and social communication deficits. J Med Genet 45: 519–524.

Kim MS, Shigenaga J, Moser A, Grunfeld C, Feingold KR (2004) Suppression of DHEA sulfotransferase (Sult2A1) during the acute-phase response. Am J Physiol Endocrinol Metab 287: 731–738.

Laurente J, Remuzgo F, Avalos B, Chiquinta J, Ponce B, Avendano R, Maya L (2007) Neurotoxic effects of thimerosal at vaccines doses on the encephalon and development in 7 days-old hamsters. Ann Fac Med (Lima, Peru) 68: 222–237.

Lawton M, Iqbal M, Kontovraki M, Lloyd Mills C, Hargreaves AJ (2007) Reduced tubulin tyrosination as an early marker of mercury toxicity in differentiating N2a cells. Toxicol In Vitro 21: 1258–1261.

Lederman SA, Jones RL, Caldwell KL, Rauh V, Sheets SE, Tang D, Viswanathan S, Becker M, Stein JL, Wang RY, Perera FP (2008) Relation between cord blood mercury levels and early child development in a World Trade Center cohort. Environ Health Perspect 116: 1085–1091.

Loosen PT, Purdon SE, Pavlou SN (1994) Effects on behavior of modulation of gonadal function in men with gonadotropin-releasing hormone antagonists. Am J Psychiatry 151: 271–273.

Lopez-Hurtado E, Prieto JJ (2008) A microscopic study of language-related cortex in autism. Am J Biochem Biotech 4: 130–145.

Lutchmaya S, Baron-Cohen S, Raggatt P, Knickmeyer R, Manning JT (2004) 2nd to 4th digit ratios, fetal testosterone and estradiol. Early Hum Dev 77: 23–28.

Manning JT, Baron-Cohen S, Wheelwright S, Sanders G (2001) The 2nd to 4th digit ratio and autism. Dev Med Child Neurol 43: 160–164.

Marques RC, Bernardi JV, Dorea JG, Bastos WR, Malm O (2008) Principal component analysis and discrimination of variables associated with pre- and post-natal exposure to mercury. Int J Hyg Environ Health 211: 606–614.

Marques RC, Dorea JG, Fonseca MF, Bastos WR, Malm O (2007) Hair mercury in breast-fed infants exposed to Thimerosal-preserved vaccines. Eur J Pediatr 166: 935–941.

Mathias JR, Clench MH, Abell TL, Kock KL, Lehman G, Robinson M, Rothstein R, Snape WJ (1998) Effect of leuprolide acetate in treatment of abdominal pain and nausea in premenopausal women with functional bowel disease: a double-blind, placebo-controlled, randomized study. Dig Dis Sci 43: 1347–1355.

Minami T, Miyata E, Sakamoto Y, Yamazaki H, Ichida S (2010) Induction of metallothionein in mouse cerebellum and cerebrum with low-dose Thimerosal injection. Cell Biol Toxicol 26: 143–152.

Montgomery KS, Mackey J, Thuett K, Ginestra S, Bizon JL, Abbott LC (2008) Chronic low-dose prenatal exposure to methylmercury impairs motor and mnemonic function in adult C57/B6 mice. Behav Brain Res 191: 55–61.

Nataf R, Skorupka C, Amet L, Lam A, Springbett A, Lathe R (2006) Porphyrinuria in childhood autistic disorder: implications for environmental toxicity. Toxicol Appl Pharmacol 214: 99–108.

Nataf R, Skorupka C, Lam A, Springbett A, Lathe R (2008) Porphyrinuria in childhood autistic disorder is not associated with urinary creatinine deficiency. Pediatr Int 50: 528–532.

Nelson BK (1991) Evidence for behavioral teratogenicity in humans. J Appl Toxicol 11: 33–37.

Olczak M, Duszczyk M, Mierzejewski P, Majewska MD (2009) Neonatal administration of a vaccine preservative, Thimerosal, produces lasting impairment of nociception and apparent activation of opioid system in rats. Brain Res 1301: 143–151.

Opitz H, Schweinsberg F, Grossmann T, Wendt-Gallitelli MF, Meyermann R (1996) Demonstration of mercury in the human brain and other organs 17 years after metallic mercury exposure. Clin Neuropathol 15: 139–144.

Palmer RF, Blanchard S, Stein Z, Mandell D, Miller C (2006) Environmental mercury release, special education rates, and autism disorder: an ecological study of Texas. Health Place 12: 203–209.

Palmer RF, Blanchard S, Wood R (2009) Proximity to point sources of environmental mercury release as a predictor of autism prevalence. Health Place 15: 18–24.

Prudova A, Albin M, Bauman Z, Lin A, Vitvitsky V, Banerjee R (2007) Testosterone regulation of homocysteine metabolism modulates redox status in human prostate cancer cells. Antioxid Redox Signal 9: 1875–1881.

Parran DK, Barker A, Ehrich M (2005) Effects of Thimerosal on NGF signal transduction and cell death in neuroblastoma cells. Toxicol Sci 86: 132–140.

Pichichero ME, Gentile A, Giglio N, Umido V, Clarkson T, Cernichiari E, Zareba G, Gotelli C, Gotelli M, Yan L, Treanor J (2008) Mercury levels in newborns and infants after receipt of Thimerosal-containing vaccines. Pediatrics 121: e208–e214.

Redwood L, Bernard S, Brown D (2001) Predicted mercury concentrations in hair from infant immunizations: cause for concern. Neurotoxicology 22: 691–697.

Rury J (2006) Links between environmental mercury, special education, and autism in Louisiana (dissertation). Baton Rouge (LA): Louisiana State University.

Ryan RA, Carroll J (1976) Studies on a 3β-hydroxysteroid sulphotransferase from rat liver. Biochim Biophys Act 429: 391–401.

Sajdel-Sulkowska EM, Lipinski B, Windom H, Audhya T, McGinnis W (2008) Oxidative stress in autism: elevated

cerebellar 3-nitrotyrosine levels. Am J Biochem Biotech 4: 73–84.

Schweikert C, Li Y, Dayya D, Yens D, Torents M, Hsu F (2009) Analysis of autism prevalence and neurotoxins using combinatorial fusion and association rule mining. Ninth IEEE Computer Science International Conference on Bioinformatics and Bioengineering, 400–404.

Stajich GV, Lopez GP, Harry SW, Sexson WR (2000) Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. J Pediatr 136: 679–681.

Strous RD, Golubchik P, Maayan R, Mozes T, Tuati-Werner D, Weizman A, Spivak B (2005) Lowered DHEA-S plasma levels in adult individuals with autistic disorder. Eur Neuropsychopharmacol 15: 305–309

Sugita M (1978) The biological half-time of heavy metals. The existence of a third, "slowest" component. Int Arch Occup Environ Health 41: 25–40.

Sweeten TL, Bowyer SL, Posey DJ, Halberstadt CM, McDougle CJ (2003) Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. Pediatrics 112: e420.

Takahashi T, Kimura T, Sato Y, Shiraki H, Ukita T (1971) Time-dependent distribution of ^{203}Hg-mercury compounds in rat and monkey as studied by whole body autoradiography. J Hyg Chem 17: 93–107.

Thompson WW, Praice C, Goodson B, Shay DK, Benson P, Hinrichsen VL, Lewis E, Eriksen E, Ray P, Marcy SM, Dunn J, Jackson LA, Lieu TA, Black S, Stewart G, Weintraub ES, Davis RL, DeStefano F; Vaccine Safety Datalink Team (2007) Early Thimerosal exposure and neuropsychological outcomes at 7 to 10 years. N Engl J Med 357: 1281–1292.

Toimela T, Tahti H (2004) Mitochondrial viability and apoptosis induced by aluminum, mercuric mercury and methylmercury in cell lines of neural origin. Arch Toxicol 78: 565–574.

Tordjman S, Ferrari P, Sulmont V, Duyme M, Roubertoux P (1997) Androgenic activity in autism. Am J Psychiatry 154: 1626–1627.

Tozzi AE, Bisiacchi P, Tarantino V, De Mei B, D'Elia L, Chiarotti F, Salmaso S (2009) Neuropsychological performance 10 years after immunization in infancy with Thimerosal-containing vaccines. Pediatrics 123: 475–482.

Umathe SN, Bhutada PS, Dixit PV, Jain NS (2008a) Leuprolide: a luteinizing hormone releasing hormone agonist attenuates ethanol withdrawal syndrome and ethanol-induced locomotor sensitization in mice. Neuropeptides 42: 345–353.

Umathe SN, Bhutada PS, Jain NS, Dixit PV, Wanjari MM (2008b) Effects of central administration of gonadotropin-releasing hormone agonists and antagonist on elevated plus-maze and social interaction behavior in rats. Behav Pharmacol 19: 308–316.

Umathe SN, Bhutada PS, Jain NS, Shukla NR, Mundhada YR, Dixit PV (2008c) Gonadotropin-releasing hormone agonist blocks anxiogenic-like and depressant-like effect of corticotrophin-releasing hormone in mice. Neuropeptides 42: 399–410.

Umathe SN, Dixit PV, Wanjari MM, Ullewar MP (2008d) Leuprolide-a GnRH agonist-prevents restraint stress-induced immunosuppression via sex-steroid independent peripheral mechanism in mice. Int Immunopharmacol 8: 71–79.

Vahter M, Akesson A, Liden C, Ceccatelli S, Berglund M (2007) Gender differences in the disposition and toxicity of metals. Environ Res 104: 85–95.

Veltman JC, Maines MD (1986) Alternations of heme, cytochrome P-450, and steroid metabolism by mercury in rat adrenal. Arch Biochem Biophys 248: 467–478.

Verstraeten T, Davis RL, DeStefano F, Lieu TA, Rhodes PH, Black SB, Shinefield H, Chen RT; Vaccine Safety Datalink Team (2003) Safety of Thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases. Pediatrics 112: 1039–1048.

Waly M, Olteanu H, Banerjee R, Choi SW, Mason JB, Parker BS, Sukumar S, Shim S, Sharma A, Benzecry JM, Power-Carnitsky VA, Deth RC (2004) Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and Thimerosal. Mol Psychiatry 9: 358–370.

White JF (2003) Intestinal pathophysiology in autism. Exp Biol Med 228: 639–649.

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK (2006) Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay area. Environ Health Perspect 114: 1438–1444.

Xu F, Suiko M, Sakakibara Y, Pai TG, Liu MC (2002) Regulatory effects of divalent metal cations on human cytosolic sulfotransferases. J Biochem 132: 457–462.

Yel L, Brown LE, Su K, Gollapudi S, Gupta S (2005) Thimerosal induces neuronal cell apoptosis by causing cytochrome c and apoptosis-inducing factor release from mitochondria. Int J Mol Med 16: 971–977.

Yochum CL, Wagner GC (2009) Autism and Parkinson's disease: animal models and a common etiological mechanism. Chin J Physiol 52: 236–249.

Young HA, Geier DA, Geier MR (2008) Thimerosal exposure in infants and neurodevelopmental disorders: an assessment of computerized medical records in the Vaccine Safety Datalink. J Neurol Sci 271: 110–118.