REVIEW

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# Autism spectrum disorder and mercury toxicity: use of genomic and epigenetic methods to solve the etiologic puzzle

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Autism spectrum disorder (ASD) is an increasingly prevalent neurodevelopmental condition of unknown etiology. Mercury is a common, highly neurotoxic heavy metal. The similarities of neurologic manifestations of mercury exposure and ASD raise an intriguing hypothetical question: Is ASD, at least partially, a manifestation of mercury toxicity? The fetus is particularly vulnerable to mercury exposure from the "double jeopardy" combination of the genetics of his mother and his own genetics, as relates to mercury toxicity. In this paper, I review the evidence suggesting relationships between ASD and mercury toxicity. I suggest ways to confirm these relationships with genetic and epigenetic research. I propose a hypothesis associating mercury toxicity with ASD. This may present opportunities for further research in prevention and treatment of ASD.

Key words: autism, autism spectrum disorder, mercury, toxicity

## INTRODUCTION

Autism spectrum disorder (ASD) is an increasingly prevalent neurodevelopmental condition of unknown etiology. Importantly, reported manifestations of low-level exposure to mercury, a highly neurotoxic heavy metal, are similar to those of ASD. In the past several years, evidence has accumulated linking the risk of toxic manifestations of low-level mercury exposure to particular genes, mutations, and epigenetic changes. The fields of genomics, genetics, and epigenetics are rapidly developing, which has led to the creation of new methods and tools that can be applied to investigate heritable conditions and diseases. These methods and tools are particularly well suited for seeking genetic predispositions to toxicity from exposures and adverse manifestations of those exposures, such as mercury and ASD. In the present paper, we examine the evidence linking ASD with mercury toxicity. We present a plausible etiologic mechanism of ASD causation, consisting of prenatal mercury exposure together with maternal and fetal genetic propensities, causing an elevated risk of subsequent neurotoxicity via a "double jeopardy". This analysis will suggest means of confirming or refuting relationships between mercury exposure and ASD through targeted clinical investigations that utilize these new genomic, genetic, and epigenetic methods and tools.

In various degrees of severity, ASD has been recognized for three quarters of a century as a constellation of neurologically debilitating symptoms in children. These symptoms begin in infancy or early childhood and progress through adulthood (Kanner, 1995). ASD includes various manifestations of neurologic symptoms and findings, with specific identifiable characteristics and various degrees of severity. ASD has been previously known as autism, Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder. Despite the large body of peer reviewed publications on ASD, a paradigm that explains the well described clinical findings of ASD is lacking.

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Mercury is a markedly toxic metal that is prevalent in our environment as a result of both natural processes and human activity. A number of factors have pointed to a potential role of mercury in ASD. Some of these factors are:

1) The marked similarity of manifestations of mercury toxicity and of ASD.
2) The ubiquity of exposure to mercury.
3) The increasing prevalence and incidence of ASD, at a time when exposure to mercury and its presence in the environment have shown similar increases.
4) The large number of peer reviewed papers published in the scientific literature demonstrating plausible and logical means and mechanisms that implicate a causal role for mercury in ASD.
5) The lack, at this time, of a competing etiologic agent or mechanism supporting a paradigm that explains the ultimate cause of ASD.
6) The Apolipoprotein E (ApoE) system being linked with both mercury toxicity and ASD.
7) Genomic studies which account for observations that similar levels of mercury affect different people to different degrees.

It is important to recognize the confounding role that economic, emotional, and social factors play in evaluating the role of mercury as an important potential cause of ASD. These potential confounding roles are exacerbated by the key role that mercury plays in industry, pharmaceuticals, dentistry, and medicine. If mercury were to be established as a dangerous substance in our environment, even at tiny doses, the costs involved in its elimination would be economically challenging to bear. The devastating consequences of ASD to patients, families, and society at large are likewise catastrophic and not bearable if there is any way to mitigate or avoid them. The presence of a - by some estimates - likely etiologic factor in ASD necessitates action to identify and combat that factor. It is the responsibility of the scientific community at large to reject economic, emotional, and social influences and to proceed in an evidence-based manner in the search for truth.

It is widely recognized that younger, more rapidly developing brains are more vulnerable to toxic exposures, particularly to mercury exposure (Hewitson et al., 2010; Ida-Eto et al., 2011; Olczak et al., 2011; Sulkowski et al., 2012; Blanusa et al., 2012; Ida-Eto et al., 2013). In a 1997 publication, Grandjean et al. note that prenatal exposure to methylmercury is associated with significant detectable neurologic performance deficits at age 7, as measured via neuropsychological evaluation. These neurologic deficits are observed in the domains of language, attention, memory, visuospatial and motor functions. Of note, levels of mercury exposure in that study were of a degree that is considered safe (Grandjean et al., 1997). Later, in a 2006 paper, Grandjean et al. summarize the toxicities of mercury and other industrial chemicals, noting their propensities to cause neurodevelopmental disorders. Also noted is the greatly increased hazard of these agents for developing brains, both *in utero* and in early childhood (Grandjean and Landrigan, 2006). In one study, in a population with low prenatal degrees of mercury exposure, there was nonetheless a mercury-related IQ decrement with higher levels of mercury exposure. However, this effect was observed only in the higher socio-economic segment of the population. In addition, genetic polymorphisms in children contributed to neurotoxic effects of mercury, although these effects were mild (Julvez et al., 2013). Another more recent study showed adverse motor outcomes at age 2 years that were related to prenatal mercury exposure. In that study, genetic predisposition for mercury toxicity or ASD risk was not assessed (Barbone et al., 2019). In a 2017 study, Prpic et al. reported a decrement in fine motor skills at age 18 months in subjects who had increased prenatal methylmercury exposure, as measured via cord blood methylmercury levels. Genetic risk for mercury toxicity was not evaluated in that study (Prpic et al., 2017).

A considerable number of papers have reported evidence suggesting that mercury toxicity is a cause of ASD manifestations. In a 2001 publication, Bernard et al., articulated the similarities between mercury toxicity and ASD manifestations. These authors concluded that "autism represents an unrecognized mercurial syndrome". In this paper, the authors also present evidence for thimerosal as a source for the brain damaging mercury (Bernard et al., 2001). Table II in the Bernard et al. (2001) paper (presented here, as Table I, with permission) summarizes those similarities.

In 2004, Geier and Geier evaluated epidemiological data from the US Centers for Disease Control and Prevention (CDC) and datasets from US Department of Education datasets. The authors demonstrated a correlation between higher mercury exposure through vaccine use, and an increasing incidence of ASDs. Based on these data, the authors claim that "there is biological plausibility and epidemiological evidence showing a direct relationship between increasing doses of mercury from Thimerosal-containing vaccines and neurodevelopmental disorders" (Geier and Geier, 2004).

Multiple studies have found evidence of abnormal oxidation, impaired methylation, and enzymatic dysfunction in ASD populations. In a 2004 publication, James et al. reported impaired methylation and increased oxidative stress in a cohort of 20 children with autism compared to 33 control children. The authors note that these metabolic abnormalities may contribute to the manifestation of autism (James et al., 2004).

Importantly, these metabolic abnormalities are similar to those of mercury toxicity. In a 2006 report, James et al. again noted that methylation capacity was reduced in autistic children, based on the ratio of S-adenosylmethionine to S-adenosylhomocysteine. Antioxidant capacity and redox homeostasis were likewise decreased based on levels of cysteine, glutathione, and oxidized glutathione. A variety of allele frequencies and polymorphisms were noted in relevant genes that would be expected to impact these capabilities. The authors noted "that an increased vulnerability to oxidative stress (endogenous or environmental) may contribute to the

Table I. Summary comparison of biological abnormalities in autism and mercury exposure.

| Mercury exposure | Autism |
|---|---|
| *Biochemistry* | |
| Binds -SH groups; blocks sulfate transporter in intestines and kidneys | Low sulfate levels |
| Reduces glutathione availability; inhibits enzymes of glutathione metabolism; glutathione is needed in neurons, cells, and liver to detoxify heavy metals; reduces glutathione peroxidase and reductase | Low levels of glutathione; decreased ability of liver to detoxify xenobiotics; abnormal glutathione peroxidase activity in erythrocytes |
| Disrupts purine and pyrimidine metabolism | Purine and pyrimidine metabolism errors lead to autistic features |
| Disrupts mitochondrial activities, especially in brain | Mitochondrial dysfunction, especially in brain |
| *Immune system* | |
| Sensitive individuals more likely to have allergies, asthma, and autoimmune-like symptoms (particularly rheumatoid-like ones) | More likely to have allergies and asthma; familial presence of autoimmune diseases (particularly rheumatoid arthritis); lgA deficiencies |
| Can produce an immune response in CNS; causes brain/MBP autoantibodies | Ongoing immune response in CNS; brain/MBP autoantibodies present |
| Causes overproduction of Th2 subset; kills/inhibits lymphocytes, T-cells, and monocytes; decreases NK T-cell activity; induces or suppresses IFNg & IL-2 | Skewed immune-cell subset in the Th2 direction; decreased responses to T-cell reduced NK T-cell function; increased IFNg & IL-12 |
| *CNS structure* | |
| Selectively targets brain areas that are unable to detoxify or reduce Hg-induced oxidative stress | Specific areas of brain pathology; many functions spared |
| Accumulates in amygdala, hippocampus, basal ganglia, cerebral cortex; damages Purkinje and granule cells in cerebellum; brain stem defects in some cases | Pathology in amygdala, hippocampus, basal ganglia, cerebral cortex; damage to Purkinje and granule cells in cerebellum; brain stem defects in some cases |
| Causes abnormal neuronal cytoarchitecture; disrupts neuronal migration, microtubules, and cell division; reduces NCAMs | Neuronal disorganization; increased neuronal cell replication increased glial cells; depressed expression of NCAMs |
| Progressive microcephaly | Progressive microcephaly and macrocephaly |
| *Neurochemistry* | |
| Prevents presynaptic serotonin release and inhibits serotonin transport; causes calcium disruptions | Decreased serotonin synthesis in children; abnormal calcium metabolism |
| Alters dopamine systems; pyridoxine deficiency in rats resembles mercurialism in humans | Either high or low dopamine levels; positive response to pyridoxine, which lowers dopamine levels. Elevated norepinephrine and epinephrine |
| Elevates epinephrine and norepinephrine levels by blocking enzyme that degrades epinephrine | Elevated glutamate and aspartate |
| Elevates glutamate | Cortical acetylcholine deficiency; reduced muscarinic receptor binding in hippocampus |
| Leads to cortical acetylcholine deficiency; increases muscarinic receptor density in hippocampus and cerebellum | Demyelination in brain |
| Causes demyelinating neuropathy | |
| *Neurophysiology* | |
| Causes abnormal EEGs, epileptiform activity, variable patterns, | Abnormal EEGs, epileptiform activity, variable patterns, including subtle, low amplitude seizure activities |
| e.g., subtle, low amplitude seizure activities | |
| Causes abnormal vestibular nystagmus responses; loss of sense of position in space | Abnormal vestibular nystagmus responses; loss of sense of position in space |
| Results in autonomic disturbance: excessive sweating, poor circulation, elevated heart rate | Autonomic disturbance: unusual sweating, poor circulation, elevated heart rate |

Reprinted with permission: Bernard et al. (2001) Autism: a novel form of mercury poisoning. Med Hypotheses 56: 462-471.

development and clinical manifestations of autism" (James et al., 2006).

Several authors have reported associations among mercury exposure, elevated levels of mercury in individuals, or definite mercury toxicity in ASD populations. In a 2006 paper, Monnet-Tschudi et al. noted that heavy metal concentrations caused delayed toxicity in an animal model. The authors stated that "heavy metals lead and mercury contribute to the etiology of neurodegenerative diseases and emphasize the importance of taking preventive measures in this regard" (Monnet-Tschudi et al., 2006). Also in a 2006 study, Palmer et al. found significantly increased rates of ASD in geographic areas with higher levels of mercury released into the environment. The authors' conclusion was carefully worded: "These results have implications for policy planning and cost analysis" (Palmer et al., 2006). Eliminating the environmental emissions of mercury could prove economically challenging.

In a 2008 study, Adams et al. compared mercury levels in the baby teeth of ASD children and controls. The authors found that ASD children had 2.1 times the level of mercury in their teeth compared to controls. Children with ASD also had used significantly more antibiotics in their first 12 months of life as compared to controls (Adams et al., 2007). In 1981, Seko et al. reported mechanisms by which antibiotics lead to significant reductions in excretion of mercury administered to mice. Excretion of inorganic mercury was reduced to 26% of control levels in mice given antibiotics, and total mercury excretion was reduced to 60% of control levels (Seko et al., 1981). Mice fed a synthetic diet compared to a milk-based diet were less able to excrete mercury (Rowland et al., 1984). In each case, the authors hypothesized that it was the impact of antibiotics and/or a less appropriate food source on the mouse intestinal microbiome that led to adverse changes in mercury excretion. In 2007, Desoto and Hitlan also reported a significant relationship between higher blood levels of mercury and ASD. This paper also reported reduced excretion of mercury in hair, suggesting impaired mercury excretion in ASD (Desoto and Hitlan, 2007).

In 2009, Adams et al., reported a correlation between the severity of ASD symptoms and the body burden of toxic metals. Dental amalgams were reportedly associated with ASD in a 2009 study. In that study, pregnant women with more than 6 dental amalgams were found to be 3.2-fold more likely to have a child with ASD than those women with five or fewer amalgams (Geier et al., 2009).

In a 2010 study, Geier et al. reported an analysis of red blood cell (RBC) mercury levels in ASD versus normal subjects. The authors found that RBC mercury levels were 1.9-fold higher in ASD subjects than in the control group. The authors further tested the data for a threshold that would indicate toxic mercury levels. They found that subjects with an RBC mercury level of 15 microgram/liter had a 6.4 times greater risk of being diagnosed with an ASD ($P<0.0005$) than subjects with an RBC mercury level less than 15 micrograms per liter. The authors stated that "the weight of scientific evidence supports Hg as a causal factor in subjects diagnosed with an ASD" (Geier et al., 2010).

In a study published in 2011, Garrecht and Austin evaluated the possibility that mercury is involved in the etiology of autism. The authors conclude: "the existing scientific literature supports the biological plausibility of a Hg-based autism pathogenesis". This comprehensive, well-referenced paper also discusses genetic propensities that would be expected to increase risk of mercury-associated neurodegenerative disease (Garrecht and Austin, 2011). In the same year, Lakshmi Priya and Geetha examined hair and nail levels of mercury and lead levels, as well as, levels of essential metals in hair and nails of ASD subjects ages 4-12. The authors found that levels of mercury, lead, and also copper were elevated in ASD subjects compared to controls. The more severely symptomatic ASD subjects had more marked mercury and lead elevations (Lakshmi Priya and Geetha, 2011). Also in 2011, Thomas Curtis et al. reported that mercury induced an increase in tumor necrosis factor (TNF-α) in the hippocampus and cerebellum in male (but not female) prairie voles. In addition to providing support for mercury induced neurodegenerative diseases in general, this study offers potential insight into the observation of male preponderance in ASD (Thomas Curtis et al., 2011).

## Genetics

Genetic factors underlie an individual's vulnerability to the toxic effects of mercury (Gundacker et al., 2010: Llop et al., 2014; Andreoli and Sprovieri, 2017; Llop et al., 2017). The incorporation of newer genetic (or genomic) and epigenetic insights into theories of causation of neurodevelopmental disease constitutes an important means to more fully understand the fundamental etiologies of these conditions. A number of genetic factors influencing mercury toxicity and risk of ASD have been, and continue to be, reported (Spalletta et al., 2007; Goodrich et al., 2011; Austin et al., 2014; Drescher et al., 2014; Engstrom et al., 2016; Parajuli et al., 2016; Spencer et al., 2018). ASD risk has a strong genetic component. The majority of investigators estimate that 38-90% of ASD risk arises from genetic components, and the remaining risk comes from unknown environmental factors. Consistent with the "double

jeopardy" of fetal genetic risk (see below), maternal inheritance appears stronger than that derived from paternal inheritance (Sandin et al., 2014; 2017; Yip et al., 2018).

The goal of the Casa Pia Children's Amalgams clinical trial was to evaluate risk of mercury exposure from dental amalgams in children. Results of the trial showed no overall deficit attributable to dental amalgams. However, genomic analyses linked several genetic polymorphisms to significantly increased toxicity from higher mercury levels, particularly in boys. Variant genotypes in 12 of the 13 identified genes were found to impart detriments in neurobehavioral outcomes. This major prospective study establishes links between risks of mercury toxicity and specific genes and gene variants. The authors stated that the mercury sources imparting the noted risks are largely from seafood, with only a small portion of the mercury exposure coming from amalgams (Woods et al., 2012; 2013; 2014).

In a 2017 report, Andreoli and Sprovieri discuss the increased vulnerability of children to the toxic effects of mercury. The authors note variable toxic manifestations in individuals who are exposed to the same level of mercury: "There exists a marked variability of personal response to detrimental mercury action". Further, the authors note that "new scientific evidence on genetic backgrounds has raised the issue of whether candidate susceptibility genes can make certain individuals more or less vulnerable to mercury toxicity." In their paper, the authors present a detailed assessment of multiple gene polymorphisms that would be expected to influence vulnerability to mercury exposure, calling these risk influencing genes "candidate susceptibility genes".

## ApoE

Apolipoproteins are glycoprotein components of lipoproteins, such as chylomicrons, LDL, and HDL. Apolipoproteins solubilize and facilitate the transport of lipids in plasma (Vance and Hayashi, 2010). One type of apolipoprotein, ApoE, regulates brain lipid metabolism and modulates the delivery of cholesterol to neurons and to the brain more generally. The brain is an organ with the highest cholesterol content in the human body, and ApoE is the most prominent apolipoprotein in the brain. The brain synthesizes ApoE and utilizes it for growth and remodeling (Hirsch-Reinshagen et al., 2009).

ApoE consists of 299 amino acids, and exists in three isoforms with variation in amino acids at positions 112 and 158. ApoE2 has cysteine (i.e., sulfur containing) amino acids at both positions, ApoE4 has arginine amino acids (non-sulfur containing) at both positions, and ApoE3 has a cysteine amino acid at position 112 and an arginine at 158 (Arrifano et al., 2018).

The ApoE system in the body is the most significant heritable factor known to confer risk of, or resistance to, Alzheimer's disease. Humans have two ApoE alleles, and these alleles can be either ApoE2, ApoE3, or ApoE4. One copy of each allele is inherited from each parent. The highest risk of Alzheimer's disease (and possibly ASD and mercury toxicity, as we describe below) is found in people with two ApoE4 alleles. The lowest risk, in contrast, is associated with two ApoE2 alleles and other ApoE combinations denote intermediate risk (Mutter et al., 2004). The body utilizes sulfur containing amino acids in detoxification pathways. Given that less detoxification capability would be expected in ApoE4 carriers due to the lack of sulfhydryl groups, deficient detoxification of heavy metals and other toxins is a plausible explanation for the ApoE-associated Alzheimer's disease (and possibly ASD and mercury risk characteristics) (Godfrey et al., 2003; Mutter et al., 2007).

Recent data indicate increased risk of toxicity from mercury in ApoE4 allele carriers (Arrifano et al., 2018). ApoE4 carriers were noted to have a higher risk of a variety of ASD-like abnormalities in neurodevelopment and emotional well-being in childhood. Impaired mercury detoxification would be anticipated and has been shown in individuals with two ApoE4 alleles (Ng et al., 2013; 2015). Increased prenatal exposure to mercury was shown to increase risks of adverse findings in cognitive and fine motor skills at 18 months of age, but only in ApoE4 carriers (Snoj Tratnik et al., 2017). On the other hand, no adverse impact of ApoE4 alleles on incidence of ASD was found in a 2004 study of over 300 ASD families (Raiford et al., 2004).

In a related recent study, ApoE hypermethylation was noted to be a risk factor for ASD in a Chinese population. Hypermethylation, an epigenetic marker, was associated with decreased levels of ApoE (Hu et al., 2018). This finding adds credence to the previously noted lower levels of ApoE in ApoE4 allele carriers (Arrifano et al., 2018). In addition to lower levels of ApoE and diminished detoxification capability, ApoE4 may exert its deleterious effects through domain interaction. In particular, this interaction may be related to the carboxyl and amino terminal domains which lead to production of neurotoxic fragments, and subsequently, mitochondrial dysfunction, cytoskeletal alterations, and tau phosphorylation (Mahley, 2016).

## Glutathione

Glutathione is the most important endogenous antioxidant, which is thought to be due to its free sulfhy-

dryl group (Coles and Kadlubar, 2003). Recent evidence supports the role of oxidative stress in the etiology of ASD. One important study found low levels of glutathione and increased levels of oxidized glutathione in ASD (James et al., 2004). Others studies have documented an increased prevalence of genetic polymorphisms that limit the effectiveness of glutathione mediated detoxification in ASD (Westphal et al., 2000; Buyske et al., 2006; Ming et al., 2010; Yochum et al., 2010; Rahbar et al., 2015). Together, these studies provide additional insight into oxidative mechanisms of ASD etiology. Glutathione plays a major role in defense against mercury toxicity (Cookson and Pentreath, 1996; Tokumoto et al., 2018). Different combinations of glutathione-related genes may be associated with ASD, even when the same genes are not individually related to ASD (Rahbar et al., 2015).

## Fetal double jeopardy

The above genetic and environmental factors lead to a proposed hypothesis that, to our knowledge, has not been previously articulated in the medical literature. This hypothesis accounts for individually specific fetal and infant vulnerability to mercury exposure, through maternal and paternal genetics combined with environmental exposure. Maternal genetic factors that increase risk of mercury toxicity would logically result in elevated *in utero*/fetal levels of mercury exposure. An additional role is played by the fetus's own genetic status, half of which is derived from maternal inheritance. Thus, the role of paternal genetics would be to increase or decrease vulnerability to mercury toxicity through impacting the fetal genome. Fetal and infant genetics determine the degree of toxicity derived from gestational and postpartum mercury exposure. The fetus or infant, therefore, is exposed to maternal genetic risks in two ways:

1) Maternally derived *in utero* mercury exposure related to maternal genetics and the mother's exposure to mercury during and prior to pregnancy.
2) Through the fetus's own maternally and paternally derived genetic makeup. Thus, the fetus or infant is exposed to paternal genetic risk only through the inheritance of genes from the father. These factors explain the observation that maternal ASD traits carry more risk to the child than paternal ASD traits.

## The fetal double jeopardy hypothesis

The fetus accumulates an amount of mercury, and the amount depends on the genetics of the mother in combination with the fetus's own genetics, as well as, maternal exposure to mercury prior to and during the pregnancy. It is the fetus or infant's own genetic characteristics – that are derived from maternal and paternal inheritance – that determine how the child handles the mercury load to which he/she is exposed. In the post-partum period and extending throughout infancy and early childhood, there is additional mercury exposure through vaccines, food, and potentially other exposures. Eventually, a threshold may be crossed that results in the neurologic manifestations that we recognize as ASD.

## Epigenetics

Modifications of DNA comprising the genome are required for the myriad types of cells in an individual to perform their varied functions. A skin cell needs to have characteristics distinct from a brain cell, for instance. These DNA modifications fall into a recently defined category called "epigenetics". DNA modifications can include DNA methylation, histone modifications, microRNA, and other changes. Identical twin studies in ASD strongly implicate epigenetic mechanisms, based on evidence that genetically identical twins are not universally concordant in expression of ASD traits (Hu, 2013).

## MicroRNA

MicroRNA (miRNA) are an emerging feature of epigenetic relationships in ASD. MiRNA are non-coding segments of RNA, and about 22 base pairs in length. MiRNA are a means of controlling gene expression, by preventing transcription of specific proteins/genes. Estimates suggest that over 60% of all human genes are subject to miRNA regulation, and each miRNA has dozens to hundreds of potential targets (Ander et al., 2015). Recent findings demonstrate the relevance of miRNA to long-term memory formation, cognition, neuronal development, and plasticity, resulting in potentially profound impacts on brain function (Butler et al., 2016).

Recent studies have found associations between abnormal miRNAs and ASD. In a 2008 report, nine miRNAs were found to be expressed at significantly different levels in post mortem cerebellar tissue from ASD as compared with normal control tissue. The genes targeted by these miRNAs are known genetic causes of ASD (Abu-Elneel et al., 2008). In another post mortem tissue analysis, six specific miRNAs were found to be expressed at different levels in ASD compared with control samples (Ander et al., 2015). Several other studies have identified miRNAs associated with ASD (Ghahramani Seno et al., 2011; Mundalil Vasu et al., 2014;

Ander et al., 2015; Hicks et al., 2016; Wu et al., 2016; Jyonouchi et al., 2017; Schumann et al., 2017; Vaccaro et al., 2018; Yu et al., 2018). Of note, the genes targeted by these ASD-associated miRNAs play a key role in a range of ASD-related functions, including but not limited to: social and language behavioral dysfunctions, TGF-beta signaling, regulation of the actin cytoskeleton, oxidative phosphorylation, focal adhesion, mTOR signaling, ASD sexual dimorphism, neuronal plasticity, and neuronal development.

The diagnosis of ASD is often hampered by lack of useful measurable markers. MiRNA detection may aid in establishing a diagnosis of ASD. One particular miRNA shows promise as a serum biomarker for ASD (Cirnigliaro et al., 2017). A salivary miRNA evaluation appears effective as a non-invasive screening method for markers for ASD (Hicks et al., 2016).

Together, it is clear that the accumulation of miRNA findings in association with ASD has been substantial over the past several years. The gene targets of the ASD-associated miRNAs add support to prevailing concepts of the pathophysiology of ASD. The unanswered, and generally unasked question is - what is the reason that these miRNAs exist? Are these ASD-associated miRNAs a root cause of the neurologic manifestations of ASD, or do they reflect a response to another, more fundamental cause of ASD?

Environmental factors have a major effect on epigenetic modifications. These modifications often allow for adaptation to these environmental factors, and the modifications tend to be inherited (Feinberg, 2018). For instance, one study showed that mercury exposure induced behavioral changes in zebrafish, and these behavioral changes were subsequently inherited by offspring who lacked direct mercury exposure (Napier et al., 2016). Other characteristic epigenetic modifications have been reported in response to toxic exposures, including mercury (Basu et al., 2014). In 2015, Bakulski et al. reported cord blood epigenetic changes in association with mercury exposure. In another 2015 study, epigenetic changes in paternal sperm were reportedly associated with increased ASD risk in offspring (Feinberg et al., 2015). Thus, miRNA abnormalities may be related to exposure to toxins, including mercury (Li et al., 2015; Sanders et al., 2015). The finding of epigenetic changes in various diseases and exposures suggests that epigenetic science will be a valuable research tool in understanding the etiology of challenging diseases such as ASD. Utilizing epigenetic methods to seek evidence for toxic exposures in ASD cohorts may produce even more evidence for a link between the two variables. Bakulski and Fallin (2014) stated that "epigenetics may represent a mechanistic link between environmental exposures, or genetics, and many common diseases".

## Vaccines and ASD

For over twenty years, mercury from vaccines has been suspected to play a causal role in ASD. The transition from a normal toddler or young child to one that is impaired, often to a severe degree, is heartbreaking to parents. If that child has recently been vaccinated, especially with vaccines containing the preservative thimerosal, the natural tendency for parents is to blame the vaccines. Thimerosal contains mercury in the form of ethyl-mercury. Fortunately, thimerosal has largely been removed from pediatric vaccines, although it remains in some multidose vials.

On the other hand, medical professionals involved in public health recognize the unparalleled benefit that vaccines have provided in preventing dangerous illnesses, many of which have fortunately faded in our collective memory. The capability of preventing a variety of infectious diseases via vaccination ranks as one of the most significant achievements in medicine. The success in vanquishing severe diseases, which were sources of considerable morbidity and mortality, has led to appropriate enthusiasm in applying the expertise gained in vaccine development and utilization to ever more diseases. An increasing numbers of vaccinations, particularly in children, have occurred at a time when ASD incidence and prevalence have increased, for reasons unknown. It is not surprising that some concerned individuals have suspected that vaccinations might be causally related to the increasing occurrence of ASD.

Vaccines and other medicines in the past have contained mercury in the form of ethyl mercury, a constituent of thimerosal and used as preservative. Compounds of mercury are known for being effective anti-bacterial agents. Multiple dose vials of vaccine are the most likely form of vaccines to contain thimerosal, since inadvertent introduction of microbes into the vial when withdrawing a vaccine dose might contaminate the other doses remaining in the vial. Potential mercury exposure, especially in children who are more vulnerable to associated neurotoxicity, has led to concerns that mercury in the vaccines could be causing neurodegenerative diseases. This concern has led to the removal of mercury from most vaccines and other medicines.

Multiple peer reviewed manuscripts have been published regarding the potential connection between vaccinations and neurodevelopmental disease. Many of these research papers support the potential toxicity of mercury in vaccines (Makani et al., 2002; Baskin et al., 2003; Burbacher et al., 2005; Geier and Geier 2003; 2006a; 2006b; 2007; Humphrey et al., 2005, James et al., 2005; Yel et al., 2005; Young et al., 2008; Geier et al., 2009; Gallagher and Goodman 2010). In a 2003 report, the Vaccine Adverse Events Reporting System (VAERS)

database demonstrated a marked increase in risk of neurodevelopmental disorders in children who received thimerosal containing DTaP vaccines compared with thimerosal-free but otherwise similar vaccines. The relative risk of ASD with the mercury containing vaccines was 6.0. The relative risk of mental retardation was 6.1 (Geier and Geier, 2003).

Other publications report ostensibly reassuring safety data regarding the risk of serious neurologic consequences from vaccinations in general, and thimerosal in particular. However, weak neurotoxicity associations with mercury in vaccines were reported in these studies, noting problems with attention, executive functioning, speech articulation, and motor tics in boys. Despite the reassurance of the authors of these studies, refuting the vaccine-ASD connection, the association of thimerosal with neurologic symptoms, albeit mild ones, is less comforting to others (Stehr-Green et al., 2003; Verstraeten et al., 2003; DeStefano, 2007; Thompson et al., 2007, McMahon et al., 2008; McGuinness 2015; Gogoi and Chatterjee 2016).

The authors of these papers expressing differing conclusions are likely sincere scientists, truly believing the assessments they have articulated. One clear fact persists: there is not unanimity of opinion on whether a vaccine might contribute to ASD risk. How can we reconcile these disparate findings? Genetics may hold an answer. The previously noted genetic factors involved in the risk of mercury toxicity were not considered in vaccine studies, given that the genetic factors were more recently reported. These genetic (or genomic) factors may be part of the reason for the conflicting conclusions of vaccine safety studies.

Of course, the above discussion of vaccine and thimerosal risk is rendered nearly moot by the removal of thimerosal from almost all vaccines. On the CDC website, the vaccine summary PDF notes that, to-date, thimerosal is present only in a limited number of vaccines: Fluvirin, meningococcal vaccine, Td (Mass Biologics), and in multi-dose vials of the influenza vaccine. Of note, the influenza vaccine is recommended for pregnant women, infants, and children. Thimerosal continues to be present in childhood vaccines that are used in developing countries (Sykes et al., 2014). The risk of inducing thimerosal-mediated neurologic toxicity persists, although removal of mercury from many vaccines has reduced that risk. It is incumbent on parents and physicians to keep that risk low through awareness and avoidance of mercury-containing vaccines, when possible.

## ASD etiology

Progress in prevention and treatment of ASD has been rendered limited or impossible by the lack of fundamental understanding of the etiology. The factors discussed in this paper point to a hypothesis that is consistent with the facts as currently understood and accounts for the clinical manifestations, heritability, genetics, and clinical course of ASD.

## The mercury hypothesis of autism spectrum disorder

To summarize the data and hypotheses presented above; when present in excess in the brain, mercury causes (via free radical and oxidative reactions) (Sarafian et al., 1994; Shenker et al., 2002) brain inflammation (Lee et al., 2010; Theoharides and Zhang 2011; Theoharides et al., 2016) and mitochondrial dysfunction (Smith et al., 2012). Inflammation and mitochondrial dysfunction, in turn, lead to dysfunction of the brain to various degrees, manifesting as the clinical picture of ASD. Children are at highest risk of developing ASD due to the increased vulnerability of the developing brain (Andreoli and Sprovieri, 2017).

Genetic factors impact the potential of each individual to experience toxicity from a given level of mercury exposure, and the consequent likelihood of clinical brain dysfunction (Goodrich et al., 2011; Parajuli et al., 2016). These genetic influences impact the degree to which a given amount of mercury will be absorbed into the body, and/or how effectively it will be eliminated or metabolized, if at all. Genetic factors can also modulate the degree of damage from a given amount of mercury in that individual. The "double jeopardy" highlights the prenatal impact of maternal gene-mediated mercury metabolism and the fetal innate genetic mercury resistance or vulnerability. This combination of factors creates a unique pattern of inheritance that is difficult to explain through other mechanisms. The prenatal period is particularly important, due to the increased risk of the developing fetal brain to damage from mercury (Bjorklund et al., 2017). In the postpartum period, the infant's own mercury management takes over and is completely dependent on the infant's own genetics - as inherited from maternal and paternal sources. The developing infant brain only very gradually becomes less at risk from mercury exposure, eventually reaching the adult level of vulnerability as adulthood approaches. Therefore, the risk from mercury exposure continues throughout infancy and onward. The source of these exposures may include dietary components (largely seafood), vaccines and medicines, dental amalgams, and random environmental exposures (for e.g., a broken mercury thermometer, or broken florescent or CFL light bulb.)

There are challenges unique to pursuing the potential role of mercury in neurologic disease. For one,

mercury impairs enzyme functioning which has the effect of limiting its own excretion. In addition, urine mercury levels are unreliable indicators of mercury levels in the body (Ely, 2001). Even hair levels of mercury are not reliable, being low at times when they are expected to be high. Mercury also exists in different phases in the body. Mercury from fish sources is in the form of methyl mercury whereas vaccines may contain ethyl mercury. Methyl mercury has a blood half-life of about 60 days (Smith and Farris, 1996) whereas the blood half-life of ethyl mercury is about 10 days or fewer (Dorea et al., 2013). In the brain, however, methyl and ethyl mercury are partially converted to the inorganic state, which has a half-life measured in several decades. This means that, once mercury reaches the brain, it is essentially there permanently. There is no assay to determine how much inorganic mercury is in a living person's brain (Bjorkman et al., 2007). There is speculation about the relative fates of methyl and ethyl mercury. The shorter blood half-life of ethyl mercury may be related to its more rapid conversion to inorganic mercury in the brain and other tissues (Harry et al., 2004). Given that methyl and ethyl mercury are cleared from the blood in a relatively short time, and that inorganic brain mercury is not measurable, it is apparent that longer term mercury exposures are much more difficult to ascertain (Mutter et al., 2010).

Why has an association between the toxic effects of mercury and neurodevelopmental disease not been previously recognized? One reason may be the heterogeneous response to a given level of mercury in different people. As previously noted, genetics may render some persons relatively resistant to levels of mercury that, in other persons, are quite toxic.

Another reason for resistance to recognizing subtle mercury toxicity is the economic consequence of that recognition. Dental amalgams are fortunately rarely used in the United States, but the removal of those already in place would entail much expense. More concerning is the mercury emitted into the environment through fossil fuel burning power plants, some types of gold mining, cement production, and other industrial processes. In addition, some vaccines may still contain low levels of mercury and seafood often contains significant amounts. The elimination of mercury contamination of our air, water, medicine, and food would entail considerable expense, and require decades or longer to substantially accomplish. A high level of evidence will be required for the societies and nations of the world to accept that such a huge effort is needed and/or economically responsible.

Perhaps the most useful approach at this time for determining the role, if any, that mercury plays in the pathogenesis of ASD, would be through genomics and the related field of epigenetics. It is well within the grasp of current research methods to detect target mutations or epigenetic modifications involved in impaired mercury handling. Should studies of ASD patients show increased numbers of such mutations or modifications compared with controls, that would constitute strong evidence for a causal role of mercury in the pathogenesis of ASD. The remarkable Casa Pia study, discussed previously in this paper, did in fact show an association between mercury and mild cognitive impairment in apparently normal children who had specific genetic polymorphisms implicated in mercury handling (Woods et al., 2014). Researchers are identifying a rapidly increasing number of such mutations in genes that modify the body's handling of mercury (Andreoli and Sprovieri, 2017). Evidence of increased mercury handling mutations or certain epigenetic characteristics in individuals with ASD and, more importantly, their mothers, would imply a causal role for mercury in ASD. Given the vulnerability of the fetal brain to low levels of mercury, a maternal genomic/epigenetic study is clearly needed. The relative strength of each of the proposed mutations or epigenetic modifications would likely vary, thereby complicating the interpretation of such findings. There is already evidence linking genetic variation in one mercury handling polymorphism - the ApoE system - to Alzheimer's disease and ASD. In particular, carriers of the ApoE4 allele demonstrate increased propensity for both mercury toxicity and neurodevelopmental disability, including Alzheimer's disease and ASD (Strittmatter et al., 1993; Godfrey et al., 2003; Ng et al., 2013; 2015). The potency of ApoE4 in expressing these effects may overshadow lesser mutations.

Finally, a comprehensive review found that, as of 2016, there were 91 original studies in humans that examined the potential relationship between mercury and ASD. Of those 91 studies, 74% found a relationship between mercury exposure and ASD, indicating that mercury exposure may play a causal and/or contributory role in ASD (Kern et al., 2016).

## CONCLUSION

Mercury is a complex element and is unique among the toxic heavy metals in that it exists in three phases in the body (i.e., elemental, inorganic, and organic). The impact of mercury on its own means of excretion complicates our attempts to understand the subtleties of its impact on human pathophysiology. Recent advances in genomics and epigenetics offer a previously

unavailable means to determine if mercury plays a role in a number of human diseases.

ASD has clinical and pathophysiological characteristics that are strongly suggestive of mercury toxicity. At present, ASD lacks a broadly accepted or identified etiology. The data presented in this paper are consistent with the notion that the etiology of ASD arises during the gestational period and beyond as a manifestation of mercury toxicity. Although beyond the scope of this paper, other heavy metals and toxic exposures that have similar toxic manifestations may eventually be incorporated into this hypothesis.

Additional research is needed to understand the potential role of mercury in ASD. Genomic and epigenetic analyses should identify populations that are vulnerable to mercury toxicity. These approaches may hold the long-awaited key that allows for the identification of a fundamental etiology of ASD, and potentially, a broad range of neurodevelopmental diseases. The prospect of finally being able to finally prevent and treat such diseases is tantalizing, indeed.

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